SCHOOL OF PH.D. STUDIES
SEMMELWEIS UNIVERSITY

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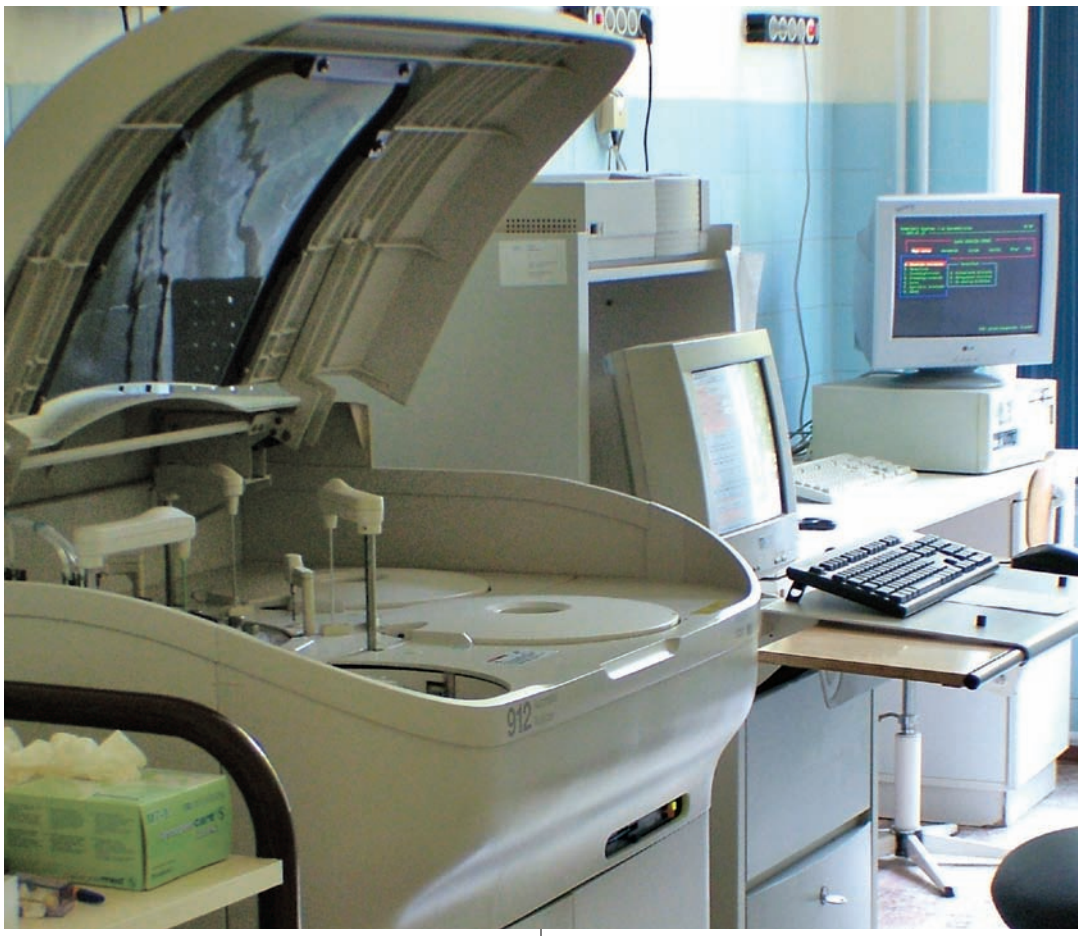
GENERAL OVERVIEW

Semmelweis University's School of Ph.D. Studies' foundations were laid in the early 1990s, following *Act LXX of 1993 on Higher Education*, which for the first time gave individual universities the right to issue doctoral degrees.

There are hundreds of Ph.D. students enrolled in one of the more than 40 training programmes offered by the University's eight doctoral schools. These students work under the supportive guidance of the School's dedicated tutors, and can choose from among the 60–120 courses that are offered by the School each semester.

The School's professors and tutors come either from one of Semmelweis University's six faculties, or from the institutes and laboratories of various other Hungarian universities. This level of interdepartmental and interuniversity collaboration has lead to the creation of new research centres that function as true melting pots.

The supportive guidance and knowledge of the School's experienced professors and talented tutors has a great impact on the research work of young candidates, whose motivation is augmented from being able to meaningfully interact with experts in their particular field. Indeed, by involving the greatest authorities from within, as well as from without the university, the School of Ph.D. Studies has, from the beginning, been able to preserve the quality and prestige of the degree.



ORGANIZATIONAL STRUCTURE

The School of Ph.D. Studies is an autonomous educational body of Semmelweis University; its activities are subject to the decisions made by the University's Doctoral Council, which meets every second month of the academic year. The Doctoral Council determines the content of the Ph.D. programmes, the admission procedures and the admission fee. The work of the Doctoral Council is supported by the Doctoral Secretariat, which is also responsible for providing detailed information about these to the applicants.

The School of Ph.D. Studies is organised around doctoral schools, which serve as umbrellas for related branches of science and their respective programmes. Each doctoral school has its own council, which serves as its central administrative body. Nonetheless, the individual programmes continue to enjoy a considerable amount of independence.

Currently the School of Ph.D. Studies at Semmelweis University has eight doctoral schools:

1. Basic Medicine
2. Clinical Medicine
3. Pharmaceutical Sciences
4. Mental Health Sciences
5. Sport Sciences
6. Neurosciences (János Szentágothai)
7. Molecular Medicine
8. Pathological Sciences.

The School of Ph.D. Studies integrates research groups and programmes from all the faculties of Semmelweis University that are entitled to issue Ph.D. degrees. The University's largest faculty, the Faculty of Medicine, is represented in almost every doctoral school. The faculties of Dentistry and Health Sciences each have their own study programmes, while the Faculty of Pharmacy and the Faculty of Physical Education and Sport Sciences are represented by their own doctoral schools.

The basic unit of the School's complex educational system is "one student—one tutor". Together, they enjoy a high level of freedom and autonomy in conducting their research, within the limits set by the School's rules and regulations.

PROGRAM OVERVIEW

The Ph.D. program at Semmelweis University consists of two parts: the Educational Phase (Phase I) and the Qualification Phase (Phase II).

Phase I: Educational Phase

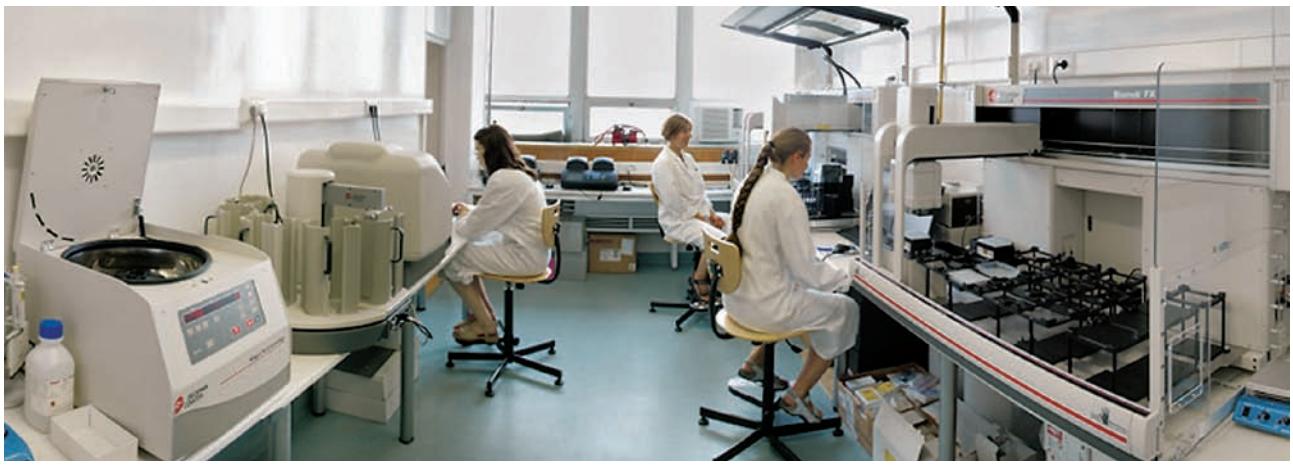
The aim of Phase I is to train students to become scientists through coursework and research activity. It is in this phase that students select the specific scientific topic that will become the core of their final dissertation. Research is conducted in collaboration with faculty members, while a qualified tutor supervises each student.

Phase II: Qualification Phase

Phase II provides students with the opportunity to evaluate the results of experiments and publish them in acknowledged scientific journals. Naturally this is, or can be, an ongoing activity in Phase I as well. At the end of this phase, students are required to pass a comprehensive examination and to write and defend a dissertation. Since dissertations are required to be made available to the public prior to its defence, an online database containing hundreds of full-text doctoral theses, and their respective synopses has been set up by the School, in order to facilitate this process. Although Phase II logically follows Phase I, students may choose to skip the first phase and go straight into Phase II, provided that the necessary prerequisites and requirements have been met.

Ph.D. Courses

There are a number of courses announced on the School's website each semester. The list of required courses, which have to be taken during the Ph.D. training period, is finalised each year a few weeks after registration. Beginning in 2002, the doctoral schools have published a study plan for the entire training period, while a database of available courses is made accessible through the School of Ph.D. Studies' website, giving students the freedom to easily construct their own individual study plan.



ADMISSION AND TUITION

The School of Ph. D. Studies offers three forms of education:

- Full-time, entering Phase I as students
- Part-time, entering Phase I as students
- Individual studies, entering Phase II as candidates.

Both full-time and part-time students are required to meet the same admission requirements. Individuals who join the programme in Phase II are not considered students, and will not be given a record book or student identity card; rather, they are considered candidates for the doctoral degree.

Admission Requirements and Process

Doctoral applicants must

- be university graduates or students registered for their final semester of university studies
- possess at least a certified C type (oral and written) state foreign-language exam or an an equivalent certificate if applying admission to the Hungarian-language program. Applicants for the English-Language program must have a good command of English.

When applying to the programme, applicants are required to state the specific training programme and research topic they wish to pursue within one of the University's doctoral schools.

The admission procedure is based on evaluating the candidate's

- general knowledge and personal ability
- topic-related knowledge and academic competence
- previous scientific activity and contribution.

The admission board of each doctoral school creates a ranked list of candidates which is submitted to the University's Doctoral Council. The Doctoral Council makes the final decision regarding admissions.

Tuition Fees

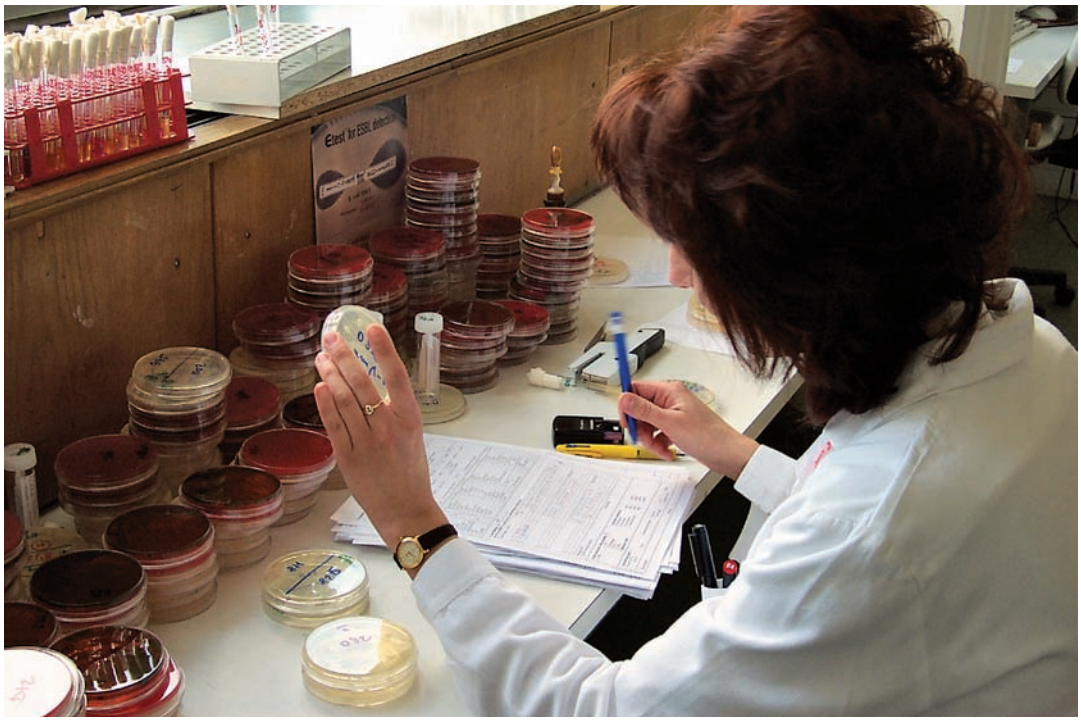
Certain costs of education, scientific training and official procedures are covered by students and candidates. Most of the fees are equal to or close to what is ordinarily paid by undergraduate students.

DOCTORAL COUNCIL

President of the Doctoral Council: **Dr. Ágoston Szél**

Members of the Doctoral Council:

Dr. Gábor Makara	Vice-President, President of the Educational Board
Dr. Veronika Ádám	Vice-Rector for Scientific and International Affairs
Dr. László Rosivall	Basic Medicine Doctoral School
Dr. Zsolt Tulassay	Clinical Medicine Doctoral School
Dr. Károly Rácz	Clinical Medicine Doctoral School
Dr. Éva Szőke	Pharmaceutical Sciences Doctoral School
Dr. István Bitter	Mental Health Sciences Doctoral School
Dr. Miklós Réthelyi	János Szentágothai Neurosciences Doctoral School
Dr. György Nagy	János Szentágothai Neurosciences Doctoral School
Dr. József Mandl	Molecular Medicine Doctoral School
Dr. Emil Monos	Basic Medicine Doctoral School
Dr. József Tihanyi	Sciences Doctoral School
Dr. Pál Magyar†	Representative of the Faculty of Medicine
Dr. Gábor Varga	Representative of the Faculty of Dentistry
Dr. Kálmán Magyar	Representative of the Faculty of Pharmacy
Dr. Péter Tamás Sótonyi	Representative of Szent István University
Dr. Zsolt Radák	Representative of the Faculty of Physical Education and Sport Sciences
Dr. János Rigó	President of the Quality Control and Evaluation Board



PERMANENT COMMITTEES OF THE DOCTORAL COUNCIL

Educational Board (EB)

The Educational Board (EB) meets at least once in a half year. It expresses opinion on course proposals and requests for financial support for courses. If needed, the EB can alter courses. It can make proposals in the following matters: (1) the structure of teaching, (2) the coordination of courses, (3) the nature of the method of course registration and registration deadlines, (4) the establishment of credit points which can be given to each course and (5) the recognition of credit points.

Students receiving tuition are advised to choose those courses which are promoted by the Doctoral Council of Semmelweis University. Theoretical knowledge and skills necessary for research are obtained from the compulsory course modules. Throughout the year the Ph. D. schools organize optional courses. Some of them organize courses which are obligatory for all students who are registered in that particular school.

President of the Educational Board: **Dr. Gábor Makara**

Members of the Educational Board:

Dr. László Rosivall	Basic Medicine Doctoral School
Dr. Béla Molnár	Clinical Medicine Doctoral School
Dr. Sylvia Marton	Pharmaceutical Sciences Doctoral School
Dr. László Tringer	Mental Health Sciences Doctoral School
Dr. Gábor Pavlik	Sport Sciences Doctoral School
Dr. Emília Madarász	János Szentágothai Neurosciences Doctoral School
Dr. Ágota Vér	Molecular Medicine Doctoral School
Dr. Ferenc Rozgonyi	Pathological Sciences Doctoral School
Áron Cseh	Representative of the Doctoral Students' Union

Members of this body debate and decide whether a candidate has a thorough grounding in academic work by examining his/her previous performance in education and research and the elected topic for research. The board decides whether the candidate will or will not be able to produce a thesis three or four years after his/her entrance into the system.

Quality Control and Evaluation Board (QCEB)

The efficiency of the doctoral training is shown in the frequency of completed doctoral theses and academic publications. The standards of these works are judged by scientometric indicators. This process evaluates both the academic competence of individual Ph.D. candidates and also the academic standards of the Doctoral School.

One of the most important acts of quality control is that everybody involved in the process complies with the instructions given in the qualification phase by the Doctoral Council (DC). The importance of this issue led to the establishment of The Quality Control and Evaluation Board (In Hungarian: VMB). This first evaluating forum controls

whether the submitted work fulfils the conceptual, structural and formal requirements of a doctoral thesis. Consequently, a formal opinion is released stating whether the Ph.D. candidate acquired the scientometric indicators prescribed in the Book of Regulations of the Doctoral School or not.

President of the Quality Control and Evaluation Board: **Dr. János Rigó**

Members of the Quality Control and Evaluation Board:

Dr. Tamás Ivanics	Basic Medicine Doctoral School
Dr. László Herszényi	Clinical Medicine Doctoral School
Dr. István Antal	Pharmaceutical Sciences Doctoral School
Dr. Katalin Hegedűs	Mental Health Sciences Doctoral School
Dr. Gyöngyi Szabó (Földesiné)	Sport Sciences Doctoral School
Dr. András Csillag	János Szentágothai Neurosciences Doctoral School
Dr. László Hunyady	Molecular Medicine Doctoral School
Dr. Janina Kulka	Pathological Sciences Doctoral School
Katalin Dezső	Representative of the Doctoral Students' Union

Considering the proposals given by the QCRB (VMB in Hungarian) the Doctoral Council decides whether the doctoral thesis can be sent to the opponents. At a first glance this process might seem unnecessary because the control of requirements looks like a simple administrative issue. However, the process of evaluation described above—which applies the general rules (sometimes with a great deal of empathy)—is highly desirable for a number of reasons: there is a great variety of research topics, the issues discussed range from molecular biology to behavioral sciences which must be able to win academic recognition in Hungary and abroad.

Apart from works which meet all the standards there are doctoral theses which are inadequate in their content and structure and are therefore unacceptable. Candidates who do not fulfill the main requirements are advised to withdraw their dissertation prior to a detailed, expert review. We are proud that only thoroughly controlled doctoral theses are given in the hands of official opponents and reviewers. It is worth mentioning some of these requirements which doctoral candidates must take into consideration.

(a) Only those candidates deserve scientific degrees who are able to publish their results intelligibly and based on a coherent analysis. Summarizing the content of two or three excellent publications is not equal to a doctoral dissertation.
(b) Some articles which were published in high-rank international journals with a sophisticated title and a high impact factor are not sufficient to create the basis for Ph.D. qualification. Only those articles are potential items for recognition which are the result of real academic work. Therefore, articles summarizing the academic literature of a particular topic or commenting on the academic work of others as "letters to the editor" are not acceptable. By contrast, articles published in a journal under the latter heading could include original scientific results. But this must become clear from the presentation of scientific methods and/or from the editor's professional opinion.

(c) The QCRB during its evaluation takes into consideration the grammatical correctness and style of the English or Hungarian language, the length as well as the external appearance of the thesis and the quality of illustrations.

These examples illustrate that the board has a high level of responsibility in defining the academic quality of Ph. D. qualifications. At the same time the board is meticulously tactful in giving criticisms. Therefore, written judgments also include constructive suggestions for correcting the deficiencies discovered. This professional opinion is sent to both the candidate and the head of the training program. This process creates grounds for the necessary corrections and gives the possibility of legal remedies.

When this evaluation process is applied in practice, for instance, nine doctoral theses out of ten are found acceptable. The other one is rejected usually because basic requirements are not fulfilled, i. e. the shortage of sufficient acceptable publications. Furthermore, it occurs that two or three theses are rejected in form (i. e. the outward appearance and/or some of the essential requirements are missing from the package which was submitted to the QCRB). In these cases the professional review of the doctoral theses will be delayed until the corrections are completed and necessary supplements are submitted.

One of the major duties of the QCRB is to report its experience to the Doctoral Council and to suggest proposals concerning the alterations in the Rules of the Doctoral School if necessary. Apart from the duties described above the QCRB has authority over any stage of the doctoral procedure in scientific matters. Furthermore, it passes judgments on applications, looks into the matters of complaints and makes decisions concerning naturalizations (e. g. the recognition of qualifications).

Since 2000 the Quality Control and Evaluation Board has had this role of quality evaluation within the Doctoral School.

Disciplinary Procedures Committee

The activity of this committee is needed only in exceptional cases, of which plagiarism and disharmony between student and tutor have given some work to the committee in the past years.

President of the Disciplinary Procedures Committe: **Dr. György Nagy**

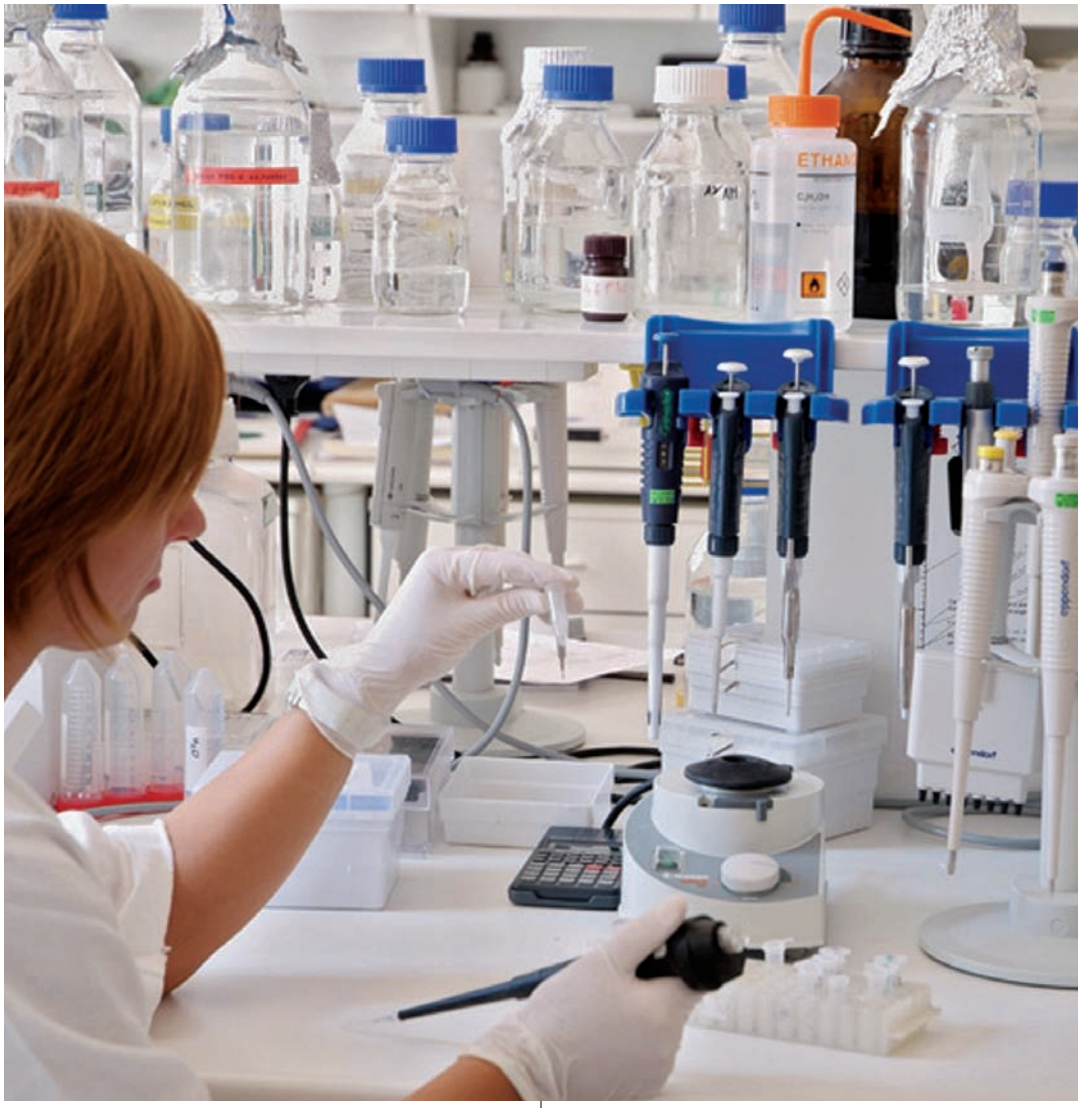
Members of the Disciplinary Procedures Committe:

Andrea Dunai	Basic Medicine Doctoral School
Dr. István Antal	Pharmaceutical Sciences Doctoral School
Emese Ficsor	Pharmaceutical Sciences Doctoral School
Orsolits Barbara	János Szentágothai Neurosciences Doctoral School

DOCTORAL SECRETARY OFFICE

Emőke Márton	Head
Anna Marádi (Pintérné)	Financial officer
Anita Marosfalvi	Adviser
Tímea Rab	Adviser

The administrative duties of the Doctoral School are managed by the Doctoral Secretary Office which creates a link between the Doctoral Council, the Ph.D. training programs and the Ph.D. students. It maintains permanent contact with the educational bodies outside of the university (e.g. Ministry of Education, Hungarian Accreditation Board, etc.). The Secretary is available for Ph.D. students on an office-hours basis, however, they are ready to help anytime in urgent cases. In one little room practically every major step of the degree obtaining process is handled, starting from the first inquiries and paper work of the entrance examination, all the way up to the preparation of the diplomas. (Address: H–1085 Budapest, Üllői út 26, ground floor Nr. 9.)



DOCTORAL STUDENTS' UNION (DSU)

The DSU (In Hungarian: DHÖK) is a body elected by the doctoral students in order to legally represent their interests both inside and outside the university; e. g. in the Doctoral Council and the National Association of Doctoral Students. Members of the union participate in the *ad hoc* commissions dealing with matters in their interest and are responsible for organizing the Scientific Ph. D. forums. The head office of the DSU is: The Students' Office of Semmelweis University (H–1089 Budapest, Nagyvárad tér 4, ground floor Nr. 18).

Members of the Union of the Doctoral Students are also accessible through the Doctoral Secretarial Office.

Members of the Doctoral Students' Union:

Andrea Dunai	Basic Medicine Doctoral School
Áron Cseh	Clinical Medicine Doctoral School
Emese Ficsor	Pharmaceutical Sciences Doctoral School
Nikoletta Bódi	Mental Health Sciences Doctoral School
Zoltán Cserháti	Institute of Psychiatry and Psychotherapy
Kinga Kiszela	Sport Sciences Doctoral School
Barbara Orsolits	János Szentágothai Neurosciences Doctoral School
Péter Szelényi	Molecular Medicine Doctoral School
Katalin Dezső	Pathological Sciences Doctoral School

INTRANET

Website: http://www.phd.sote.hu

The website of the Doctoral School at Semmelweis University is an essential means for organizing doctoral education. Hundreds of Ph.D. students take the courses as part of more than thirty training programs of eight doctoral schools under the supervision of hundreds of tutors in different locations. Every half year the school organizes 60–120 courses; the lectures and seminars are given in more than 50 locations within the university.

There are great advantages stemming from the intensity and variety of our education system. Therefore, it is a high priority to publish information which is clear-cut and accessible by everybody. The gradual augmentation of the website leads to the accumulation of information about every training program and sub-program and of every teacher and doctoral defense since 2000. Importantly, the website provides information about on-going courses and application possibilities. All application forms and documents, which are necessary for the administration of doctoral matters and the resolutions of the Doctoral Council, are also accessible on the website. Other detailed information and curricula are also available electronically. The website provides addresses, telephone numbers and e-mail addresses.

The regulations, the forms, the course and program data, the decisions of the Doctoral Council and the invitations to all defense ceremonies are accessible via the Internet. News on important conferences, university events, calls for proposals are also not missing from the repertoire.

The website opens a possibility for course leaders to put the information about their courses directly to the website. The Doctoral Secretarial staff manages and publishes all the relevant information: e.g. general news, advertisements and Ph.D. defenses without the assistance of a web supervisor.

The website has considerable web traffic according to statistical figures. The average number of visitors a day was 2000 between 1 March and 30 June 2003, in May it went up to 3500 daily visits. The number of downloaded files was 800–1000 every day and in May it reached a daily 2000. Since then this figure then has increased due to the high demands for electronic admission information. (These figures represent only the general turnover not the actual numbers of visitors. Repeated visits by the same person are registered each time, therefore, the real numbers of inquirers are unknown.)

The database system of presenting doctoral theses on the internet was set up. It is required that doctoral theses be available to the public before the defense so they may be accessed electronically in full through the internet simultaneously with the announcement of the Ph.D. defense.

The “Dissertation Abstracts”, a trademark of ProQuest, allows for the availability of each recently defended thesis all over the world. Older works are not available yet, however we try to put out as many of our precious creations as possible.

'VERITAS ET VIRTUS' AWARD IN MEMORY OF DR. ZSOLT FARKAS JR.

After the tragic death of Dr. Zsolt Farkas Jr., a Ph.D. student of the Doctoral School, his parents, Dr. and Mrs. Zsoltné Farkas established a foundation in memory of their late son. The aim of the foundation is to support financially research by Ph.D. candidates.
Some of the aspects of the charter are: *"The aims of the Foundation are to subsidize the work of Ph.D. students under thirty-five years who are concerned primarily with physiological research in the Doctoral School of Semmelweis University. Additionally, it contributes to the realization of the aims of the Doctoral School, i.e. to improve the quality of doctoral education, to facilitate the acquisition of widely recognized scientific degrees, to provide financial support for the expansion of accredited doctoral research programs, to establish pre-doctoral scholarships and to improve scientific communication. (…) Those Ph.D. students are able to benefit from the payments of the foundation whose submitted work wins a public competition advertised by the trustees of the Foundation. The type of work submitted can be in the process of publication but the candidate must be its first author. Other details are defined by the committee of trustees who are responsible for both advertising and reviewing the submitted work. (…) The awards must be transferred ceremonially to the winners every year on the Dies Academicus (first Saturday of November)."*
The Office of the Foundation is: H–1085 Budapest VIII., Üllői út 26. The trustee committee is the Advisory Board of the Foundation. The president of the Advisory Board of the Foundation is always the current head of the Doctoral Council at the Semmelweis University, at present, university professor, Dr. Ágoston Szél.

Recipients of 'Veritas et Virtus' Awards

2006	**Áron Lazáry**	Ph.D. School for Clinical Science in Medicine
	Lilla Reininger	Ph.D. School of Pathology
2007	**Katalin Dezső**	Ph.D. School of Pathology
	Balázs Sármán	Ph.D. School of Clinical Science in Medicine
2008	**Judit Lazáry**	Ph.D. School Mental Health Sciences
	Anikó Ludányi	János Szentágothai Neurosciences Ph.D. School

PH.D. COURSES

Every semester there are a number of courses (60–120) announced on the website. Previously, the Educational Board of the Doctoral School filtered down the number of courses to between sixty and seventy. The Doctoral Council of Semmelweis University took into consideration the views the Educational Board and restricted the number of courses which can be run by each departmental doctoral school in one semester to between five and seven.

Since then the following procedure has been established concerning Ph.D. courses, i.e. the Doctoral Committee of each Departmental Doctoral School proposes between five and seven courses at the beginning of each semester which are entered the database of current courses. However, the Educational Board can recognize and award credit points for participation in Ph.D. courses of the appropriate standard at other universities. In this case the Educational Board needs the recommendation of the tutor and the head of the departmental doctoral school of the student in question.

The database of available courses is accessible at the website of the Doctoral School. Consequently, students are able to access the database and construct their own individual study plan.

Compulsory courses	**Course leaders**	**Semester**
Elements of molecular biology	András Váradi, Mária Sasvári, László Buday	2007/2008/1
Isotope use methodology and radiation protection	István Voszka	2006/2007/2, 2007/2008/2, 2008/2009/2
Research methodology and ethics	Péter Csermely	2006/2007/1
Introduction to biometry	Elek Dinya, Gábor Makara	2006/2007/1, 2007/2008/1, 2008/2009/1
Planning and evaluation of experiments	Gábor Makara	2007/2008/2
Experiments with laboratory animals	Piroska Anderlik	2006/2007/1, 2007/2008/1, 2008/2009/1
Methods of literature research	Lívia Vasas	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
General methods of scientific research	Pál Tomcsányi	2006/2007/2
Clinical biometry	György Füst	2006/2007/2, 2008/2009/2
Industrial law protection for researchers	Ferenc Török	2006/2007/1–2, 2007/2008/1–2

Science and project management	Zita Takács (Vácziné)	2006/2007/1–2, 2007/2008/1–2
Hungarian and European competition systems	Zita Takács (Vácziné)	2006/2007/1–2, 2007/2008/1–2
Biometrics	Zsolt Csende	2006/2007/1–2, 2007/2008/1, 2008/2009/1–2
Pharmacometrics and applied clinical biostatistics	László Tóthfalusi	2006/2007/1
Hungarian medical language	Péter Bösze	2006/2007/2
Psychosomatic and behavioral medicine in everyday practice: "the difficult patient"	Márta Novák	2006/2007/2
Application for grants, project financing and management in EU and Hungary in the field of health sciences	Gábor Pörzse	2008/2009/2
Bioinformatics and study of antibiotic resistance and its epidemiology	Sebastian Amyes, Ferenc Rozgonyi	2008/2009/2
Methods in vascular diagnostics and inventional radiology	Viktor Bérczi	2006/2007/1
Electrophysiological mechanisms and non-pharmacological treatment of arrhythmias II.	Béla Merkely	2006/2007/2
In vivo and *in vitro* evaluation of cardiovascular function in animal models: theory and practice	Violetta Kékesi	2007/2008/1
Echocardiography in practice	Márk Kollai	2007/2008/2
Cardiovascular pharmacogenomics	Zsolt Szelid	2008/2009/1
XVII. International Semmelweis Symposium 2008: building bridges from basics to invasive cardiology	Béla Merkely	2008/2009/1
ECG in theory and at bedside	Béla Merkely	2008/2009/1
Clinical cardiovascular physiology	Emil Monos, Márk Kollai	2006/2007/2, 2007/2008/2, 2008/2009/2
Current methods of biosignal processing	András Eke	2006/2007/1
Modern research and measuring methods in experimental and clinical medicine (selected chapters)	László Dézsi	2006/2007/2, 2007/2008/1–2, 2008/2009/1
Regulation and clinical significance of vascular adaptation during pregnancy	Péter Tóth	2007/2008/2, 2008/2009/1–2
Financial planning of innovative biotechnological projects	Zsombor Lacza	2008/2009/1–2

Clinical and physiological basis of respiration and respiratory disorders	Ildikó Horváth, Márk Kollai	2008/2009/1
Phototherapy	Gabriella Csík Györgyi Rontó	2006/2007/2
Investigation of molecular motions and interactions in biological systems by optical and spectroscopical methods	Pál Gróf	2006/2007/1, 2008/2009/1
Budapest Nephrology School (Nephrology, Hypertension, Dialysis, Transplantation)	László Rosivall	2006/2007/2, 2007/2008/2, 2008/2009/2
Nephrology—from molecules to bedside	László Rosivall	2008/2009/1–2
Normal and pathologic function of the heart and coronary circulation from the aspects of basic and clinical research	Ákos Koller	2006/2007/2, 2007/2008/1, 2008/2009/1–2
Theoretical and practical studies for a successful Ph.D. degree	Anna Blázovics	2006/2007/1, 2007/2008/1, 2008/2009/1
Pain management	Katalin Darvas	2006/2007/1
Free radicals in biological systems	Anna Blázovics	2008/2009/2
Hepatology	János Fehér†	2008/2009/2
Introduction to clinical genetics	Zoltán Papp	2008/2009/1
Postgraduate course in gastroenterology	Zsolt Tulassay	2006/2007/2, 2007/2008/2, 2008/2009/2
Perioperative management of patients with endocrine disorders	Katalin Darvas	2006/2007/2, 2007/2008/2
3rd obligatory postgraduate course in internal medicine	Zsolt Tulassay	2008/2009/1
Metabolic bone diseases	Péter Lakatos	2006/2007/2, 2007/2008/1–2, 2008/2009/1
Postgraduate course in endocrinology	Károly Rácz	2006/2007/1
Physiology and pathology of the musculo-skeletal system's function	Miklós Szendrői	2006/2007/2
Asthma bronchiale	Pál Magyar†	2008/2009/2
Lung disorders with airway obstruction (physiological and clinical aspects)	György Losonczy, Pál Magyar†	2007/2008/1, 2008/2009/1
Radiation biology for radiologists	Olga Ésik	2006/2007/1, 2007/2008/1
Radiogen late effects of normal tissues	Olga Ésik	2006/2007/2, 2007/2008/1

Significance of radiation biology in the diagnosis and treatment of malignancies	Géza Sáfrány	2008/2009/2
Problems of endovascular graft implantation in the treatment of vascular aneurysms	Kálmán Hüttl	2006/2007/2
Diagnostic and therapeutic techniques in the clinical practice	Attila Nemes	2006/2007/1, 2007/2008/1, 2008/2009/1
Pathogenesis of atherosclerosis and the role of risk factors	Albert Császár	2006/2007/1
Perioperative cardiorespiratory risk assessment, monitoring and evaluation of treatment option of patients undergoing vascular and cardiac surgery	János Gál	2008/2009/1
Immunoendocrinology—theory and clinical aspects	Péter Igaz	2006/2007/1
Genetics and genomics in endocrinology	Péter Igaz	2007/2008/1
Clinical endocrinology	István Szabolcs, Miklós Góth	2006/2007/2, 2008/2009/1
Dietotherapy	István Szabolcs	2006/2007/1, 2007/2008/1–2, 2008/2009/2
Clinical hemotology: physiological, pathophysiological and molecular biological aspects	Judit Demeter	2006/2007/2
Challenge of diabetes for manual specialists in medicine–diabetes education	Anikó Somogyi	2008/2009/1
Diabetes education	Anikó Somogyi	2006/2007/1
Lipid and carbohydrate metabolism	Károly Cseh	2006/2007/2, 2007/2008/2
19th Congress of the Hungarian Diabetes Society	György Jermendy	2007/2008/2
Pharmacogenomical aspects of adverse drug reactions	Sarolta Kárpáti	2006/2007/2, 2007/2008/2
Health economics and pharmaceutical economics	László Gulácsi	2008/2009/2
Science-based health care and health care technology	László Gulácsi	2008/2009/2
Industrial pharmaceutical technologies	István Antal	2008/2009/2
Pharmaceutical biotechnology	Éva Szőke	2007/2008/2
Pharmaceutical aspects of quality assurance	Romána Zelkó	2006/2007/2, 2007/2008/2, 2008/2009/2

Phytochemistry	Éva Lemberkovics	2006/2007/1, 2007/2008/1, 2008/2009/1
Interactions, side effects in phytotherapy	Ágnes Kéry	2007/2008/1, 2008/2009/2
Pharmacodynamics	Klára Gyires, Kornélia Tekes	2006/2007/1–2, 2008/2009/1–2
Clinicopharmacological investigation and rational application of analgesics	András Telekes	2006/2007/2
The fate of drugs in the body: drug metabolism and pharmacokinetics	Imre Klebovich	2006/2007/2
Phytotherapy and biocosmetics	Mária Then	2008/2009/2
Bioanalytic methods in pharmacokinetics	Imre Klebovich	2006/2007/1, 2007/2008/1, 2008/2009/1
Clinical pharmacology and rational use of anticancer drugs	András Telekes	2007/2008/1
Introduction into drug research	Béla Noszál	2006/2007/2, 2007/2008/2, 2008/2009/2
Toxic plants, plant poisoning	Gabriella Maczal	2008/2009/1
Interfacial behavior of macromolecular colloids	Ferenc Csempesz	2006/2007/1
Biopharmacia	Sylvia Marton	2006/2007/2
Plant fermentation in producing active substances of medicinal plants	Miklós László	2007/2008/1
Approaches used in structural proteomics	Judit Fidy	2006/2007/1
Pharmacoeconomics	Zoltán Vincze, Ágnes Mészáros	2006/2007/2
Chromatographic methods and their use in pharmacology	Huba Kalász	2008/2009/1
Nanosystems in biomedicinal sciences	Ferenc Csempesz	2008/2009/1
Role of ion transports and ion channels in neurochemical transmission	Tamás Török	2008/2009/2
Cardiometabolic risk and its treatment	Csaba Farsang	2008/2009/1
The role of family in causing, maintaining and treating disease	János Füredi	2006/2007/1
Methodology of behavioural sciences	Mária Kopp	2006/2007/2
Basis of behavioural medicine	Mária Kopp	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2

Introduction to the clinical practice of family therapy	Tamás Kurimay	2006/2007/2
Evidence based medicine in behavioral medicine	István Mucsi	2007/2008/1, 2008/2009/1
Clinical neuropsychology	Ilona Pataky	2007/2008/2
Application of psychological methods	Dóra Perczel-Forintos	2006/2007/2, 2007/2008/1
Neuropsychiatry of cognitive disturbances of organic origin: clinical EEG studies	Péter Rajna	2006/2007/1
Recognition, measurement of expressed emotion based on universal features	Lajos Simon	2006/2007/2, 2008/2009/2
Psychotherapy in medical practice	László Tringer	2006/2007/2, 2007/2008/1
Clinical psychopharmacology	István Bitter	2006/2007/1, 2008/2009/1
Psychopathologic and psychiatric aspects of ways for artistical expression	Péter Rajna	2007/2008/1, 2008/2009/1
Therapeutic possibilities of insomnia	Márta Novák	2007/2008/2 2008/2009/1–2
Role of obstacles and helping resources in family functioning	Katalin Horváth Szabó	2008/2009/1
Cognitive neuroscience	Szabolcs Kéri	2008/2009/2
Sociological and social political evaluation of underprivileged groups	Roger Csáky-Pallavicini†, Péter Török	2008/2009/2
Research methods	János Mészáros†, Miklós Zsidegh	2006/2007/1, 2007/2008/1, 2008/2009/1
Research methods	Gyöngyi Szabó (Földesiné)	2007/2008/1–2
Sport in contemporary society	Gyöngyi Szabó (Földesiné)	2006/2007/1–2, 2008/2009/1–2
Research methods in social sciences	Edit Nagy (Bíróné), József Bognár	2006/2007/1–2
Physiology of fitness	Gábor Pavlik	2007/2008/2, 2008/2009/2
Excercise physiology	Tamás Szabó	2006/2007/1, 2007/2008/1, 2008/2009/1
The pedagogical aspects of training theory and methods	Endre Rigler†	2006/2007/1

The pedagogical aspects of training theory and methods	Zsolt Radák	2006/2007/2
Physiology of physical fitness	Gábor Pavlik	2006/2007/2
Sportpsychology for the performance	Csaba Nagykáldi	2006/2007/2, 2007/2008/1, 2008/2009/1
Sports law	András Nemes	2007/2008/1–2, 2008/2009/1–2
Basis of community mental hygiene	Katalin Horváth Szabó, Péter Török, Teodóra Tomcsányi	2007/2008/2
Research methodology in sport science	József Bognár	2007/2008/1–2, 2008/2009/1–2
Sports pedagogy	Edit Nagy (Bíróné), József Bognár	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Theoretical and methodological fundamentals of PE curricula	Pál Hamar	2006/2007/2, 2007/2008/2, 2008/2009/2
Theory in action—efficacy	Csaba Nagykáldi	2006/2007/1, 2007/2008/2, 2008/2009/2
Human biology	János Mészáros†	2007/2008/2, 2008/2009/1
Free radicals, physical excercise and aging	Zsolt Radák	2008/2009/2
Nutritional science	Róbert Frenkl†, Csaba Nyakas	2006/2007/2, 2007/2008/2, 2008/2009/2
Biomechanics of the musculoskeleton system	József Tihanyi	2006/2007/1–2, 2007/2008/1, 2008/2009/2
Medical physiology	Róbert Frenkl†	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Biometrics	János Mészáros†, Miklós Zsidegh	2006/2007/2, 2007/2008/2, 2008/2009/2
Physiology and pathophysiology of aging	Csaba Nyakas	2008/2009/2
Research methods	Zsolt Csende	2008/2009/2
Free radicals, excercise and aging	Zsolt Radák	2006/2007/1
Motor learning and control	Zsolt Csende	2006/2007/1–2, 2007/2008/1, 2008/2009/1

Research methods	Zsolt Csende, János Mészáros†	2006/2007/1–2
Recreation	László Jakabházy	2007/2008/2
Endocrinology and sport	Róbert Frenkl†, Csaba Nyakas	2007/2008/1, 2008/2009/1
Neuromechanics of movements	József Laczkó	2007/2008/1–2, 2008/2009/2
How to prepare a scientific publication	Zsolt Radák	2007/2008/1
History of philosophy	Ferenc Takács	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Research methodology	Károly Ozsváth	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
History of philosophy	Katalin Vermes	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Management of events (organizational aspects of major sport events)	Mihály Nyerges	2007/2008/1–2, 2008/2009/1
Social structure, social mobility	Gyöngyi Szabó (Földesiné)	2006/2007/1–2, 2008/2009/1–2
Theory of sport games	Mariann Reigl	2006/2007/2, 2007/2008/1–2, 2008/2009/2
Theoretical aspects of science	János Farkas	2007/2008/1, 2008/2009/1
Modern history of sports	Katalin Szikora	2008/2009/1–2
Interpretation of the physical body in phylosophy and psychology	Katalin Vermes	2008/2009/2
Sport aesthetics	Ferenc Takács	2008/2009/2
Neurochemistry	Veronika Ádám	2006/2007/1
Functional organization of the cerebral cortex	Tamás Freund	2006/2007/1
Function of glial cells	Mihály Kálmán	2007/2008/2
Neuronal cell differentiation	Emília Madarász	2008/2009/1–2
In vitro cell technology	Emília Madarász	2006/2007/2, 2008/2009/2
Gene technology in neurosciences	Gábor Balázs Szabó	2006/2007/2
Human neuromorphology II.: experimental and clinical neuromorphology	Miklós Palkovits	2006/2007/1, 2007/2008/2, 2008/2009/1

Bioenergetics and oxidative stress in the brain: physiological and pathological relations	Veronika Ádám	2006/2007/1
Biology and potential clinical application of stem cells	Emília Madarász	2007/2008/2
Structure biology	Attila Ambrus	2008/2009/2
Neurochemistry, pharmacology and models of behavior	György Bagdy	2006/2007/2, 2007/2008/2, 2008/2009/2
Neuroendocrinology	Zsolt Liposits	2007/2008/2
Mechanism and clinical types of epilepsy	Péter Halász	2007/2008/2
Neuropharmacology	Szilveszter Vizi E.	2008/2009/2
Neurobiology and clinical aspects of anxiety and mood disorders	Gábor Faludi	2008/2009/2
Neurobiological basis of stroke	Zoltán Nagy	2008/2009/1
Electrophysiological methods in studying disturbances in movements and thinking	Anita Kamondi	2006/2007/1
Parkinson's disease and Parkinson's syndrome	Anita Kamondi	2008/2009/1
Electrophysiological assays and protheses	György Karmos	2006/2007/2
Neurogenetics in clinical practice	Mária Judit Molnár	2007/2008/1
Neural prostheses	György Karmos	2007/2008/1
Application of radioisotope technological methods in cell physiology	István Voszka	2006/2007/1
Structure and function of membrane receptors	László Hunyady	2007/2008/2
Separation techniques of biomolecules, proteomics	Gábor Juhász	2006/2007/1
Functional cytology	Edit Oláh	2008/2009/1
Receptors and signal transductions in the immune system	Gabriella Sármay	2008/2009/1
Role of phagocytes in natural immune defence	Erzsébet Ligeti	2007/2008/1
Networks and system stability	Péter Csermely	2006/2007/2
Stem cell biology in regeneratory medicine	Balázs Sarkadi	2006/2007/1
Molecular biological strategies in cell physiology	András Váradi	2006/2007/2
Selected chapters from molecular cell biology	László Homolya	2006/2007/2, 2007/2008/1–2, 2008/2009/1–2
Discussion of published scientific papers	László Homolya	2006/2007/2

Application of transgenic technologies in biomedical research	Attila Mocsai	2008/2009/2
Rational drug design and signal transduction therapy	György Kéri	2008/2009/1
Separation and analitical methods	György Mészáros	2006/2007/1
Structure and function of biological membranes	Balázs Sarkadi	2007/2008/2
Rational drug design. Application of high performance separation techniques	Miklós Idei, György Kéri	2006/2007/1
Signal transmission therapy and rational drug design	György Kéri	2007/2008/1
Biotransformation	Miklós Csala	2007/2008/2
Developmental biology	Imre Oláh	2006/2007/1, 2007/2008/2, 2008/2009/1–2
Histamine biology	Zsuzsa Darvas	2007/2008/1
Chemotaxis: Its biological and clinical significance	László Kőhidai	2006/2007/2, 2007/2008/2, 2008/2009/2
Genetics of sex	Sára Tóth	2006/2007/1, 2007/2008/1, 2008/2009/1
Genomic background of multifactorial diseases	Csaba Szalai	2007/2008/2
Genomics	Csaba Szalai	2006/2007/2
Bioinformatics and pathway analysis	István Miklós	2008/2009/1
Autoimmunity	György Nagy	2008/2009/1
Novel questions in medical genomics	Csaba Szalai	2008/2009/2
Clinical immunology and allergology	Miklós Benczúr, Péter Gergely	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Clinical and biological use of antibodies	László Cervenak, Zoltán Prohászka	2008/2009/2
Experimental pathology	Tibor Kerényi	2006/2007/1
Clinical oncology	László Kopper	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Impaired cell regulation in cancer	Ilona Kovalszky	2006/2007/2
Molecular oncology	Ilona Kovalszky	2008/2009/2
Pathology	László Kopper	2006/2007/1
Theoretical basis and application of flow cytometry	Gábor Barna	2007/2008/2

Role and detection of cell junction structures and molecules	Zsuzsa Schaff, András Kiss	2006/2007/1–2, 2007/2008/2, 2008/2009/1–2
Clinicopathological approach in research	Zsuzsa Schaff	2006/2007/2, 2007/2008/2, 2008/2009/2
Aspiration cytology in practice	Balázs Járay	2006/2007/1, 2008/2009/1–2
Hemopathology—Lymphomas	Zsuzsa Schaff	2006/2007/2
Organ transplantation: present and future	Jenő Járay	2007/2008/2, 2008/2009/1
Breast pathology	Janina Kulka	2007/2008/2
Soft tissue and bone pathology	Zsuzsa Schaff	2008/2009/2
Cell adhesion molecules/The FISH technique in pathological diagnosis	András Kiss	2008/2009/2
Molecular virology: human retroviruses (HIV, HTLV) and their roles in immuno-pathological disorders	Károly Nagy	2006/2007/2, 2007/2008/2, 2008/2009/2
Health economics, pharmacoeconomics and economic consequences of communicable disease	László Gulácsi	2006/2007/2, 2007/2008/1–2
Evidence based medicine and health technology assessment with particular emphasis on prevention and treatment of communicable diseases	László Gulácsi	2006/2007/2, 2007/2008/1–2
Molecular virology and molecular epidemiology	György Berencsi	2006/2007/1, 2007/2008/1–2, 2008/2009/1
Studies on the process of becoming a nurse	Judit Mészáros	2008/2009/2
Changes in the professional role of nurses in the era of nursing graduates	Sándor Hollós	2007/2008/1–2, 2008/2009/1–2
Disorders of adaptation to society	József Rácz	2006/2007/1, 2007/2008/2, 2008/2009/1–2
Basics of society medicine	Iván Forgács	2006/2007/1, 2007/2008/1, 2008/2009/1
Role of profession in the practice of health care	Péter Balázs	2006/2007/1–2, 2007/2008/1–2, 2008/2009/1–2
Diet therapy	Mária Barna	2008/2009/2
Health education in infant and child care in hospital and outpatient practice	György Harmat	2008/2009/2

Methodology and programme planning for the evaluation and management of subjects with food allergy	Mária Barna	2008/2009/2
Health education and health promotion	Tamás Simon	2006/2007/1, 2007/2008/1
Methodological and programme planning examinations in teaching nutrition management	István Szabolcs	2006/2007/1, 2008/2009/2
Pediatric nutrition	István Szabolcs, Friedman B. J.	2006/2007/2
Current trends in dietetics and nutritional sciences	István Szabolcs, Friedman B. J.	2006/2007/2
Effects of globalization on diseases development	Anna Tompa	2006/2007/2, 2007/2008/2, 2008/2009/1–2
History of health and science	Judit Forrai	2006/2007/2, 2007/2008/2, 2008/2009/1–2
Molecular epidemiology of viral infections	József Ongrádi	2006/2007/2, 2007/2008/2, 2008/2009/1
Public health sciences (health care systems, health policy)	Iván Forgács	2007/2008/2
Associations between nutritional state and life style in the elderly	István Szabolcs	2008/2009/2

PH.D. SCIENTIFIC DAYS

The Ph.D. training program provides opportunities for every candidate to acquire practical knowledge of the methodology of presenting results gained in scientific research. Ph.D. students therefore are required to present their work regularly both among fellow workers and in a wider professional environment. The need for an overall Ph.D. conference of the Doctoral School was promoted even though the departmental doctoral schools organize scientific forums for their own Ph.D. students. The primary objective was that participants would be able to familiarize themselves with the scientific work of each program. On these occasions Ph.D. students and candidates had the opportunity to present their work in severeal sections with a jury. Candidates with works of a high standard gained awards in each section.

Every academic year highly regarded professionals, normally holders of the distinction "Excellent Ph.D. Supervisors" have been invited to give plenary lecture with great success.

Plenary lecturers

2006 **Zoltán Nagy** (János Szentágothai Neurosciences Doctoral School)
Novel aims in medical treatment od cerebral ischemia

György Füst (Pharmaceutical Sciences Doctoral School)
A specific region of the human genom, the central part of the major histocompatibility complex (MHC) located on the short arm of chromosome 6. Medical aspects.

2007 **Gyöngyi Szabó Földesiné** (Sport Sciences Doctoral School)
Post-transformational trends in Hungarian sport

István Bitter (Mental Health Sciences Doctoral School)
Research in the Department of Psychiatry and Psychotherapy of Semmelweis University

†**Sándor Juhász-Nagy** posthumus (Basic Medicine Doctoral School)

2008 **József Tihanyi** (Sport Sciences Doctoral School)
Causes and magnitude of increased stretch due to muscle extension

György Bagdy (Mental Health Sciences & János Szentágothai Neurosciences Doctoral Schools)
Serotonin in the central nervous system: journey from neurobiology and genetics to pharmacology, psychiatry and neurology

Péter Gergely (Molecular Medicine Doctoral School)
Significance of immunological studies in the evaluation of activity and prognosis of systemic lupus erythematosus.

The plenary speakers are carefully selected from among those who have been awarded with the distinction of the "Excellent Ph.D. Supervisors".

EXCELLENT PH. D. SUPERVISOR AWARD

Nominations for the Excellent Ph. D. Supervisor Award are made by the heads of individual Ph. D. Schools, and the University Doctoral Council decides on the final list of awardees. The number of awardees is limited.

2006	**Zoltán NAGY**	János Szentágothai Neurosciences Doctoral School
	György FÜST	Pharmaceutical Sciences Doctoral School
2007	**Gyöngyi Szabó FÖLDESINÉ**	Sport Sciences Doctoral School
	Balázs SARKADI	Molecular Medicine Doctoral School
2008	**József TIHANYI**	Sport Sciences Doctoral School
	György BAGDY	János Szentágothai Neurosciences Doctoral School & Mental Health Sciences Doctoral School
	Péter GERGELY	Molecular Medicine Doctoral School

AWARDING OF THE DOCTORAL DEGREE WITH DISTINCTION

The President of the Republic consented to the awarding of the doctoral degree to **Mariann Budai** *(2006)*, **Kristóf Kóczián** *(2007)*, and **Dorottya Kiss** *(2008)* with the distinction *'Promotio sub auspiciis praesidentis Rei Publicae'*. It was Dr. László Sólyom, the President of the Republic in person who handed over the Diploma and the traditional Golden Ring to the inaugurated doctors. This ceremonial program was part of the Dies Academicus of Semmelweis University.

SCHOOL OF PH. D. STUDIES

1. BASIC MEDICINE

Chairman:
László ROSIVALL M. D., Ph. D., D. Sc.
Institute of Pathophysiology
4 Nagyvárad sq, Budapest H–1089
Tel: +36 1 210 2956
Tel./fax: +36 1 210 2956
E-mail: rosivall@net.sote.hu

General overview

The Doctoral School of Basic Medicine at Semmelweis University consists of five multidisciplinary research and training Ph. D. Programs. These Programs are closely related to the physiological sciences, and are chaired by internationally recognized professors as coordinators. At first, each Program was accredited individually in 1994, then all the Ph. D. Programs were integrated into a Doctorate (Ph. D.) School in 2002.

The major aims of the Ph. D. Programs are focused on investigating the mechanisms of diseases with high morbidity and mortality statistics in Hungary (e. g. cardiovascular and renal diseases, hypertension, obesity), and to study those environmental effects (UV and X-Radiation) which may influence the whole society. Investigating the molecular-cellular background of physiological and pathophysiological processes, and integration of knowledge at organ and organism levels lead us to new scientific results and discoveries which may promote the development of up-today methods for health prevention, diagnostics and therapy. In addition to several basic research projects offered to Ph. D. students, applied clinical studies are also incorporated into the Programs of the School.

PROGRAM 1/1.

PHYSIOLOGY AND CLINICS OF THE HEART AND CORONARY DISEASES

Coordinator:
Sándor JUHÁSZ-NAGY† M. D., Ph. D., D. Sc. (1993–2007)
Béla MERKELY M. D., Ph. D., D. Sc. (2007–)
Heart Center
68 Városmajor st, Budapest H–1122
Tel./fax: +36 1 458 6844
E-mail: titkarsag@kardio.sote.hu

Program overview

The complex program is directed to well-qualified students who are interested in cardiovascular research. The spotlight is on regulatory aspects and treatment of different cardiovascular diseases. According to the scientific interest of most of coordinators, the main

problems are connected to pathophysiology (clinical physiology) of myocardial function, coronary regulation and arrhythmogenesis. (However, as the list of the topics shows, other circulatory topics are included, too.) The program prepares students for careers in either clinical science (especially invasive and non-invasive cardiology, anesthesiology, and cardiovascular surgery) or basic sciences. Preference is given to those who are ready to study overlapping territories of these sciences. Although the individual postgraduate trainings have an overall general similarity in their logistic aspects, the main characteristic of the entire educational process is the flexibility. Consequently, that research work can be tailored to the tutor's mutual interest within the territory covered by the general aims.

Titles of research projects	***Supervisors***
New aspects in the non-pharmacological therapy of tachyarrythmias	Béla Merkely
Heart failure: pathomechanisms and new methods in the pharmacological and non-pharmacological treatment	Béla Merkely
Electrophysiology of ventricular arrhythmias	Béla Merkely
Role of endogenous agents in arrhythmogenesis	Béla Merkely
Challenge in the field of interventional cardiology	Béla Merkely
Interventional radiology in the treatment and follow-up of patients with vascular diseases	Viktor Bérczi
Causes of recurrent stenosis after carotid surgery or other vascular surgical approaches. Researches in vascular surgery	László Entz
Pathophysiology of the visceral circulation	János Hamar
Vasoactive peptides in heart diseases and their experimental models	Ferenc Horkay
Cardiovascular and cardioprotective effects of endogenous peptides in myocardial ischemia: Experimental and clinical studies	Violetta Kékesi
Local myocardial interactions of cardiogenic agents: experimental studies	Violetta Kékesi
Mechanism of metabolic autoregulation in the coronary circulation	Violetta Kékesi
Mechanisms of the actions of cardiovascular regulatory agents: *in vitro* investigations	Violetta Kékesi
Diastolic dysfunction and heart failure	Mária Lengyel
Cardiovascular effects of brain death. Donor management and selection	Gábor Szabó
Heart insufficiency in the era of modern cardiac surgery	Gábor Szabó
Tissue injury during and after cardiac surgery. Novel strategies to prevent reperfusion injury and acute and chronic rejection.	Gábor Szabó
Molecular mechanisms of cardiac hypertrophy especially in the early and decompensated phase of the disease	Miklós Tóth
External and internal noxa-induced secondary circulatory damage: clinical symptoms and therapy	András Csókay
Nuclear cardiology in the diagnosis of ischaemic heart disease	István Szilvási
Identification of origin and mechanisms of wide QRS complex tachycardias using new algorithm combined with neuronal network schemes	András Vereckei
Role of cardiac and endothelial progenitor cells in the remodeling and regeneration of the myocardium: *in vivo* and *in vitro* studies.	Gábor Földes

Pharmacogemonic investigations in the cardiovascular system		Zsolt Szelid
Non-cardiac risk factors in cardiac surgery		Andrea Székely

Ph. D. students		***Supervisors***
Astrid Apor	pt	Péter Andrássy
György Bárczi	pt	Péter Andrássy
Balázs Berta	ft	Béla Merkely
Mónika Dénes	ft	Mária Lengyel
Tamás Erdei	ft	Mária Lengyel
Gábor Fülöp	pt	Béla Merkely
Zoltán Gonda	pt	László Entz
Máté Kerekes	ft	Violetta Kékesi
Zsolt Béla Kozma	pt	Ferenc Horkay
Árpád Lux	ft	Zsolt Szelid
Mónika Moravszki	pt	István Szilvási
Balázs Sax	ft	Violetta Kékesi
Zoltán Kőhalmi	pt	Béla Merkely
Attila Somorjai	pt	László Entz
György Szabó	pt	Béla Merkely
Katalin Túri	ft	Violetta Kékesi
Gabriella Veress	ft	Béla Merkely
Katalin Túri	ft	Violetta Kékesi
Gabriella Veress	ft	Béla Merkely
Krisztina Szendrei	pt	Béla Merkely
Pál Maurovich-Horvath	ft	Béla Merkely
Irén Etelka Szalai	pt	János Hamar
Bálint Kozman	pt	András Vereckei
Éva Kósa	pt	Viktor Bérczi

Ph. D. candidates		***Supervisors***
Dávid Becker	i	Béla Merkely
Tamás Breuer	ft	Miklós Tóth
Kristóf Hirschberg	ft	László Entz
Lídia Kun	ft	Miklós Tóth
Andrea Nagy	ft	Violetta Kékesi
Attila Róka	ft	Béla Merkely
Szabolcs Szilágyi	ft	Béla Merkely
Gábor Szűcs	ft	Béla Merkely
Tímea Kováts	i	Miklós Tóth
Andrea Ágnes Molnár	pt	Viktor Bérczi
Valentina Kutyifa	ft	Béla Merkely
Gábor Veres	pt	Gábor Szabó
Csaba András Dézsi	pt	Béla Merkely

Ph. D. graduates		***Supervisors***
Terézia Bogdána Andrási	pt	Sándor Juhász-Nagy
Edit Dósa	ft	László Entz

Tamás Radovits	ft	Gábor Szabó
Pál Soós	ft	Ferenc Horkay
Leila Seres	ft	Ferenc Horkay
Attila Szabó	pt	László Entz
Andrea Szűcs	ft	Béla Merkely
Ildikó Toma	pt	Violetta Kékesi
Béla Turchányi	pt	János Hamar
Hajnalka Vágó	ft	Béla Merkely
Endre Zsima	ft	Violetta Kékesi
András Zsáry	i	Sándor Juhász-Nagy

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

TERÉZIA BOGDÁNA ANDRÁSI (2006)

Factors influencing vascular reactivity in the mesenteric bed subjected to ischemic and cardiosurgical stress

Supervisor: Sándor Juhász-Nagy

Acute occlusion of the superior mesenteric artery alone almost always results in intestinal infarction and rapid demise. Prompt diagnosis and aggressive treatment are essential before deterioration becomes irreversible. However, reperfusion—the restoration of blood flow after a period of ischemia—can place the ischemic intestine at risk of further cellular necrosis and thereby limit the functional recovery. To a better understanding of the patho-physiological mechanisms we performed animal experiments on 55 narcotized dogs. The endothelial dysfunction—as disruption of the vasoconstrictor (ET-1)/vasodilator (NO) balance—represents the first step and perchance the highlight of the mesenteric vascular injury. Increased production and release of endogenous ET-1 mediate the reperfusion damage after acute occlusive mesenteric ischemia. As we demonstrated, the inhibition of ET-1 by blocking the ET-A receptors with LU135252 significantly improves the regional vascular circulation in the postischemic period, effect mediated in part by an enhancement of the scavenger capacity of the mesentery. If blood flow decrease occurs due to some functional or/and structural insult like cardiopulmonary bypass (CPB), it might cause formidable microvascular derangements leading to the development of non-occlusive mesenteric infarction. The central event in the post-CPB acute non-occlusive mesenteric ischemia remains the endothelial vascular dysfunction. We also showed that the development of heart failure significantly reduces the adaptive capacity of the mesentery to the non-physiological conditions induced by CPB leading to systemic hemodynamic disturbances followed by an enhancement of ischemia/reperfusion injury in the mesentery. The increased release of free radicals in the presence of HF overwhelms the scavenger capacity of the mesentery during CPB, accounting for the higher degree of vascular dysfunction including damage of the smooth muscle reactivity. Our results demonstrate that systemic supplementation of L-arginine at reperfusion protects the mesenteric

vascular circulation by restoring the basal vascular tone and endothelial dependent vasodilatation. By increasing extracellular L-arginine concentration, the physiological NO release synthesized by eNOS form L-arginine is restored. Extracellular L-arginine may induce direct vasodilatation, may reduce neutrophil activation and directly scavenge cytotoxic free radicals in the mesentery. PARP dependent processes are also being triggered during CPB in the mesenteric blood vessels. Inhibition of PARP with PJ34 restores the physiologic blood supply to the mesentery after CPB, increases endothelial NO production without altering its physiologic metabolism and blocks neutrophil adhesion. Due to multiple pathways of the peroxynitrite-induced cytotoxicity PJ34 reduces the endothelial damage without a complete restoration of the normal vascular circulation in the mesenteric territory. Improved heart pump function after CPB seems to be an indirect mechanism by which systemic intravenous supplementation of both L-arginine and PJ34 increase mesenteric blood flow and maintain intestinal perfusion. The practical usefulness of exogenous L-arginine, PARP inhibitors and ET-A receptor antagonists requires further investigations to test their beneficial effects in other models prone to develop postoperative occlusive/non-occlusive mesenteric ischemia due to regional/global circulatory instability.

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EDIT DÓSA (2007)

C-reactive protein, fibrinogen, soluble thrombomodulin, and vascular diseases

Supervisor: László Entz

Atherosclerosis is a chronic inflammatory disease of worldwide prevalence. No longer regarded as a bland, mechanical process, plaque evolution is now best understood as a pitched battle between proinflammatory and anti-inflammatory cellular and molecular elements. Certain mediators and systemic acute-phase reactants implicated in the pathogenesis of the disease in the future may prove to be (1) useful surrogate markers of disease severity and activity, (2) potential targets for intervention, and (3) end points for titration of therapy and timing of intervention. C-reactive protein is currently the most important of these biomarkers, and may become more so as it appears to identify asymptomatic patients at risk and directly mediate endovascular tissue injury.

We determined the distribution of CRP in a healthy Hungarian population. A representative population distribution of CRP was based on analysis of 207 Hungarians without apparent vascular disease. In our study median CRP level was 1.87 mg/L and ranges of CRP for those with lowest (quintile 1) to highest (quintile 5) vascular risk were 0.05 to

0.73, 0.74 to 1.50, 1.51 to 2.56, 2.57 to 4.77, and >4.78 mg/L. As risk estimates appear to be linear across the spectrum of inflammation, these sequential quintiles can be considered in clinical terms to represent individuals with low, mild, moderate, high, and highest relative risks, respectively, of future cardiovascular disease. According to this supposition, 40% (n= 83) of the examined subjects belonged to the high and highest cardiovascular risk group. Diseases of the blood vessels are among the most frequent cause of serious neurological disorders, ranking third as a cause of death in the adult population in Hungary and probably first as a cause of chronic functional incapacity. Randomized trials have verified the efficacy of carotid endarterectomy for treating and preventing stroke in patients with extracranial cerebrovascular occlusive disease.

To our knowledge, no data on longitudinal measurements of any acute-phase proteins with such long follow-up period after carotid endarterectomy have been published so far, and the effect of TNF-α polymorphism on sTM levels was not tested either. Therefore, we examined 117 patients with severe carotid artery stenosis, who were undergoing eversion endarterectomy at our Department. During the follow-up period (14 months) we observed a sharp, highly significant drop ($p<0.0001$) in the serum and plasma concentrations of both acute-phase proteins (CRP, fibrinogen). Serum CRP levels decreased from 7.90 (3.20–14.25) mg/L measured preoperatively to 3.00 (1.23–7.93) mg/L at the last follow-up visit. Plasma fibrinogen levels were 410 (346–479) mg/dL and 352 (285–410) mg/dL, respectively. The drop in the CRP levels during the follow-up period was mainly due to the decrease in the highest tertile of the baseline levels. Strong negative correlation ($R= –0.418$, $p=0.0006$) was found between the plasma sTM concentrations and the preoperative duplex scan values. Patients with –308 A TNF-α genotype had significantly lower ($p=0.0415$) preoperative sTM values than their counterparts with no such polymorphism. Fourteen months postsurgery the sTM levels were significantly higher ($p=0.0002$) compared to the preoperative state.

Internal carotid artery restenosis of 50% or greater was detected in 15 patients (13%), but only 4 patients (3%) had severe (≥70%) restenosis in the operated region during the follow-up period. Neither CRP nor fibrinogen levels changed significantly compared to the preoperative values till the end of the observation period in the restenosis group. By contrast, in patients with no restenosis CRP and fibrinogen levels significantly decreased as compared to the levels measured before surgery already at the first follow-up visit, and the drop continued till the end of the follow-up. In addition, early postoperative changes in fibrinogen levels predicted restenosis.

Our findings indicate that removal of atherosclerotic plaques from carotid arteries markedly decreases the production of the two acute-phase proteins in patients. This change can be due to the decrease of the inflammatory burden postsurgery or the removed advanced plaques able to produce acute-phase proteins. Then again, sTM may be adsorbed to the atherosclerotic plaques or inflamed endothelium in carotid arteries, but the pathological significance of this adsorption remains to be determined.

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TAMÁS RADOVITS (2008)

Novel antioxidant therapeutic strategies for cardiovascular dysfunction associated with ageing

Supervisor: Gábor Szabó

Overproduction of oxidants and free radicals in ageing tissues induces nitro-oxidative stress, which has recently been implicated in the functional decline of the cardiovascular system at advanced age. Cytotoxic oxidants like hydrogen peroxide or peroxynitrite damage proteins and DNA and activate several pathways causing tissue injury, including the poly(ADP-ribose) polymerase (PARP) pathway.

First, we tested whether the inhibition of the PARP enzyme can improve the endothelial dysfunction induced by hydrogen peroxide, in a simple *in vitro* model of vascular oxidative stress. In turn, our main aim was to investigate the effects of acute PARP inhibition and rapid catalytic decomposition of peroxynitrite on ageing-associated cardiac and endothelial dysfunction.

In vascular reactivity measurements on isolated rat aortic rings we investigated the phenylephrine-induced contraction, and endothelium-dependent and -independent vasorelaxation by using acetylcholine and sodium nitroprusside. Endothelial dysfunction was induced by exposing the rings to H_2O_2. In the treatment group, rings were preincubated with the potent PARP-inhibitor INO-1001. In the *in vivo* rat model of ageing-associated cardiovascular dysfunction, young and ageing rats were treated with vehicle, with a single dose of PARP-inhibitor INO-1001, or with the peroxynitrite decomposition catalyst FP15. Using a pressure-volume conductance catheter, left ventricular pressure-volume analysis of the rats was performed. Endothelium-dependent and -independent vasorelaxation of isolated aortic rings of the rats were investigated by using acetylcholine and sodium nitroprusside. DNA strand breaks were assessed by the TUNEL method. Immunohistochemical analysis of vessel wall and myocardium was performed for nitrotyrosine ("footprint of peroxynitrite"), for poly(ADP-ribose) (the enzymatic product of PARP) and for apoptosis inducing factor (a pro-apoptotic factor regulated by PARP).

In our *in vitro* model, exposure to H_2O_2 resulted in a dose-dependent impairment of endothelium-dependent vasorelaxation of aortic rings which was significantly improved by PARP-inhibition. The dose-response curves of endothelium-independent vasorelaxation to sodium nitroprusside did not differ in any groups studied. In the H_2O_2 groups immunohistochemical analysis showed enhanced PARP-activation and nuclear translocation of apoptosis inducing factor, which were prevented by INO-1001. Ageing animals showed a marked reduction of systolic and diastolic cardiac function and loss of endothelium-dependent relaxant responsiveness of aortic rings. Both acute PARP-inhibition and FP15-treatment significantly improved cardiac performance and endothelial function. Immunohistochemistry for nitrotyrosine and poly(ADP-ribose) confirmed enhanced nitro-oxidative stress and PARP-activation in ageing animals, which were reversed in the treatment groups.

Our results demonstrate the importance of endogenous peroxynitrite-overproduction and the activation of the PARP-pathway in the age-related functional decline of the cardiovascular system. Rapid catalytic decomposition of peroxynitrite by FP15 and acute inhibition of PARP may represent a novel therapeutic utility to improve cardiac and vascular dysfunction associated with ageing.

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PÁL SÓS (2007)

Cardioprotective role of vasocactive agents during myocardial ischemia

Supervisor: Ferenc Horkay

The aim of our human and experimental studies was to examine the local (paracrine) and systemic (endocrine) components of the complex protective mechanism thatactivates during myocardial ischaemia. Recently the role of pericardial fluid in cardiacregulation became certain, that's why we have paid special attention on its function. We characterized the atrial natriuretic peptide (ANP) concentrations of human myocardial tissues, in blood plasma and in pericardial fluid, moreover we analyzed the relationship between ANP levels and some special clinical conditions. Theoretically, pericardial fluid may reflect the composition of interstitial fluid, thus concentrations measured in pericardial fluid samples may indirectly provide information about the interstitial space. To present opportunity even to esteem the composition of interstitial fluid we composed a mathematical model using blood plasma samples and pericardial fluid samples to calculate interstitial concentrations. Moreover we characterized the role of the well known endocrine factor parathyroid hormone in coronary dilation and concluded that in addition to the activation of the intracellular cAMP system and inactivation of the L-type Ca channels described in connection with parathyroid hormone-induced coronary responses ATP-sensitive potassium channels may play an integrative role in the intracellular mechanisms of action of the peptide. In further studies we demonstrated the cardioprotective effect of the NO precursor L-arginin after reversible deep hypothermic ischaemia and reperfusion even if applied systemically. In addition, beneficial effect on endothelial function was proved during ischemic-reperfusion injury, as well as during endothelial dysfunction following brain death. According to our results, the role of vasoactive agents in the mechanism of cardioprotection is considerable, therefore clinical applications based on these molecules may provide new therapeutic potentialities in the near future.

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LEILA SERES (2007)

Oxidative stress in cardiovascular diseases and in experimental models

Supervisor: Ferenc Horkay

The majority of cardiovascular diseases (CVD) results from complications of atherosclerosis and hypertension. Oxidative stress is the unifying mechanism for many CVD risk factors (diabetes, obesity, cigarette smoking), that additionally supports its central role in CVD. Oxidative stress is the imbalance between antioxidants and overproduction of reactive oxygen or nitrogen species. (1) We assessed the antioxidant capacity in the serum and pericardial fluid of patients undergoing heart surgery for coronary artery disease or valvular heart disease. The antioxidant capacity in the pericardial fluid was lower than in the serum but still relatively high. The pericardial fluid may contribute to the local antioxidant defense of the myocardium. No major differences were seen in serum or pericardial fluid antioxidant capacity between the two patient groups. In the overall patient population uric acid and albumin were significantly lower in the pericardial fluid of female than of male patients. (2) An oxidative stress biomarker, referred to as advanced oxidation protein products (AOPP) reference values were measured in plasma samples from healthy volunteers. We describe in detail an automated version of the originally published microplate-based technique. We measured AOPP concentrations in many cardiovascular diseases for assessing and monitoring oxidative stress. Our experiences appear to demonstrate that this technique is especially suitable for monitoring oxidative stress in critically ill patients (sepsis, reperfusion injury, heart failure). In the acute phase of acute coronary syndrome oxidative stress is not apparent, except in patients with diabetes mellitus, chronic renal disease or cardiac decompensation. Plasma AOPP concentrations were high—presumably due to progressed atherosclerosis—in patients undergoing surgery for various peripheral diseases. Complications such as inflammation or gangrene further enhanced AOPP concentrations. (3) We investigated the effects of poly (ADP)ribose polimerase enzyme inhibitors (PJ34 and INO-1001) in experimental canine model of cardiopulmonary bypass. PARP inhibition prevented cardiopulmonary bypass-induced heart and tissue damage and mesenteric endothelial dysfunction.

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ATTILA SZABÓ (2008)

Early resetnosis after carotid endarterectomy: the role of growth factors and the genotype of mannose-binding lectin

Supervisor: László Entz

Thirty five to thirty six thousand new stroke events are diagnosed in Hungary each year, 80–85% of which are of ischaemic origin. Surgery or stenting of the supraaortic arteries became important methods of stroke prevention in both symptomatic and asymptomatic cases. Mid-term and long-term results are modified by restenosis formation, which can be myointimal hyperplasia or progression of the atherosclerosis on the operation field. Restenosis rate following eversion carotid endarterectomy is described as 2 to 34% in the literature. In the first prospective study the incidence of restenosis after eversion endarterectomy was determined. The data of 171 eversion carotid endarterectomy procedures on 151 patients, performed between 1991 and 1993, were analysed at the Department of Cardiovascular Surgery, Semmelweis University Budapest, Hungary. Perioperative stroke morbidity and mortality rate was 0.8%, five year survival rate was found 85%. Significant (>70%) restenosis developed in 11 patients (9%), restenosis >50% was found in 12% of all inspected cases. Until today we could not to find a single study in the literature referring for a longer period of follow-up time (56 months), than our study. Our results regarding to eversion carotid endarterectomy are similar to those in the literature, therefore conclusions of further studies regarding to the same patient population seem to give internationally acceptable results. The denudation of the arterial endothelium during carotid endarterectomy and ischaemia reperfusion injury following crossclamping together initiate the inflammation process of the arterial wall. In consequence, growth factor production is upregulated and myointimal hyperplasia may initiate early restenosis. Experimental and cilical datas suggest that the inflammatory process may triggered by mannose binding lectin and the lectin pathway of the complement system. In a former study we proved, that restenosis following eversion carotid endarterectomy is partially genetically determinated, and that genetic polymorphism of mannose-binding lectin (MBL) has a key role in the pathophysiology of this process. We could not find a study in the literature, that describes a correlation between the frequency of carotid restenosis and the preoperative serum level or postoperative serum level changes of various circulating growth factors. The total of 82 patients (55 males, 27 females, 66.2±8.9 years [mean±SD age]) were included and followed-up in the second prospective study. The patients underwent elective eversion carotid endarterectomy between October 2000 and March 2003 because of severe (mean 83.1±9%) internal carotid artery stenosis. Carotid duplex scan sonography was undertaken at all patients preoperatively and three times postoperatively, 6 weeks, 7 and 14 months after the operation. Restenosis higher than 50% was found at the 7- and at the 14-month follow-up in 9 and 12 patients, respectively. Clinically significant restenosis (>70%) was measured in the same 4 patients (4/82=4.9%) postoperatively both at the 7- and 14-month follow-up. Two patient groups were formed according to the presence of considerable (>50%) restenosis found in postoperative control examinations. An increase of the serum levels of the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) was found significantly more frequent ($p=0.0044$ and $p=0.0653$, respectively) in those patients, who developed restenosis at the 7th postoperative month, than in those without restenosis. The patients were subdivided into two groups according

to MBL2 genotype. Group 1 comprised patients homozygous for the normal (wild) MBL2 allele (genotype A/A), and group 2 comprised patients with 1 or 2 variant alleles (MBL genotypes A/O or O/O). When the relations between VEGF and PDGF value changes and 7-month restenosis or 14-month CDS values were analyzed, highly significant correlation were found at both time points in group one. By the contrast, no correlation was found in the patients of group two. We could not find any siginificant correlation regarding to restenosis and the early postoperative changes of serum levels of the endothelian growth factor (EGF). In conclusion, our present findings definitely indicates that after eversion carotid endarterectomy restenosis occurs primarily in patients who are homozygous for the normal MBL2 (A/A) allele, a genotype associated with high MBL levels, and a marked complement activating capacity in conjunction with upregulation of PDGF and VEGF production occurs in the early postoperative period. This is clearly a hypothesis generating study, the conclusion of which should be tested by further studies in different cohorts. Such studies are under way in our departments.

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ANDREA SZŰCS (2006)

Changes of serum levels of endothelin-1 following spontaneous and induced arrythmias and biventricular resynchronisation therapy

Supervisor: Béla Merkely

Malignant ventricular arrhythmia causing sudden cardiac death is the leading factor for cardiovascular mortality. Endothelin-1, one of the best-known endogenous arrhythmogenic substances, has an indirect arrhythmogenic effect based on its vasoconstricting ability, and also an independent direct arrhythmogenic effect. However, although serum levels of endothelin-1 (ET-1) are elevated in several cardiovascular diseases, the exact relationship between its release and arrhythmias is less-known.

Our aim was to observe the changes of serum levels of ET-1 during spontaneous and induced ventricular tachycardias and ventricular fibrillations (VT/VFs) and induced supraventricular tachycardias (SVTs). Endothelin serum levels were also studied in patients with cardiac resynchronisation therapy (CRT) that was applied for drug resistant chronic heart failure with intraventricular conduction delay. Serum levels of ET-1 increased during spontaneous, incessant VTs. The precursor of ET-1, bigET levels increased significantly after SVT/VT induction while ET-1 levels did not change significantly. Control ET-1/bigET levels were higher in patients with inducible VT/SVTs com-

pared with patients without inducible arrhythmias.This finding suggests the potential role of endothelins in the inducibility of arrhythmias.
Patients with primary dilated cardiomyopathy have better clinical status and lower ET-1 levels after CRT therapy compared with patients with ischemic etiology. Control bigET levels proved to be an independent negative predictor of a positive response to CRT therapy. Our results show, that higher serum ET-1 levels correlate with the inducibility of ventricular arrhythmias. Changes of endogenous ET-1 and bigET levels due to arrhythmias suggest a close correlation between the activity of the ET-system and endogenous arrhythmogenesis. The local concentration of endothelins acting via a paracrine mode can be much higher than we observed.

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ILDIKÓ TOMA (2006)

Interactions between cardioregulatory agents in the pericardial space: an experimental study

Supervisor: Violetta Kékesi

The life saving capacity of appropriate medical management in cardiovascular diseases has inspired intensive research into the regulation of cardiac function. While most of the relevant anatomical compartments within this most critical organ have been well characterized, surprisingly little attention has been given to the compartment in which the entirety of the heart is enclosed: the pericardial space and the pericardial fluid (PF). The general consensus on the physiological role of the pericardial fluid assigns it the function of providing continuous lubrication for the epicardium of the beating heart. In addition to those constituents which serve a hydration function, the pericardial fluid also contains a large number of locally produced and active endogenous agents such as endothelin-1 (ET-1), adenine nucleosides, catecholamines, natriuretic peptides, and angiotensin II (AT II). Even more importantly, the concentrations of these regulatory factors were found to be several magnitudes higher in the pericardial fluid than in the plasma, reflecting their derivation from the myocardial tissue and suggesting a teleological role for the pericardial space as a functionally distinct compartment with a potential function in cardiac regulation.
Some information is readily available from isolated, microphysiological systems regarding the signals and effects of these local interactions. However, a legitimate concern remains about ways to interpret the well described mechanisms of the *in vitro* experiments when attempting to apply the information to understanding overall organismal *in vivo* conditions. At the same time, it is extremely important in cardiovascular research to

be able to construe the associations between individual regulatory relationships and the ways in which they coordinate together in living physiological systems.

Our investigations of the composition and dynamic changes of the pericardial fluid provide a remarkable opportunity to study these interactions within *in vivo* systems because of two critical principles: these agents exert their effects through the interstitial space and pericardial fluid composition portrays the changes in myocardial function. Taking this information together, we aimed to investigate in the comparatively closed system of the intrapericardial space the cardiovascular effects of several vasoactive agents (which are present in the pericardial fluid in higher concentratios than that in the plasma) and the dynamic interactions influencing their production by the myocardium. Our investigations belong to a comprehensive series of explorations into pericardial agents and their effects. In these particular studies, we examined the relationships of the following agents: the classic cardiostimulatory agents the catecholamines (dopamine and norepinephrine), the aggressive vasoconstrictor peptide endothelin-1, its compensatory metabolite adenine nucleosides and the two major representatives of the myocardial tissue hormones, the natriuretic peptides and angiotensin II.

In our results, we found that dopamine and norepinephrine applied intrapericardially exerted characteristic cardiovascular effects and elicited increasing myocardial release of ET-1 and adenosine, which, could be detected as dose-dependent elevations of their concentrations in the pericardial fluid (i); the elevation of intrapericardial level of ET-1 induced myocardial ischemia with concomitant ST segment elevations on ECG—without significant depression of different cardiovascular variables—and had an increasing effect on pericardial atrial natriuretic peptide concentrations, but not on brain natriuretic peptide levels (ii); beside its weak or moderate cardiovascular effects the intrapericardially applied ATII stimulated the ET-1 and ANP production in the heart, which was detected as increases of big-ET-1 and ANP concentrations. Meanwhile, the plasma levels of these agents did not change in any of the experiments.

We know that by examining the effects of and interactions between certain endogenous regulatory agent in the pericardium we could not describe a coherent intramyocardial regulatory pattern of these processes, but we think that these investigations may serve information for the characterization of the agents possibly involved in the local myocardial regulatory processes, their stimulatory signals and which cloud be iniciated from the pericardial space, and the local responses detectable as alterations in composition of the pericardial fluid. Understanding the signals, mechanisms, and consequences of functioning local regulatory systems in the pericardium may provide some bases for the development of new diagnostic tools and, possibly therapeutic modalities in the treatment of cardiovascular diseases.

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BÉLA TURCHÁNYI (2006)

Injury of the skeletal muscle connected with the use of tourniquet (morphological, functional and clinical trials)

Supervisor: János Hamar

Consequences of the disturbed blood supply to the limb are determined by the degree of muscle injury, which can lead to complications of the whole body. The advantages of routinely used pneumatic tourniquet are: more safe preparation, less blood loss and shorter operating time. The commonly accepted limit for continuous tourniquet time is 2 hours, and it is allowed to use it for another plus hour after a 15–20 minutes long transient reperfusion (R). The secondary tissue injury, provoked by the tourniquet itself, could be at the cause of postoperative complications. In our experiments we tried to determine duration of tourniquet, which is harmless. We investigated the role of the afferent neurons and the damage of NMJ in reperfusion injury, as well as the systemic inflammatory response generated in the affected limb. In our animal model the injury of the extensor digitorum longus muscle was characterized by the loss of contractile force after 0.5, 1 and 2 hour (h) of ischemia (I) and 1, 24, 72 and 168 h of reperfusion (R). The loss of muscle contraction force after 0.5 and 1 h ischemia was more impaired after the first day of reperfusion, the worst values were 80% and 50% respectively. After a week of reperfusion the recovery of the muscle contraction force of 0.5 h ischaemia group was complete, but there was no restoration in the group of 1 h ischemia. There was no measurable muscle contraction force after 2 h ischemia. The loss of contractile force after 2 h ischemia is significantly reduced after selective deafferentation (i.e. blocking the peptidergic fibers by capsaicin pretreatment). We did not find any effect of ischaemia/reperfusion, with or without deafferentation, on the muscle contraction force of the contra-lateral side. Reperfusion injury, combined with artificial inflammation, caused loss of muscle contraction force in the contra-lateral side and the protective effect of deafferentation could not develop. Analyzing the ultrastructure of NMJ after ischemia/reperfusion we found significant damage of presynaptic elements, the axon terminal was fragmented and replaced by Schwann cells, while the postsynaptic membrane remained intact. The above changes got more aggravated after the first hour, but there were signs of regeneration at the end of first week. The regeneration of MNJ was delayed after selective deafferentation. We found sequestration of polymorphonuclear cells after minor surgery of upper limb in humans operated by 1 hour long tourniquet at 10 minutes reperfusion. We also found elevated IL-6 concentration after 24 hours, which is characteristic for major operations. The NCV of the forearm decreased significantly 48 hours after the use of tourniquet and even it was not normal after 30 days in most cases. We have shown that 30 minutes long tourniquet provokes measurable, but within a week reversible changes even in healthy organisms. The effects of tourniquet lastin for one and two hours are different in quality. The loss of muscle contraction force after 2 hours tourniquet can be reduced by selective deafferentation. The effect of half hour ischemia in inflamed milieu is significant and can be observed even in the contra lateral side. One hour tourniquet ischemia and reperfusion can elicit cell sequestration elevated cytokine concentrations, characteristic for tissue damage and long lasting axonal damage. We recommend that tourniquet time should not be longer than 1 hour at intact tissues and only 30 minutes in inflamed milieu.

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HAJNALKA VÁGÓ (2006)

Investigation of mechanisms and non-pharmacological therapy of cardiac arrythmias

Supervisor: Béla Merkely

Learning of mechanisms of arrhythmias may contribute substantially to the development of effective pharmacological and non-pharmacological therapeutic methods. Clinical relevance of endothelin-1 (ET-1), a strong vasoconstrictor and arrhythmogenic endogenous substrate, is not clarified yet. In our experimental studies, performed in the in situ canine heart, electrophysiological effects and the role in the pathomechanism of malignant ventricular tachyarrhythmias of endogenous and exogenous ET-1 was investigated. It has been proven in the *in vivo* ischaemia-reperfusion canine heart model, that during reperfusion ET-1 and big-ET levels increase in the coronary sinus, however there was no correlation between endothelin levels and electrophysiological changes. ET_A-receptor antagonist darusentan does not prevent electrophysiological changes and development of ventricular tachyarrhythmias during ischaemia and reperfusion. On the contrary, during ischaemia endogenous ET-1 tends to show a balancing effect. It has been proven that administration of high dose intracoronary ET-1 bolus has dual, ischaemic and direct, electrophysiological effect. It has been shown for the first time, that ET-1 causes monophasic action potential (MAP) and T-wave alternant. Our clinical study leads to the conclusion that previous atrial fibrillation, absence of preoperative β-blocker treatment and combined heart surgery are strong predictors of atrial fibrillation following open heart surgery. The basis of new nonpharmacological therapies is the learning of pathomechanisms of arrhythmias and in some cases heart failure, which is an arrhythmogenic substrate. In our experimental study reliable MAP measurements, suitable for investigation of arrhythmogenesis, were performed for the first time using fractally coated ablation catheters during spontaneous rate and during stimulations. It has been proven that radiofrequency ablation affects significantly MAP parameters. In Hungary, we were the first to apply effectively biatrial pacemaker and biatrial cardioverter defibrillator for the prevention of paroxysmal atrial fibrillation. In the majority of patients frequency of paroxysmal atrial fibrillation decreased significantly due to biatrial stimulation or combined pharmacological and resynchronisation therapy. Parasymphathetic cardiac neurostimulation is a promising new non-pharmacological treatment option in certain types of arrhythmias. In our clinical study we were able to stimulate cardiac parasympathetic nerves innervating atrioventricular node achieving ventricular rate control during atrial tachyarrhythmias with

chronically implanted coronary sinus lead. In our study biventricular pacemakers and cardioverter defibrillators were applied successfully in the treatment of drug refractory congestive heart failure combined with inter- and/or intraventricular conduction disturbances. AV sequential left sided chronic pacing using a single lead located in the coronary sinus has not been previously reported. Left sided DDD pacing was effective chronically in the improvement of the functional stage of patient suffering from congestive heart disease combined with left bundle branch block and binodal disease. Parallel with the investigation of pathomechanism of life-threatening ventricular tachyarrhythmias and the most common, clinically relevant atrial fibrillation due to recent technical development, we were able to support nonpharmacological therapeutic modalities, gaining popularity in clinical management, with novel observations.

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ENDRE ZIMA (2006)

Pericardial activation of metabolic adaptive processes: the effect of endothelin-1

Supervisor: Violetta Kékesi

The purine metabolites, adenosine (ADO) and inosine (INO) playing an important role in the metabolic coronary adaptation, and the most potent endogenous vasoconstrictor endothelin-1 (ET-1) exert opposite effect on the coronary blood flow. These agents show several fold higher pericardial concentrations than those of measured in the venous plasma in physiological conditions, and their concentrations show further increase in cardiac diseases. It was supposed that alterations of pericardial concentrations of adenine nucleosides reflect the activation of adaptive processes in the heart tissue. Moreover, it was also hypothesised that elevated levels of these endogenous regulatory agents in the pericardial space may affect cardiovascular function and may modulate production and effects of each other. On the basis of the above considerations we examined changes in pericardial concentrations of purine metabolites (ADO, INO and hypoxanthine—HXA) in myocardial ischaemia induced by intracoronary and intrapericardial administration of ET-1 to the *in situ* canine heart. The potential role of nitrogen monoxide (NO) in compensating the ET-1-induced ischaemic stress, and in modulating the myocardial purine metabolite deliberation was also studied by measuring changes of pericardial purine metabolite concentrations after intrapericardial ET-1 administration with or without systemic nitrogen monoxide synthase (NOS) blockade. The intracoronarially and intrapericardially administered ET-1 has significantly elevated the concentrations of the pericardial adenine nucleosides, while no changes were observed in the systemic plasma levels of these agents. The ischaemic effect of ET-1 was shown by ST segment elevations in each experiment. More-

over, the significant shortening of the left ventricular epicardial monophasic action potential (MAP) after the ip. administered ET-1 proved the ischemic effect of ET-1 that was exerted mainly in the subepicardial myocardium. The haemodynamic variables showed marked decrease to intracoronary administration of ET-1, but no significant changes occurred in either series of intrapericardial ET-1 studies. In the NOS inhibition (L-NAME iv. administration) setting the ET-1 elevated the pericardial purine metabolite concentrations significantly both in the control and L-NAME treated groups compared to basic values. However, no differences were detected in ischaemic elevations of pericardial purine metabolite levels, when compared the results obtained in the L-NAME treated or untreated experimental groups. The results suggest that the alterations of purine metabolites in the pericardial space reflects the dynamic changes of the nucleoside concentration in the adjoining interstitium i.e. the activation of the coronary adaptive mechanisms, which could be evoked either by intracoronary or intrapericardial ET-1. Considering the fact, that systemic NO synthase blockade did not aggravate the intrapericardial ET-1-induced myocardial ischaemia, and did not change the concomitant release of purine metabolites it is supposed that endogenous NO is not a supplementary factor to adenosine and inosine in this type of coronary adaptive responses.

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ANDRÁS ZSÁRY (2006)

Anthracycline induced cardiomyopathy and endothelin-1

Supervisor: Sándor Juhász-Nagy

The best-known side effect of adriamycin is development of cardiomyopathy. Besides the direct DNA destroying effect, free radicals may play a causative role in this process. Endothelium regulates the circulation and the homeostasis through the production of numerous compounds. Cardiac endothel cells modulate the myocardium in a paracrine manner. We investigated the effect of anthracycline treatment (max.dose: 450 g/m2) on left ventricular systolic function (i.e. ejection fraction (EF), aortic outflow velocity-time integral [VTI]), on diastolic function (i.e. mitral inflow E and A wave, E/A ratio, deceleration time [DT]), on Doppler index, which combines the systolic and diastolic left ventricular perfomance, and on plasma endothelin-1 (ET-1) level in 31 lymphoma patients. Also, we analyzed the correlation between the above variables. The echocardiographic assessment of the left ventricular function and the measurement of the plasma ET-1 level by ELISA was performed before and after the anthracycline treatment. 20 patients were followed up at one-year as well. For statistical analysis we used the Wilcoxon and ANOVA tests. The

plasma ET-1 level decreased after treatment (5.47±3.34 vs 3.44±0.69 pg/ml, $p<0.02$) and remained decreased at one year (3.43±0,57 pg/ml $p<0.008$). The EF also decreased after treatment (57.80±4.73% vs 48.05±5.65%, $p<0.0001$) and after one year (50.65±8.87% $p<0.0007$). The E/A ratio decreased significantly (1.35±0.40 vs 1.15±0.40 $p<0.01$) and further decrease was observed at one year (1.10±0.34 $p<0.003$). The DT increased significantly (177.0±44.96 vs 209.50±66.25 ms, $p<0.04$) and this was more pronounced at the follow-up (223.25±46.85 ms, $p<0.0022$). No significant changes were observed in other echocardiographic parameters (DI, VTI). The deterioration of both systolic and diastolic left ventricular function was observed following the completion of the adriamycine treatment and this effect persisted for one year. The decrease of ET-1 level is attributable to the direct cytotoxic effect (mRNA inhibition) and the shortage of the physiologically necessary ET-1 may play a role in the development of cardiomyopathy.

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- *Zsáry A, Szűcs S, Keltai K, Schneider T, Rosta A, Sármán P, Fenyvesi T, Karádi I (2004) Endothelins: A possible mechanism of cytostatics-induced cardiomyopathy. Leukemia & Lymphoma 45: 351–355.*
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PROGRAM 1/2.

MECHANISMS OF NORMAL AND PATHOLOGICAL FUNCTIONS OF THE CIRCULATORY SYTEM

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Program overview

The program consists of 12 research sub-programs completed with appropriate theoretical courses for postgraduate students. Different aspects of normal and disturbed regulatory processes of the cardiovascular system are in the focus. Each Ph.D. student is working on his/her own individual research project under the guidance of a qualified scientific advisor. Successful completion of the Program including publications in recognized international journals provides an opportunity to summarize the results in a Ph.D. thesis.

Titles of research projects	*Supervisors*
Changes in network- and biomechanical properties of intramural coronary resistance arteries with age, hypertension and other angiogenetic processes	György Nádasdy, Emil Monos
Changes in biomechanical properties of extremity arteries and veins during angiogenetic processes	Emil Monos, György Nádasdy
Pathophysiology of the cerebral circulation	Zoltán Benyó
Cardiovascular adaptational mechanisms in the whole body, as well as the myocardium and the brain cortex	László Dézsi
Role of bradykinin receptors in the circulatory adaptation under normal and pathological conditions; interactions with other mechanisms affecting blood pressure	László Dézsi
Spatio-temporal correlation of coupled hemodynamics and neuronal activities in the brain	András Eke
Role of postmenopausal hormonal deficiencies in altering the fractal structuring of hemodynamic fluctuations in the brain cortex	András Eke
Impact of cerebralsclerosis in altering the fractal structuring of cerebrocortical hemodynamic fluctuations	András Eke
Effects of blood substitutes on tissue hemodynamics and oxygenation	András Eke
Regulation of calcium homeostasis in the myocardial tissue	Tamás Ivanics
Alterations of the intracellular calcium homeostasis in progressive heart failure	Tamás Ivanics
Comparative evaluation of clinical and epidemiological diagnostic methods in the assessment of cardiovascular autonomic and peripheral sensory neuropathy	Péter Kempler
Investigation of vascular functions affecting the autonomous cardiovascular tone and reflex activity	Márk Kollai
Cardiovascular autonomous neural system	Márk Kollai
Study of promoting and inhibiting factors in cardiovascular aging	Béla Székács
Ischemia-induced molecular-biological changes of the blood-brain barrier	Péter Sándor
The role of the female sex hormones in the regulation of the cerebral blood flow	Péter Sándor
Role of oxidative stress in pathophysiology of cardiovascular system	Csaba Szabó
Videomicroscopic analysis of ureteral movements. Pharmacological and pathological effects.	György Nádasdy
Alterations of the biomechanical properties of extremity arteries and veins during angiogenetic processes	György Nádasdy
Alterations of biomechanical and network properties of intramural coronary resistance arteries with aging, hypertension and in other angiogenetic processes	György Nádasdy
Adaptation mechanisms of hemodynamic functions and network properties of the vascular system to physiological and to pathological loading	György Nádasdy
Functional integrity of the cardiopulmonary system	Ildikó Horváth

Videomicroscopic analysis of ureteral movements. Pharmacological and pathological effects	Imre Romics
The role of mitochondria in ischemic and degenerative diseases	Zsombor Lacza

Ph. D. students		***Supervisors***
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Péter Paragi	ft	Péter Tóth
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Éva Ruisanchez	ft	Zoltán Benyó
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Gábor Lenzsér	ft	Péter Sándor
Miriam Leszl-Ishiguro	ft	Zoltán Benyó
Petra Őrsy	ft	Zoltán Benyó
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Ph. D. graduates		***Supervisors***
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Béla Horváth	ft	Zoltán Benyó
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Zoltán Máthé	i	László Gerő
Beatrix Mersich	ft	Márk Kollai
Tamás Mersich	pt	Péter Sándor
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Gábor Raffai	i	Emil Monos
Orsolya Szenczi	ft	László Ligeti

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph.D. theses successfully defended in 2006, 2007 and 2008

BÉLA HORVÁTH (2008)

The role of the heme – heme-oxygenase – carbon monoxide pathway in the regulation of cerebral blood flow

Supervisor: Zoltán Benyó

Heme-oxygenase (HO)-mediated heme degradation is the primary cellular mechanism for production of endogenous carbon monoxide (CO). Previously thought to be simply a waste product, endogenously formed CO is now known to serve as a messenger in numerous physiological and pathophysiological processes. The HO–CO pathway has been reported to evoke direct vascular effects and to influence other vasoregulatory mechanisms like the cyclooxygenase (COX) and nitric oxide synthase (NOS) systems. However, the participation of CO in the regulation of the cerebral circulation has received little attention. Therefore, the aims of our studies were fivefold: (1) to investigate the involvement of endogenous CO in the regulation of the resting hypothalamic circulation, (2) to clarify its possible interaction with the L-arginine-NO pathway, and (3) its possible interaction with the COX pathway. Furthermore, (4) we aimed to determine the influence of the HO pathway on the cerebrocortical blood flow in the presence and absence of a functional NOS system, and (5) to test the presence of these interactions *in vitro* in isolated middle cerebral artery (MCA) segments.

Regional cerebral blood flow changes were determined using the Aukland's H_2-gas clearence method in the hypothalamus, and using laser Doppler flowmetry in the parietal cortex of rats. In vitro tension recording of the rat MCA has been performed using a conventional myograph system. The hypothalamic tissue NOS activity was measured on the basis of the formation of labeled citrulline from labeled L-arginine, and prostanoid levels in the cerebrospinal fluid were determined by gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS).

HO blockade by zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) increased hypothalamic NOS activity without changing hypothalamic blood flow (HBF). Furthermore PGE_2 concentration in the CSF of animals treated with ZnDPBG was found to be significantly lower as compared to saline-treated controls. Interestingly HO-blockade failed to influence the levels of other prostanoids such as PGD_2, the PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$. After NOS blockade, however, inhibition of HO induced a further significant reduction in the HBF, indicating that in the absence of NO, CO becomes responsible for the maintenance of cerebral blood perfusion. After COX blockade by diclofenac the inhibiton of HO increased NOS activity and blood flow in the hypothalamus. In animals subjected to simultaneous inhibition of COX and NOS ZnDPBG failed to alter HBF. In the parietal cortex HO blockade increased blood flow, which effect was abolished by NOS inhibitor pretreatment. Under *in vitro* conditions, ZnDPBG had no effect on the tension of isolated MCA segments.

Endogenous CO production appears to increase the HBF via a PGE_2-dependent mechanism, but at the same time suppresses NOS activity which leads to the reduction of the HBF. These effects of CO may neutralize each other under physiological conditions. In pathophysiological states, however, which are associated with altered COX and NOS

activity, the HO pathway may significantly influence the resting HBF. In the parietal cortex, however, the primary effect of carbon monoxid is the inhibition of the NOS activity, which effect seems to be mediated either by nonvascular carbon monoxide sources, or by the inhibition of nonvascular NO release. In conclusion, the HO-pathway appears to regulate the cerebral circulation of adult rats via interactions with the COX- and NOS-systems in a regionally heterogenous manner.

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KATALIN KERESZTES (2006)

The prevalence and risk factors of autonomic and sensory nerve dysfunction in patients with newly diagnosed Type-1 diabetes mellitus and primary biliary cirrhosis

Supervisor: Péter Kempler

Autonomic and sensory nerve dysfunction is a serious complication of both diabetes mellitus and chronic liver diseases. This is the first study that evaluates the frequency and predisposing factors of autonomic and peripheral sensory neuropathy in patients with newly diagnosed Type-1 diabetes mellitus and with primary biliary cirrhosis (PBC).

Our data confirm that autonomic and sensory nerve dysfunctions are frequent complications in newly diagnosed Type-1 diabetes mellitus and seem to be closely related to each other. Autonomic and sensory neuropathy may be significant even when the disease is diagnosed, involving both the parasympathetic and the sympathetic systems, as well as small and large fibres. Traditional cardiovascular risk factors (smoking, hypertension and serum cholesterol) should be considered as potential risk factors for the development of neuropathy, even in newly diagnosed Type-1 diabetic patients. These observations may confirm the role of vascular factors in the pathogenesis of neuropathy. There is a relationship between autonomic nerve dysfunction and QT-interval prolongation even in newly diagnosed Type-1 diabetes, which justifies that the standard autonomic reflex-tests should be performed on patients with a prolonged QT-interval.

Autonomic and sensory nerve dysfunction are frequent complications in patients with PBC and seem to be mutually related. As a novel finding a significant reduction of both time domain and frequency domain parameters of heart rate variability (HRV) were proven in PBC. The HRV analysis was found to be more sensitive for detecting autonomic impairment than standard reflex-tests and revealed the synchronic impairment of parasympathetic and sympathetic systems. Autonomic neuropathy is closely related to the severity and duration of liver disease as well as to the markers of hepatocellular dys-

function. We provided the first evidence that hyperesthesia is a characteristic feature of sensory neuropathy, and might contribute to the itching, a typical symptom of PBC. The sympatho-vagal imbalance is significantly related to the reduction of casual, and 24-hour mean blood pressure, as well as to lower blood pressure variability. These observations indicate that autonomic neuropathy may play a role in the development of systemic vasodilatation characteristic of hyperdynamic circulation.

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LÁSZLÓ KOCSIS (2007)

Theoretical investigation of the quantification problems of near infrared tissue spectroscopy (NIRS)

Supervisor: András Eke

Near infrared spectroscopy (NIRS) measures the absorption of near infrared light passing through the tissue, and calculates the mean tissue concentration of absorbers (absolute methods) or its change (relative methods). The main absorbers examined this way are oxy- and deoxyhemoglobin.

1. The relative technique (continuous wave NIRS) is based on the assumption that light scattering in the tissue is constant throughout the measurement. However, this assumption does not hold in case of neural and muscle tissue, where scattering follows the alterations of the membrane potential. I showed that neglecting scattering changes always leads to a relatively small systematic error, the size thereof being substantially determined by the oxy- and deoxyhemoglobin concentration of the tissue, independently of the wavelength combination used. Some special combinations (not applied in practice) are exceptions to this rule, where the error converges asymptotically to infinity.

2. Absolute techniques (time and frequency domain NIRS) are able to determine total tissue hemoglobin concentration, which is proportional to blood content and therefore related to hemodynamics, and mean tissue saturation, which characterizes oxygenation. The problem is that these variables provide only indirect information about the state of tissue level hemodynamics and oxygenation. In order to clarify this issue, I developed a novel mathematical model, which is based and extends on previous models. I showed that the physiological information content of data measured by NIRS, i.e. the whole set of background hemodynamic and oxygenation variables causing their alterations and being calculable from their values, can be unambiguously determined on the grounds of the formulas derived.

- *Kocsis L, Hermán P, Eke A (2006) The modified Beer-Lambert law revisited. Phys Med Biol 51: N91–N98.*

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ZOLTÁN MÁTHÉ (2007)

Factors influencing the early results of pancreatic islet transplantation: studies on isolation enzymes and islet revascularisation

Supervisor: László Gerő

The success of islet transplantation is hampered by variability of islet isolations and the considerable loss of islets early after transplantation. Causes of these are the lot-to-lot variability of isolation enzymes and the inadequate revascularisation of islets. It has been shown that VEGF plays a crucial role in this process. The aim of my thesis is to study a new two-component enzyme blend for human islet isolations and to assess the effects of VEGF-A upregulation in beta-cells on islet cell revascularisation and function after transplantation. We compared the results of human islet isolations performed either with *Collagenase NB1*/*Neutral Protease NB1* enzyme combination (group I, n=9) or with traditional Liberase HI (group II, n=9). Islet yield, morphology, apoptosis, *in vitro* and *in vivo* functions were analysed. Islets from RIP-VEGF transgenic mice were isolated, studied *in vitro*, then transplanted into chemically diabetic mice. Moreover, we modified the CDM3D beta cells using a lentiviral vector to promote secretion of VEGF-A in a Tetracycline (TC)-controlled manner (CDM3D-TET-VEGF cells). *In vitro* VEGF secretion, angiogenesis and stimulated insulin secretion were assessed. The cells were transplanted into syngeneic STZ-diabetic mice to assess the effects of this controlled VEGF expression *in vivo*. Time to normoglycaemia, IPGTT, graft vascular density were evaluated. Total IEQ/gr of pancreas was higher, islet morphology was improved and there was a higher proportion of free and intact islets with less apoptosis when the new NB1 enzyme was used (232). The time for diabetic mice to return to normoglycemia and the stimulated plasma glucose clearence were significantly accelerated in mice grafted with RIP-VEGF islets or CDM3D-TET-VEGF cells (233). VEGF delivery resulted in well defined TC controlled angiogenesis *in vitro*. There was a significant increase in vascular density of grafted CDM3D-TET-VEGF cells versus control cells. VEGF was only needed during the first 2–3 weeks after transplantation, when removed, normoglycaemia and graft vascularisation were maintained (234). The most important benefit of the studied new enzyme blend is that it makes the human islet isolations adjusted to the characteristics of a given pancreas possible. On the other hand, TC-regulated temporal expression of VEGF using a gene therapy could present a novel way to improve early revascularisation and engraftment after islet cell transplantation.

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BEATRIX MERSICH (2006)

Large artery elasticity in pregnancy and in patients with corrected transposition of great arteries

Supervisor: Márk Kollai

Stiffening of large elastic arteries is known to be an independent risk factor in cardiovascular diseases: increased arterial stiffness—due to the consequent left ventricular hypertrophy and impairment in baroreflex-sensitivity (BRS)—leads to increased morbidity and mortality.

In healthy human pregnancy and in preeclampsia (PE) the carotid elastical properties and their relation to BRS is barely investigated. We performed a longitudinal study with 12 healthy pregnant women: carotid elastic parameters and carotid-femoral PWV were determined in each trimesters (T_1, T_2, T_3) and 12 weeks after delivery.

In the cross-sectional study we investigated 12 PE pregnants and 12 normotensive controls: carotid elasticity-BRS relationship was determined in T3 and 12 weeks after delivery. Pulse pressure in the carotid artery and continuous pressure recordings on radial artery were measured by applanation tonometry. Carotid diastolic diameter and pulsatile distension were measured by echo wall-track system. Carotid-femoral PWV was determined by SphygmoCor system. BRS was calculated from the spontaneous fluctuation of systolic blood pressure and heart rate. We found that in healthy human pregnacy the carotid artery stiffens, but the aorta is becoming more compliant; there is no relationship between carotid elasticity and BRS in PE pregnancy.

In the other study we investigated carotid elasticity in children with transposition of great arteries (TGA). Based on animal studies, aorticopumonal septation and large artery elastogenesis are closely related events. We hypothesized, that in congenital heart defect with abnormal septation the elasticity of large arteries is reduced. We studied 48 TGA-patients and 48 age- and gender-matched controls. The elastic parameters of the carotid artery were determined by the above detailed methodology. We found that TGA-patients had markedly reduced carotid elastic parameters; thus, in human embrional development abnormal septation might be associated with impaired large artery elastogenesis.

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TAMÁS MERSICH (2008)

Effect of painful afferent stimulation on regional cerebral blood flow and global cerebral blood volume in anesthetized rats

Supervisor: Péter Sándor

Pain is a phenomenon positioned on the border of physiology and pathology. It is a well-known phenomenon that cerebral blood flow is coupled to neural activation induced by non-noxious somatosensory stimulation. However, basic questions related to pain-induced cerebral blood flow and hemispheric blood volume changes remain unanswered. The aims of our investigation were fourfold: (1) to measure the effect of noxious, painful stimulation in three different regions of the anesthetized rat brain (hypothalamus, thalamus and cortex); (2) to determine the possible involvement of different vasoactive substances like: potassium ion, free radical NO, prostaglandins or endogenous opioid peptides in pain-related blood flow changes; (3) to estimate gCBV changes during painful stimuli (4) to determine the role of the activated sympatic nervous system in the pain-induced regional blood flow and gCBV changes.

In the present study, the sciatic nerve of anesthetized rats was subjected to electric stimulation with noxious and non-noxious parameters. Changes in regional cerebral blood flow in the sensory cortex, in the thalamus by laser-Doppler flowmetry, in the thalamus and hypothalamus by H_2-gas clearance method. Changes in global cerebral blood volume (gCBV) were estimated by using Tomita's photoelectric method and neuronal activity was controlled by c-*fos* immunohistochemistry, respectively.

Noxious stimulation resulted in significant enhancement of neuronal activity both in the thalamus and in the somatosensory cortex indicated by marked c-*fos* expression in these areas. Increase in regional cerebral blood flow and simultaneously in MAP was observed in all regions (cortex, thalamus, hypothalamus) during the stimulation.

Similar changes in MAP induced by intra-arterial transfusion had no effect on rCBF, these result suggest, that elevated MAP is not resulted in the increase of regional blood flow in these areas. Blockade of ATP sensitive potassium channels (K^+) and sympathetic α-receptors significantly ATP attenuated the pain-induced blood flow increases in tBF and cBF, while inhibition of nitric oxide synthase was effective in the thalamus and hypothalamus while its role in cortex is not significant. The blockade of the sympathetic β-receptors, opiate receptors, and the cyclooxygenase enzyme had no effect on the pain-induced cerebral blood flow elevations. These findings demonstrate that during noxious stimulation, cerebral blood flow is adjusted to the increased neural activity by the interaction of vasoconstrictor autoregulatory and specific vasodilator mechanisms, involving the activation of sympathetic β-receptors, K^+-channels and the release of nitric oxide.

Our results show for the first time that (1) gCBV remains unaltered in both brain hemispheres during 2.5 min noxious stimulation of the sensory C-fibers of the sciatic nerve, (2) blockade of the L-arginine–nitric oxide system reduced significantly the steady-state control level of gCBV, (3) NOS blockade, however, did not affect the steadiness of gCBV during the stimulation.

Beside effective and well-demonstrated rCBF increase the steadiness of global cerebral blood volume may suggest a possible existing autoregulatory mechanism in global cerebral blood volume in rat. This question is addressed to further investigations.

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ZSUZSANNA MIKLÓS (2006)

The role of intracellular Ca^{2+}-homeostasis in the development of ischemic and diabetic cardiac disorders

Supervisor: László Ligeti

The main goal of our study was to elucidate the alterations in intracellular Ca^{2+}-handling, their relation to cardiac function and their role in the pathogenesis of two distinct cardiac pathophysiological disturbances, the ischemic/reperfusional damage and the diabetic cardiomyopathy. Several data indicate that the Ca^{2+}-homeostasis is disturbed under both pathological conditions. However, it is not clear yet which of the processes regulating Ca^{2+}_i-concentration ([Ca^{2+}_i]) in the disturbed Ca^{2+}_i-handling are involved. Also, little is known about the time sequale of these pathological events. In the ischemia/reperfusion experiments we seeked answers for the following questions: how do the beat-to-beat changes in [Ca^{2+}_i] (the Ca^{2+}_i-transients) change during a 30 min ischemic period and after the restoration of flow, what are the underlying Ca^{2+}_i-regulating mechanisms and how do they influence myocardial performance; what is the relation between the changes in [Ca^{2+}_i] and the development of ischemic myocardial contracture, and membrane-degradation. In the streptozotocin-treated hyperglicemic animal model we aimed to clarify whether at early stage of diabetes when no apparent signs of cardiac failure are present, disturbances of the Ca^{2+}_i-handling processes are already manifest and if so, which of the Ca^{2+}_i-regulatory mechanisms are responsible for this. Our experiments were performed on isolated perfused rat hearts. Their mechanical performance and Ca^{2+}_i-transients were recorded. For the determination of [Ca^{2+}_i] Indofluorescence technique was used. The alterations in the Ca^{2+}_i-handling mechanisms were assessed by the analysis of the Ca^{2+}_i-transients. Our results showed, that disturbances in Ca^{2+}_i-homeostasis appeared early in ischemia. After the restoration of flow following 30 min of ischemia the Ca^{2+}_i-transients remained stunted due to depressed activity of Ca^{2+}_i-handling processes. The tissue arachidonic acid content used for assessment of membrane degradation showed tight correlation with [Ca^{2+}_i]. In the diabetes experiments, though under resting conditions no difference could be detected between 4-week diabetic and healthy hearts regarding cardiac function, after β-adrenergic stimulation the contractile response of the diabetic hearts was diminished. Parallel to this, disturbances in Ca^{2+}_i-handling could be observed.

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MARIANN MURÁNYI (2006)

Influence of diabetes mellitus on cerebral ischemia and reperfusion injury

Supervisor: Zsombor Lacza

Diabetes mellitus aggrevates and worsens the brain damage caused by global or focal cerebral ischemia. The purpose of our study was to explore what is the underlying mechanism of the exaggerated brain damage caused by diabetic ischemia. In our experiments we investigated whether there is a difference in the activation of the mitochondria-dependent apoptotic pathway after ischemic injury in diabetic rats compared to non-diabetic animals. In further experiments we examined whether increased free radical production and activation of astrocytes contribute to the exaggerated brain damage after diabetic ischemia compared to non-diabetic ischemia. Finally, we investigated the heat shock protein expression and synthesis in hyperglycemic ischemia. Early activation of apoptotic cell death pathway in diabetic animals was observed. The results show that the production of free radicals such as superoxide anion, nitric oxide and peroxynitrite are stimulated in neurons after diabetic ischemic injury. In astrocytes only nitric oxide production was enhanced. These data suggest that diabetic ischemia increases peroxynitrite production in neurons by enhancing the formation of superoxide since peroxynitrite is derived from the reaction of NO and superoxide. As a reaction to the increased stress caused by hyperglycemia enhanced the heat shock protein expression and synthesis in neuronal cells. Finally, we detected reactive astrogliosis in both ischemic groups, although in the diabetic animals damage of astrocytes was observed early after the ischemic injury. These results draw attention to the enhanced intracellular events leading to cell damage may further limit the time-window of the effective therapy, if the patient suffer from diabetes mellitus.

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GÁBOR RAFFAI (2006)

Adaptation of the cardiovascular system to gravitational loading: systemic hemodynamic and regional vascular responses to acute and chronic orthostasis

Supervisor: Emil Monos

As compared with other species, human race has to be adapted to several orthostatic challenges due to the erect body position. By assuming standing position, high blood columns are generated in the longitudinal large vessels of the body. The weight of these columns in vertical position of the body exerts a substantial increase in transmural pressure. The rate of pressure increase is especially large in the very distensible lower extremity veins. Several mechanisms have been evolved (e.g. baro- and vestibulosympathetic reflexes, myogenic response, hormonal components etc.) protecting the whole organism against deteriorating consequences of such gravitational challenges. The significance of this issue is accentuated by the fact that the incidence of orthostatic disorders of the cardiovascular system has shown a tendency to increase in the past decades. Orthostatic disturbances affect both arterial and venous circuits and thus influence healthy functioning of the whole organism. For example, orthostatic hypertension, which is frequently associated with diabetes and/or neuropathy, can substantially contribute to the rise of systemic blood pressure also increasing the risk of other cardiovascular diseases. This is the reason why studying chronic adaptive mechanisms of the extremity vessels has been standing in the focus of our laboratory. In addition to general conduit functions the role of extremity blood vessels in supporting orthostatic tolerance can be significant as well. The accessibility of saphenous and brachial vessels makes it possible to carry out comparative studies of these two regions and also between arterial and venous vessels both in animal models of experimental orthostasis and, with substantially more restrictions, in humans. In this Ph.D. study we intended to reveal unknown mechanisms of cardiovascular adaptation activated either by short-term or long-term orthostatic body position. Short-term responses of systemic hemodynamics on one hand and local long-term responses of the endothelium on the other hand were investigatedin conscious rats using a novel experimental head-up tilt model. For the purpose of short-term studies we combined the tilt technique developed by us with modern cardiovascular telemetry. It was found that 5 min experimental orthostasis does not induce significant changes in arterial blood pressure, while orthostasis sustained 8 for 120 min resulted in hypertensive responses in both normotensive and hypertensive animals. Hypertensive action of sustained orthostasis was prevented by administering α1-receptor antagonist prazosin and was augmented when non-specific stress effects were inhibited by the application of chloralose in subanesthetic dose. In the case of chronic studies rats were exposed to orthostatic load for two weeks. To examine responses of the endothelial cells of extremity vessels the tilt technique was combined with light and electron microscopy, immunohistochemistry and immunocytochemistry, as well as morphometry. Vacuolization of pathological extent developed in the endothelium of saphenous and brachial veins, but not in the corresponding arteries when immersion fixation of the cylindrical tissue segments was applied. The extent of vacuolum development was not influenced by chronic experimental orthostasis. Following careful whole body fixation no vacuolization appeared, however approximately four times more electron dense vesicles were observed in the endothelium of the saphenous

vein than in the corresponding artery or in any of the brachial vessels. Neither the vacuoles nor the dense vesicles could be linked to apoptotic or any other kind of cell damage. In the endothelium of saphenous vein chronic orthostasis significantly suppressed the size of the dense vesicular system without altering that in the other extremity vessels. Endothelin and platelet derived growth factor were identified in these vesicles. Chronic tilt did not influence the endothelin level of blood plasma. In conclusion, short-term sustained orthostasis results in a small but significant arterial blood pressure elevation via increased sympathetic discharge. This response is probably inhibited by non-specific stress, but not inhibited by the elevation of arterial blood pressure via NO deprivation. This protocol seems to be applicable for modeling orthostatic hypertension. Results related to the endothelium of extremity vessels suggest that endothelin and platelet derived growth factor are released from the dense vesicles to the abluminal side in response to chronic orthostatic load, where they may exert their vasoconstrictor and smooth muscle proliferative actions, supporting orthostatic tolerance of the whole organism. In addition, these results highlight the importance of careful selection of the type of fixation when endothelium is under investigation. Thus, we demonstrated evidences of unknown mechanisms of gravitational adaptation at both systemic and cellular organization levels. Our results may help in opening new directions of practical applications, such as the development of effective methods to treat orthostatic hypertension, and to prevent orthostatic intolerance as well as venous varicosity.

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ORSOLYA SZENCZI (2006)

Intracellular calcium handling of myocardium: alterations in ischemia and cardiomyopathy

Supervisor: László Ligeti

Cardiac contractility is dependent upon the precise regulation of intracellular Ca^{2+}_i cycling, i. e. the Ca^{2+}_i transient. In the present study disturbances of Ca^{2+}_i handling in ischemic/reperfused heart after heat shock (HS) treatment and in doxorubicin (DOX) induced cardiomyopathy have been studied.

Over the past two decades, numerous studies have shown that HS preconditioning enhances the tolerance of the heart against ischemia/reperfusion insult. One of the features of ischemia/reperfusion-induced cardiac injury is the persistent increase of intracellular free Ca^{2+} level. Experimental data on Ca^{2+}_i handling in the ischemic/reperfused heart after HS treatment are, however, scarce. The available information mainly pertains alter-

ations in SR calcium uptake and is contradictory. No experimental data regarding HS preconditioning and Ca^{2+}_i handling during ischemia/reperfusion have been obtained in isolated cardiomyocytes or perfused heart preparations, hence one of the main aims of the present study was, therefore, to determine the effect of *in vivo* HS pretreatment on Ca^{2+}_i handling in the intact ischemic/reperfused rat heart in relation to contractile performance and to disclose the nature of the alterations in Ca^{2+} release and sequestration processes, if there is.

Development of cardiomyopathy is multifactorial. However, it appears that oxidative and nitrosative stress—such as induced by DOX—plays a clear role in this process. Doxorubicin undergoes redox cycling to generate free radicals that are responsible for mediating the various cytopathologies associated. One of the more recently identified pathways of doxorubicin cardiotoxicity is related to poly (ADP ribose) polymerase (PARP) activation. When activated by DNA single-strand breaks, PARP initiates an energy consuming cycle slowing the rate of glycolysis and mitochondrial respiration eventually leading to cellular dysfunction and death. Very little is known however how these processes affect Ca^{2+}_i handling of the cardiomyocyte, prompting the secondary aim of the present study.

In the first part of our study we have shown for the first time in intact hearts "*in vitro*" that heat shock pretreatment prevents a further increase in end-diastolic Ca^{2+}_i after an ischemia/reperfusion insult and, hence, mitigates post-ischemic calcium overload. Our results also suggest that improved postischemic myocardial performance in response to HS pretreatment is at least partly due to a relatively preserved sensitivity of the myocardial contractile machinery towards Ca^{2+}. However, delineation of the precise mechanisms responsible for the observed phenomena needs further experimental work.

In the second part of this study we made the following observations (1) demonstration of an increase in end-diastolic calcium levels in doxorubicin-treated hearts; (2) development of a decline in calcium sensitivity of the contractile machinery in the diseased hearts; (3) the enhanced susceptibility of the doxorubicin-treated hearts towards a subsequent acute oxidative stress and (4) the protective effects of PARP inhibitor, strongly suggesting that activation of the nuclear enzyme PARP is involved in the functional alterations of the doxorubicin-treated heart. Overall, the results of the current study are consistent with the notion that reactive oxidant species and the PARP pathway play a pathogenetic role in the development of doxorubicin induced cardiac dysfunction. Normalization of cellular calcium handling appears to be an additional mode of the cardio protective effects of PARP inhibitors.

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PROGRAM 1/3.

BIOLOGICAL EFFECTS OF IONIZING AND NON-IONIZING RADIATION

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Program overview

Biological effects, induced by different physical and chemical environmental factors (e.g. ionizing and non-ionizing radiations, pollutants) endanger the whole biosphere including humans. The conscious environmental protection and the prevention of several human diseases due to these environmental factors can be effective only knowing and quantifying the sources (natural radiation background, nuclear disaster, solar radiation, ozone depletion, chemical pollution, etc.). In this Program field and laboratory measurements of monitoring of ionizing and ultraviolet radiations are offered with particular interest to the quantification of their biological effects on global, cellular and molecular level.

Titles of research projects	*Supervisors*
Self-assembling amyloid filament systems	Miklós Kellermayer
Self organizing and nanomechanical properties of the myosin motor protein	Miklós Kellermayer
Nanobiotechnology	Miklós Kellermayer
Nanomechanics of nucleoprotein systems	Miklós Kellermayer
Molecular biophysics of the giant muscle protein titin	Miklós Kellermayer
Measurement of the environmental UV (ultraviolet) radiation, evaluation of health risk of the population caused by environmental and artificial UV radiation	Györgyi Rontó
Study of protein conformational dynamics related to function: Experimental and computational approaches	Judit Fidy
Studies of the effects of antioxidants and photosensitizers on cell cultures and liposomes	Pál Gróf
Mechanism of action of photosensitizers and their application in microbial inactivation	Gabriella Csík

Ph.D. student		*Supervisor*
Dániel Sándor Veres	ft	Pál Gróf

Ph.D. candidates		*Supervisors*
Georgina Fröhlich	ft	Tibor Major
Csilla Pesznyák	i	Györgyi Rontó

Ph. D. graduates		*Supervisors*
Csaba Böde	ft	Judit Fidy
Marianna Budai	ft	Pál Gróf
Márton Hegedüs	ft	Andrea Fekete
József Lövey	pt	György Köteles
Jenő Pálvölgyi	i	Györgyi Rontó
Kristóf Zupán	pt	Gabriella Csík

ft, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

CSABA BÖDE (2006)

The role of the quaternary structure in the chaperone function of small heat-shock proteins

Supervisor: Judit Fidy

In our work we investigated the chaperone function and quaternary structure formation of small heat-shock proteins. We have chosen two members of the small heat-shock family: βγ-crystallin, an abundant mammalian sHSP, and MjHSP16.5 from archebacteria *Methanococcus jannaschii*. With various physical and chemical methods (high pressure, change of the environmental pH) we modified the structure of these proteins, and investigated the related structural and functional changes.

The secondary structure changes of the examined proteins were small, or after the extreme acidic pH shock the secondary structure quickly regenerated. On the contrary, the quaternary structure changes were large, and after the perturbation there was no, or only very slow rearrangement of the quaternary structure.

We observed that after the preturbation, presumably the change in the quaternary structure caused an enhancement of the chaperon activity of the proteins. In case of βγ-crystallin we observed more quaternary structures belonging to the active state of the protein. The existence of more, active quaternary structures might be important in the adjustment of small heat-shock proteins to different stresses and substrates.

The high pressure experiments revealed a possible dissociation mechanism for MjHSP 16.5, in which first water molecules enter the inner core of the protein. The experiments showed that the weak, secondary binding forces have an important role beside the intermolecular sheets in maintaining the oligomeric structure of small heat-shock proteins.

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MARIANNA BUDAI (2006)

Investigation of the effects of drugs and UV radiation on biological and artificial membranes

Supervisor: Pál Gróf

Investigation of the effects exerted by different physical and chemical agents on biological and model membranes is of prominent importance. The uptake of the different drugs, their transport to the target occur through the biological membranes. Recently, a general trend can be observed in the formulation of drugs: incorporation of the drugs into liposomes. Knowledge of the molecular interactions between the transporting lipids and the incorporated agents is therefore very important.

Nowadays, the increased environmental UV radiation requires investigation of the effects of the UV radiation exerted on biological membranes. Beside of modeling biological effects, studies on the effects of the UV radiation on model membranes can result in new knowledges on the stability of the liposomes containing phototoxic drugs.

During my study, three different methods (EPR spectroscopy, DSC and light scattering measurements) have been applied to investigate the molecular interactions between drugs and the lipid molecules. Derivatives of the morphine as well as the (fluoro)quinolones mainly interact with the headgroups of the lipid molecules resulting in an increase of the molecular ordering of the lipids. Studying the effects of these drugs as the function of the temperature I observed, that the rigidizing effect is more pronounced below the phase-transition temperature. My observation, that drugs with protonable/de-protonable groups can modify the membrane-fluidity due to specific, local interactions with the lipid/stearic acid molecules of a membrane depending on the pH as well, call attention to choose optimal pH-interval for such drug formulations.

Investigating the UVA effect on human fibroblast cell line I concluded that a decrease in the membrane fluidity due to UVA radiation can be detected only at doses higher than 150 kJ/m^2, and close to the lipid head groups. The relative redox capacity of the cells determined by EPR method compared to some viability tests does not prove the protective effect of the vitamin-E treatment at the membrane level. According to our measurements, increasing amount of the unsaturated lipids, moreover the presence of phototoxic drugs in the model membranes increase the sensitivity of the membranes against the UV radiation.

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MÁRTON HEGEDÜS (2007)

Extension of DNA based dosimetry to broad-band UV radiation

Supervisor: Andrea Fekete

Human activity is leading to a changing radiation environment, including the broadening spectrum of ultraviolet radiation. The main target of ultraviolet rays in the living organisms is the genetic material, therefore it seems reasonable to use DNA itself as a biological dosimeter. The DNA damage in skin cancer makes UV radiation the most ubiquitous environmental carcinogenic agent.

The aim of this work was the development and application of a method for the quantitative determination of DNA damages caused by a wide spectrum of UV radiation by polymerase chain reaction (QPCR). QPCR was optimized for two fragments of bacteriophage T7 DNA. Based on the amount of the PCR product Poisson-equation was used to calculate the average lesion frequency. The QPCR works also on intact phages and its sensitivity depends on the fragment length used. Routine use of the 555 bp fragment is possible for the determination of large doses, while the 3826 bp fragment is suited for smaller ones. In the case of five various sources of UVA–B–C radiation a good agreement was found between the lesion frequencies determined by QPCR and calculated from biological activity data. This indicates that the QPCR method is capable of detecting practically all UV photoproducts and it can be used for the validation of the phage T7 biological dosimeter. The comparison of total lesion frequencies in intact phage and isolated DNA led to the conclusion that protein binding to DNA can increase its UV sensitivity due to local structural changes, where protein binding is the decisive factor and not DNA conformation. High-energy ultraviolet radiation is also one of the deleterious parameters in the space survival of biomolecules. Our T7 phage/DNA thin layers to be sent to the EXPOSE unit of ISS proved to be suitable for space simulation experiments. The dehydrating effect of vacuum and on-board temperature fluctuation resulted in low phage/DNA lesion frequencies. Interestingly, UVC irradiation led to a saturation tendency in lesion formation both in phage T7 and DNA, which can be explained by the photoreversion of dimer photoproducts. A broader spectrum of extraterrestrial radiaton caused a significant amount of other photoproducts as well. UVC radiation combined with vacuum treatment showed a synergistic effect. However, shielding by thicker layers and e.g. mineral material may result in substantial protection of phage/DNA from extraterrestrial damages.

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JÓZSEF LÖVEY (2006)

Studies on radiosensitivity of malignant tumors in experimental and clinical conditions

Supervisor: György Köteles

Approximately half of the patients diagnosed with cancer receive radiotherapy during the course of the disease. Irradiation plays important role as a definitive-curative treatment alone or in conjunction with chemotherapy, as adjuvant treatment accompanying surgery and as a very effective palliative modality. Studies about the radiosensitivity of tumors as well as modification of their radiosensitivity are in the mainstream of cancer research.
In our studies we examined different aspects of radiation sensitivity and radiosensitization of human cancers *in vitro, in vivo* and in clinical circumstances.
We investigated the effect of paclitaxel (PTX) and radiation on cytoskeleton, proliferation, clonogenecity, tumorigenicity and metastatic potential of human squamous cancer cell lines *in vitro* and *in vivo*, and the effect of motility of human glioblastoma cell lines with long-term video-microscopy.
We examined the effect of low dose PTX administration concurrently with radiotherapy in locally advanced head and neck cancer.
In frame of a prospective trial we evaluated the effect of irradiation on microvascular density and its predictive value in patients with oropharyngeal cancer treated with radiotherapy.
12-lipoxygenase metabolic pathway of arachidonic acid has been showed to promote prostate cancer progression, angiogenesis and cell survival. We studied how specific 12-lipoxygenase inhibitors influence the radiation sensitivity of human prostate cancer cell lines *in vitro*.
We found that PTX and radiotherapy interferes with each other in both human squamous cancer and glioblastoma cell lines.
Both low dose radiation and short exposure PTX induced bundling the tubulin of the cells and both could suspend each other's effect. Short exposure PTX enhanced the metastastic potential of carcinoma cell lines while employing in combination with radiation the metastatic potential was re-duced. Low dose radiation enhanced the motility of giloblastoma cell lines while pretreatment with low concentration PTX reversed this effect.
Low dose PTX administration concurrently with radiation proved to be feasible, resource effective and very well tolerable for the patients with locally advanced head and neck cancer.
Recently *in vitro* and animal experiments proposed that radiation effects through the killing of endothelial cells. Our results showed that decrease of microvasulcar density following radiation is a strong predictor of objective response and overall survival in patients with oropharyngeal cancer. This result supports in clinical circumstances the revolutionary findings of animal experiments on radiation and endothelial cell killing. However these results await confirmation by trials on a larger patient population.
Based on our *in vitro* experiments, 12-lipoxygenase inhibitors are promising candidates to be radiation enhancers for prostate cancer. Our results showed that 12-LOX inhibitors could increase apoptosis, decrease clonogenecity in supraadditive manner of prostate cancer when applied together with radiation.

Our studies brought new results in connection with the effect of PTX and radiotherapy and their combination. The unexpected effects we found like the prometastatic potential of short exposure PTX or the increase of motility of glioblastoma cell following low dose irradiation point out the importance to consider all effects and relationships of the combined treatments when designing new clinical trials.
Low-dose PTX administration concurrently with radiation is a promising alternative for patients with locally advanced head and neck cancer in poor general condition. The value of this treatment and the possible sensitizing effect of low dose PTX should be investigated in the frame of a randomized controlled trial.
Our results showing the predictive value of decrease in intratumoral microvessel density has a paramount significance. It also supports the preclinical findings, which showed that endothelial cell killing is important in the antitumoral effect of radiation.
As radiotherapy has a major role in the curative treatment of prostate cancer, our findings that combination of 12-LOX inhibition and radiation has supraaditive effect on prostate cancer cell lines makes these inhibitors promising candidates for clinical use. First, *in vivo* experiments have to prove the *in vitro* data and in case of confirmatory results, development toward clinical trials can be initiated.

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JENŐ PÁLVÖLGYI (2008)

On the use of a non-isocentric C-arm as a brachytherapy localiser and the multiparametric fit method in reconstruction of the Fletcher-Suit-Delclos applicator

Supervisor: Györgyi Rontó

The non-isocentric C-arm X-ray fluoroscopy unit is not typically used as a brachytherapy localizer. The aim of the dissertation was to examine the feasibility of a mobile C-arm fluoroscopy unit (Carm) as a brachytherapy localizer in treatment planning. We take the reconstruction images with a non-isocentric C-arm, located in the brachytherapy treatment room. The patient lies in the same position during the treatment planning and the dose delivery. The brachytherapy treatment planning is based on a pair of digital posterior-anterior (PA) and posterior oblique (PO) images instead of the usual lateral ones. The main advantage of this method is that the posterior oblique images are less affected by the shielding of the pelvic bony structures and the quality of the images are better, than the quality of the lateral ones. The main problem with the non-isocentric machine is that the magnification factor is not known exactly and varies with the C-arm's orbital angle. We describe our method for taking isocentric images with the non-isocentric C-arm. For generation of treatment plans with a commercial brachytherapy planning system the magnifi-

cation factors of the reconstruction images have to be determined previously. We describe our method in the determination of the magnification factor. The dedicated treatment table's shielding limits range of the possible posterior oblique angle. We tested the reconstruction accuracy under these circumstances [*Pálvölgyi J. (2003) Radiother Oncol 67: 107–112*].

We present an alternative reconstruction method that is suitable for reconstruction of FSD applicators and that utilizes pairs of posteri-anterior and lateral or preferably posterior oblique reconstruction images obtained by a C-arm and the determination of the magnification factors is incorporated. The MPF reconstruction relies on the known geometry of the tandem and the ovoid. The actual FSD insertion is reconstructed by adaptation of the geometry of tandem and ovoid by translations and rotations. The fixation mechanism limits the possible excursions of the FSD applicator's parts. The limited freedom of excursions and the determination of the magnification factors are also incorporated. The accuracy tests performed with a tandem phantom and FSD insertion with different tandem-ovoid geometries showed that the method is suitable for the clinical use [*Palvolgyi J. (2006) Med Phys 33(1): 69–75*].

We use the reconstruction images obtained by the C-arm in treatment planning of the cervix carcinomas with the FSD applicator and the endometrial carcinomas using the modified Heyman packing method. We describe our treatment planning methods based on the reconstruction images obtained by the C-arm [*Palvolgyi J. (2006) Physica Medica 22(4): 127–130*].

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KRISTÓF ZUPÁN (2008)

The role of photoactivation in the genotoxicity of drugs and chemicals: photosensitization of DNA and nucleoprotein complex with a cationic porphyrin derivative

Supervisor: Gabriella Csík

Physico-chemical virus inactivation procedures are potential tools in the prevention of transfusion-transmitted viral infections. As the blood products usually lack nucleic acids as valuable components, the destruction of the genetic material should be primarily considered for the pathogen inactivation strategies. One promising approach is the application of the photodynamic effects with photosensitizers binding selectively to nucleic acids. In my work, I studied the utility of tetrakis-(4-N-methyl-pyridyl)-porphin (4MPP) in the nucleic acid-specific photosensitization. As a model for the human pathogen viruses, I used an icosahedral, double stranded DNA bacteriophage of *Escherichia coli*, the phage T7. The binding of 4MPP to the bacteriophage and to DNA isolated from the virus was studied using absorption and fluorescence spectroscopy, fluorescence lifetime and optical

melting experiments. High affinity of the porphyrin toward both systems was demonstrated. It was shown that the dye binds to the DNA within the phage nucleoprotein particle; there exists no form of 4MPP bound to the protein part of the system. The effect of various environmental factors on the binding was also studied in details. The inactivation of the bacteriophage upon 4MPP + visible light irradiation was assessed by determining the plaque-forming activity of the virus samples. It was shown that under appropriate circumstances, the infectivity of the phage can be virtually abolished. The mechanisms of the inactivation were characterized via absorption spectroscopy, optical melting, agarose gel electrophoresis and polymerase chain reaction. It was found that the damage of the phage nucleic acid, the destruction of the capsid proteins and the formation of DNA-protein cross-links are all partially responsible for the loss of the biological function of the phage. Based on these results, 4MPP is a promising agent for nucleic-acid specific photosensibilization.

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PROGRAM 1/4.

FLUID AND ELECTROLYTE BALANCE IN HEALTHY AND PATHOLOGICAL REGULATION OF BLOOD PRESSURE AND CIRCULATION

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Program overview

Our Ph. D. Program in pathophysiology/nephrology received accreditation in 1993. The goal of this Program is to foster the continued development of traditionally and internationally recognized basic and clinical nephrology research in Hungary. Participating experts in this Program represent various fields of physiology, pathophysiology, internal medicine, pediatrics, transplantation and clinical nephrology, and share a complex, multidisciplinary view of nephrology research and education. In our research activities, special emphasis is placed on the control of fluid and electrolyte balance, blood pressure and regulation of kidney function. With the discipline of translational research, modern experimental techniques are used at various levels from molecule to bedside. Research topics for doctorate degree graduates in Nephrology and Hypertension (35 Ph. D.-s so far) are quite extensive. Our research team has gained international recognition and is a leading expert on the regulation of renal hemodynamics and microcirculation, the mechanisms and progression of various kidney diseases including chronic renal failure, diabetic nephropathy, fibrosis and kidney allograft rejection. We are studying intracellular signal mechanisms, cell-cell communication, TGF-beta and the renin-angiotensin system, and their interaction with the control of renal hemodynamics. By studying the morphology and function of the afferent arteriole and juxtaglomerular apparatus, we have described a novel regulatory mechanism of glomerular filtration.

Titles of research projects	*Supervisors*
Risk factors in diabetes nephropathy. Analysis of the correlations among serum relaxin, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β) and angiotensin II levels. Prevention of cardiovascular death	László Rosivall
Renin-angiotensin system (RAS)	László Rosivall
Intracellular signalling mechanisms of transforming growth factor-beta and angiotensin II	László Rosivall
Factors associated with the outcome of kidney transplantation	László Rosivall
Novel concepts in the regulation of blood pressure and kidney function	László Rosivall
Inhibition of progressive renal fibrosis by genetic factors. Exploration of molecular transcriptic as a mechanism of the anti-fibrotic effects of TGF-beta	László Rosivall
Effects of VEGF, Angiotensin II, relaxin and renin (prorenin) on endothelial fenestration and permeability	László Rosivall

Dialysis therapy, biocompatibility, quality of life	László Rosivall
Characterization of leucocyte subpopulation to follow/prevent the rejection of kidney in transplanted patients	László Rosivall
Dialysis therapy, biocompatibility, quality of life	István Mucsi
Intracellular signalling mechanisms of transforming growth factor-beta and angiotensin II	István Mucsi
Diagnosis and treatment of renal osteodystrophy	István Mucsi
Obstructive sleep apnea syndrome as a cardiovascular risk factor in chronic kidney disease patients	István Mucsi
Factors associated with the outcome of kidney transplantation	István Mucsi
Cell-cell and cell-matrix interactions in the progression of chronic renal fibrosis	István Mucsi
Cardiovascular risk, calcium, phosphorus and bone metabolism in chronic kidney disease patients	István Mucsi
Cardiovascular and renal pathophysiology of aging	Zoltán Ungvári
Pathophysiology of nano-medicines with particular focus on nephrology and circulation	János Szebeni
New prognostic and morphological approaches to diagnosing HIV	János Szebeni
Pathophysiology of the complement system, with particular focus on the mechanism of drug-induced acute activations, their consequences and inhibition	János Szebeni
Novel concepts in the regulation of blood pressure and kidney function	János Peti-Peterdi
Endogeneous diuretic substances in the development of cardiac hypertrophy: experimental and clinical studies	Miklós Tóth
Effect of hypertension on microcirculation	Ákos Koller
Therapeutic utilisation of RNA interference to prevent ischemia-reperfusion injury in the kidney	Péter Hamar
Investigating the dual role of TGF-beta in atherosclerosis in a double gene-modified mouse strain	Péter Hamar
Molecular mechanisms of renal allograft rejection	Péter Hamar
The role of zinc in regulation of intracellular Ca^{2+} and cAMP concentrations. Effects of zinc in the regulation of transepithelial ion transport	Ákos Zsembery
Cardiovascular diseases and renal failure. Prevention of renal hyperparathyroidism and osteodystrophy in early stage renal failure	András Szabó
SPECT analysis of cerebrovascular dysfunction induced by free radicals following cerebral trauma	Kinga Karlinger
Hypertension in pregnancy and molecular mechanisms of toxicosis	Miklós Molnár
The role and mechanisms of epithelial-mesenchymal transition during fibrosis and tumor progression	Attila Sebe

Ph. D. students		***Supervisors***
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Csaba Révész	ft	Péter Hamar
Csaba Mihály Rikker	a	László Rosivall
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Ph. D. graduates		***Supervisors***
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Péter Komlósi	i	László Rosivall
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Zoltán Lengyel	pt	László Rosivall
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Attila Sebe	pt	István Mucsi
Nikoletta Szücs	pt	Károly Rácz
Tamás Terebessy	ft	László Rosivall, István Mucsi
Zhi-Ren Zhang	i	László Rosivall

a, absolutorium; ft, full-time; pt, part-time, i, individual

Abstracts of Ph.D. theses successfully defended in 2006 and 2008

GÁBOR ANDRÁSSY (2008)

The effect of different stressors on the QT interval and the T wave

Supervisor: László Rosivall

The duration of the QT interval on the surface ECG is a global measure of the time the heart takes to depolarize and repolarize. Prolonged QT interval is associated with the generation of life-threatening rhythm disturbances and sudden cardiac death. The QT duration is principally influenced by heart rate (RR, cycle length), so heart rate correction is required in the analysis of repolarization duration. Based on mathematical modeling of the QT/RR relationship several correction equations have been published, including the most commonly used Bazett formula that was a methodological exception because it was purely observational and did not involve any regression modeling. Not surprising that amongst all, the Bazett formula performs the worst: because of its profound inherent heart rate dependency, QTc values incorporate excess distortion. Several clinical circumstances have been reported to be associated with QTc prolongation, but the use of the Bazett method questions their relevance. One such condition with confounding reports is smoking, that has been found both to prolong or either to shorten the QT interval. Consequently, we conducted a placebo controlled trial and clarified the effect of acute smoking on the QT interval: as an effect of smoking the Bazett corrected QT interval prolonged but corrected QT-s obtained by more reliable methods did not change. Further, in another model when exercise ECGs obtained at different heart rates were compared, we demonstrated that the study specific method of QT correction (fitting the correction method to the studied data set) is superior to any other preformed formula. In addition, Bazett method was inferior to all the other formulae. These findings underscore the importance using a reliable QT correction method when comparing QT-s measured at different heart rates: the Bazett method is clearly inappropriate, its use may lead to erroneous conclusions.

The autonomic nervous system, which can act directly at the cellular level or indirectly through modulation of heart rate, is another important source of QT changes. Both chronic and acute mental stresses induce cardiovascular and neuroendocrine responses including QT changes and lethal arrhythmias through alterations of the neural transmissions to the heart. Epidemiologic evidence also suggests that there is a relationship between stress and cardiac morbidity and mortality in susceptible individuals. However, the effect of psychological stress on the QT interval is subject to speculation: previous reports provided conflicting data on the effect of mental stress on the QT interval duration. Therefore, we have accomplished four trials assessing the effect of various mental stressors on the QT interval. To overcome controversy about the use of fixed equations for QT correction, QT adjustment in these studies included the study and subject specific methods that were reported to perform best. These studies yielded important results and new findings. First, we found that the mental stress induced QT response is not generic, substantial individual differences exist. We have shown that these differences are linked to the individual's cardiovascular reactivity. Second, we have first demonstrated under laboratory circumstances that mental stress prolongs the corrected QT in stress-responders. This effect is

most pronounced at stress initiation. Third, we have also first report that in otherwise healthy subjects mental stress and isometric exercises may induce T wave notching, a sign of nonhomogenous repolarization, that may link emotional stress with arrhythmia.

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PÉTER KOMLÓSI (2008)

Macula densa-dependent and non-macula densa-dependent signaling in the juxtaglomerular apparatus

Supervisor: László Rosivall

The juxtaglomerular apparatus plays an important role in the regulation of renal hemodynamics and in the control of renin release. Elevations in distal tubular salt delivery are sensed by the tubular epithelium (including the macula densa) which leads to the constriction of the adjacent afferent arteriole. In the present studies we utilized the isolated double perfused afferent arteriole-glomerular preparation with attached cortical thick ascending limb. To study changes in cell volume and the intracellular calcium concentration, basolateral membrane potential, we used multiphoton fluorescence microscopy and fluorescent dyes calcein, fluo-4, $DiBAC_4(3)$, respectively. We concluded that concomitant elevations in luminal sodium chloride concentration and osmolality produce macula densa cell shrinkage. This change in cell volume is maintained, suggesting the cells' limited ability to regulate their cell volume. The intracellular calcium concentration in the macula densa cells is relatively low and unresponsive to physiological challenges in the tubular lumen. On the other hand, cells in the vicinity of the plaque, called perimacular cells produce spontaneous oscillations in intracellular calcium concentration and basolateral membrane potential and produce characteristic changes in the pattern of intracellular calcium signaling. The early distal tubule and the adjacent afferent arteriole establish a close anatomical region of contact and functional relationship, suggesting that the perimacular cells and the connection of the early distal tubule and the afferent arteriole contribute to the paracrine signaling machinery of the juxtaglomerular apparatus.

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GÁBOR KÖKÉNY (2008)

Molecular mechanisms of chronic experimental inflammatory renal diseases

Supervisor: László Rosivall

Progressive renal fibrosis due to ongoing inflammation is the final common pathway of chronic renal failure (CRF), regardless of the etiology. The reninangiotensin system is a key mediator of kidney fibrosis, and ACE inhibitors are widely used in the treatment of CRF patients. Blood pressure independent blockade of the sympathetic nervous system (SNS) also ameliorated kidney fibrosis in experimental and clinical studies. Although these therapeutic modalities have non-hemodynamic antiinflammatory effects, monotherapies failed to halt disease progression. We investigated whether combined inhibition of the two systems provides additive renoprotection in experimental progressive glomerulosclerosis. In our model of subtotal nephrectomy (SNX), SNS blockade was achieved by dorsal rhizotomy and ACE was inhibited using Quinapril for 3 months. Blood pressure was not significantly influenced, but combination therapy markedly reduced both glomerulosclerosis and albuminuria. In the combination group, hypertrophy and oxidative stress of podocytes as well as glomerular TGF-β production was reduced to sham levels. In conclusion, combination of ACE inhibitor plus SNS blockade provideded additional renoprotection to single interventions, demonstrating the independent contribution of these systems to progressive renal fibrosis. Glomerulonephritis in systemic lupus erythematosus (SLE) may also lead to CRF. As the majority of patients are young women, the possible effect of pregnancy on progression is of great importance. Clinical data are contradictory, and little is known about the pathophysiology of nephritis in pregnancy. We investigated the effect of pregnancy on systemic autoimmunity of SLE prone MRL/lpr mice. Multiparous mice underwent 3 consecutive pregnancies. Kidney function, organ pathology and cytokine expression was compared to virgin mice. Survival and kidney function was dramatically reduced and accompanied by hypertension in multiparous group, associated with glomerular IgG and C3 deposition and increased local IFN-γ and IL-10 expression. However, serum IFN-γ level was reduced. We conclude that local cytokine production may play an important role in the aggravation of nephritis due to pregnancy, independently of systemic cytokine response. This study supports the hypothesis, that the extent of renal involvement is influenced by local factors in systemic lupus.

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- *Kokeny G, Godo M, Nagy E, Kardos M, Kotsch K, Casalis P, Bodor Cs, Rosivall L, Volk HD, Zenclussen AC, Hamar P (2007). Skin disease is prevented but nephritis is accelerated by multiple pregnancies in autoimmune MRL/lpr mice. Lupus 16: 465–477.*
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ZOLTÁN LENGYEL (2006)

Diurnal blood pressure variation and albuminuria in normotensive patients with insulin-dependent diabetes mellitus

Supervisor: László Rosivall

Abnormalities of the systemic blood pressure are closely associated with the development of diabetic nephropathy. Our aim was to examine the relationship between diurnal blood pressure pattern and albuminuria in insulin-dependent normotensive diabetic patients before the development of overt nephropathy.

Urinary albumin excretion rates were determined by radioimmunoassay, a 24-h ambulatory blood pressure monitoring was performed. Means and diurnal index was calculated for systolic, diastolic and mean arterial pressure, for day-time, night-time and the whole day. The results of the normoalbuminuric (n=39) and microalbuminuric (n=29) groups are compared, and correlation of the blood pressure patterns is analysed.

Twenty-four hours and night-time mean blood pressures were significantly higher, diurnal indices characterizing the nigth-time blood pressure drop were smaller in the microalbuminuric group. In the normoalbuminuric group 1 (2.6%) patient, in the microalbuminuric group 7 (24.1%) were "non-dippers". With multiple regression analysis a significant positive correlation (r^2=0.40) was found between albumin excretion rates and 24-hour mean systolic blood pressure and a significant negative correlation between albumin excretion rates and the diurnal index of mean arterial blood pressure ($p<0.0001$).

We conclude that in normotensive insulin-dependent diabetic patient the night-time decrease of blood pressure is smaller if microalbuminuria is present. Higher nocturnal blood pressure load is associated with the increase of albuminuria, even before the onset of overt diabetic nephropathy or hypertension.

Diurnal blood pressure pattern may predict the increase of urinary albumin excretion in normotensive normoalbuminuric type 1 diabetes mellitus patients.

To characterise the relationship between diurnal blood pressure and the subsequent increase of urinary albumin excretion in normotensive normoalbuminuric type 1 diabetic patients, ambulatory blood pressure monitoring was performed in 53 patients, who were then followed for five years.

Albumin excretion rate changed from 12.4 (8.9–17.2) to 29.3 (15.2–47.0) mg/day. Macroalbuminuria developed in 2 (3.8%), microalbuminuria in 22 (41.5%) patients, 29 (54.7%) remained normoalbuminuric. Night time diastolic blood pressure was significantly higher (64.3±6.5 vs. 60.9±5.5 mmHg; $p<0.05$), diastolic diurnal index significantly lower (15.5±9.7 vs. 22.3±6.2 %; $p<0.01$) in patients who later progressed to micro- or macroalbuminuria. Diastolic diurnal index ($r=-0.40$; $p<0.01$) and nocturnal diastolic pressure ($r=0.35$; $p<0.01$) were correlated to the change in albumin excretion.

In a multivariate analysis model with the change of albumin excretion as dependent, and means and diurnal indices of systolic and diastolic blood pressure, baseline urinary albumin excretion, cholesterol, triglycerides, HbA_{1c} and retinopathy as independent parameters ($r=0.68$; $p=0.001$), diurnal index for diastolic blood pressure ($\beta=-0.30$; $r=0.013$), baseline HbA_{1c} ($\beta=0.32$; $p=0.010$) and retinopathy ($\beta=0.44$; $p=0.001$) were significant independent correlates. We conclude that the relative increase of nocturnal blood pressure is associated with the subsequent increase of albuminuria, which in turn is predictive of overt diabetic nephropathy.

Plasma lipids may have a role in the development of the early stages of diabetic nephropathy in type 1 diabetes mellitus.

Accepted promoters of diabetic nephropathy only partially explain why the disease only develops in some type 1 diabetic patients. Our aim was to study the connection between the change of albumin excretion and mean plasma lipid levels observed over a long period in type 1 diabetic patients initially without nephropathy.

In 68 initially normoalbuminuric type 1 diabetic patients blood pressure, cholesterol, triglycerides, haemoglobin A_{1c}, were followed for ten years. Three separate urinary albumin excretion measurements were performed at the beginning and at the end of the follow-up. Correlation was analysed using the change of albumin excretion as dependent parameter.

During the follow-up mean urinary albumin excretion changed from 13.7±0.9 to 152.5± 42.2 mg/day, mean serum creatinine from 89.9±1.6 to 102.7±5.1μmol/l. Ten year mean serum cholesterol was 5.16±0.12 mmol/l, triglyceride 1.49±0.10 mmol/l, haemoglobin A_{1c} 8.84±0.15%. Upon univariate regression haemoglobin A_{1c} ($r=0.31$; $p<0.01$), total cholesterol ($r=0.37$; $p<0.01$), triglycerides ($r=0.25$; $p<0.05$) and diastolic blood pressure ($r=0.24$; $p<0.05$) were correlated significantly with the change of the albumin excretion, while mean systolic blood pressure, initial albumin excretion, age, sex, diabetes duration, age at onset of diabetes and ACE inhibitor administration were not. In a multivariate regression model ($r=0.47$; $p<0.01$) with the change of albumin excretion as dependent and cholesterol, triglycerides, haemoglobin A_{1c} and diastolic blood pressure as independent variables, haemoglobin A_{1c} ($r=0.24$; $p<0.05$), cholesterol ($r=0.28$; $p<0.05$) showed significant correlation.

We conclude that lipids appear to have a role in the development of the early stages of diabetic nephropathy in type 1 diabetic patients.

Urinary albumin excretion is correlated to fibrinogen levels and protein S activity in type 1 diabetes mellitus patients without overt diabetic nephropathy.

The aim of the study was to test the hypothesis that in diabetic patients without overt nephropathy a correlation may exist between the activity of natural anticoagulant proteins and glomerular dysfunction. Functional protein S and C activity assays, measurements of urinary albumin excretion, lipid parameters and haemoglobin A_{1c} were performed in 91 type 1 and 85 type 2 diabetes mellitus patients.

Type 1 diabetes mellitus patients with microalbuminuria had significantly higher mean age (44.1±10.9 vs. 37.9±12.7 years; $p<0.05$), fibrinogen level (3.75±1.0 vs. 3.21±0.8 g/l; $p<0.01$), protein S activity (92.3±17.6 vs. 84.5±15.5 %; $p<0.05$), higher prevalence of retinopathy ($p<0.01$) and macrovascular disease ($p<0.01$) compared to those with normoalbuminuria. Urinary albumin excretion was significantly correlated to age ($r=0.25$, $p<0.05$), fibrinogen level ($r=0.39$, $p<0.01$), protein S activity ($r=0.27$; $p<0.05$), total cholesterol ($r=0.23$; $p<0.05$), apoprotein B ($r=0.22$; $p<0.05$), retinopathy ($r=0.33$; $p<0.01$) and macrovascular disease ($r=0.33$; $p<0.01$). Type 2 diabetes mellitus patients with microalbuminuria had significantly higher apoprotein B levels (1.17±0.3 vs. 1.06±1.2 mg/dl; $p<0.05$) compared to those to those with normoalbuminuria, and apoprotein B was significantly correlated to urinary albumin excretion ($r=0.22$; $p<0.05$).

In a multivariate model of type 1 diabetes mellitus patients with fibrinogen, protein S and C activity, cholesterol, triglycerides, haemoglobin A_{1c}, retinopathy, and macrovascular disease as independent parameters ($r=0.53$; $p<0.003$) significant independent correlation of fibrinogen ($\beta=0.28$; $p<0.01$), protein S activity ($\beta=0.27$; $p<0.05$) and retinopathy ($\beta=0.21$; $p<0.01$) was found with urinary albumin excretion.

We conclude that in type 1 diabetes a relative elevation of fibrinogen level and protein S activity appear in the early stages of the development of diabetic nephropathy, and may be related to the pathogenesis of diabetic kidney disease.

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ANDRÁS MASSZI (2006)

Intracellular regulation of epithelial-mesenchymal transformation induced by transforming growth factor-β: role of RhoA G-protein and β-catenin

Supervisors: László Rosivall, István Mucsi

Myofibroblasts, smooth muscle actin (SMA) positive scar tissue cells are known to play a pivotal role in the progress of chronic renal failure. However the origin of these cells is remained to be elucidated. Recent results prove that tubular epithelial cells in response to transforming growth factor (TGF)-β undergo epithelial-mesenchymal transformation (EMT) and express SMA. The importance of this phenomenon was supported by both experimental and human clinical data, yet subcellular steps are to be clarified.

As an *in vitro* model of EMT proximal tubular epithelial cell (LLC-PK1) were treated with TGF-β1. After 24 hours features of EMT were detected such as 1) appearance of mesenchymal morphology, 2) cytoskeletal reorganization and stress fibre assembly, 3) down-regulation of E-cadherin, 4) up-regulation of fibronectin. Further, transformed cells expressed SMA. We showed that TGF-β1 activated RhoA GTP-ase in a biphasic manner. Also we showed that RhoA regulates 1) cytoskeleton reorganization via Rho-kinase (ROK) and 2) SMA expression via serum response factor (SRF).

Since contact disassembly is an early step in EMT we hypothesized that components of cell-contacts can play a role in the regulation of EMT. We found that TGF-β1 is unable to induce EMT in a confluent monolayer. However in three different models (low confluence, wounding and calcium free medium) the absence of cell contacts restored EMT in response to TGF-β1. It is known that β-catenin, a component of adherent junction regulates gene expression via T cell factor /lymphocyte enhancer factor (TCF/LEF) family. We found that β-catenin was translocated to the cell nucleus and TCF/LEF dependent gene expression was activated during EMT. Further, chelation of β-catenin reduced SMA promoter activation and protein expression.

Based on our results we suggest a two hit model: Namely both injury of tubular epithelium and the accumulation of TGF-β are required to induce EMT in tubular cells. EMT is regulated by several intracellular pathways including Rho GTP-ase and the β-catenin/TCF/LEF pathway.

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ATTILA SEBE (2008)

Intracellular signaling pathways regulating alpha-smooth muscle actin expression in renal tubular cells during epithelial-mesenchymal transition

Supervisor: István Mucsi

Epithelial-to-mesenchymal transition (EMT) of tubular cells into α-smooth muscle actin (SMA) expressing myofibroblasts is a central mechanism in the pathogenesis of tubulointerstitial fibrosis. Tubular epithelial cells that undergo EMT express SMA in response to an injury or the absence of intercellular junctions and transforming growth factor-β (TGFβ). The complex regulation of EMT requires the interplay of several intracellular signaling pathways.

We demonstrated that TGFβ regulates SMA expression through several signaling molecules, such as the Smad family of signaling proteins and p38 mitogen activated protein kinase family (p38 MAPK).

In our experiments cell contact disruption activated Rho and induced Rho kinase (ROK)-mediated myosin light chain (MLC) phosphorylation. Rho, ROK and MLC were found to regulate SMA expression. Contact disassembly enhanced nuclear accumulation of the serum response factor (SRF) as well. Contact injury-dependent Rho activation also resulted in the nuclear translocation of myocardin-related transcription factor (MRTF), a cofactor of SRF. Our results suggest that MRTF and SRF act together to induce SMA promoter activation in a cell contact- and TGFβ-dependent manner in renal tubular cells.

We showed that two additional Rho-family GTPases, Rac and Cdc42 also participate in the contact- and contractility-dependent regulation of the SMA promoter. Constitutive active Rac1, Cdc42 and their downstream effector p21-activated kinase (PAK) activated the SMA promoter in an SRF- and MRTF-dependent manner. Moreover, p38MAPK was also found to mediate cell contact disassembly-induced SMA promoter activation through MRTF.

Based on our results MRTF emerged as a key regulator of SMA expression in renal tubular cells. Its nuclear-cytoplasmic shuttling is regulated by both cell contact disassembly and TGFβ, through several downstream effectors including Rho, Rac1, Cdc42, PAK, MLC, p38. We showed here that the Rho-ROK-MLC-MRTF-SRF and Rac1/Cdc42-PAK-p38-MRTF-SRF pathways are important regulators of SMA expression and EMT in renal tubular cells.

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NIKOLETTE SZÜCS (2006)

Corticosteroid biosynthesis and metabolism in adrenal tumors

Supervisor: Károly Rácz

In this work characteristics of corticosteroid biosynthesis and metabolism in hormone producing and nonhyperfunctioning human adrenocortical adenomas was studied. It was found that leptin inhibited *in vitro* the basal and ACTH-stimulated corticosteroid secretion in cells from adrenocortical adenomas causing primary aldosteronism, Cushing's syndrome and nonhyperfunctioning adrenocortical adenomas. Based on these findings leptin may have a pathophysiological role in the regulation of corticosteroid secretions of these tumors. In other studies the secretion of 6β-hydroxycortisol was found to be under the regulatory effect of ACTH, and following ACTH administration the plasma concentration of this steroid metabolite showed an exaggerated response in patients with alcoholic liver disease after an at least 2 weeks of ethanol abstinence. Measurements of cortisol and 6β-hydroxycortisol in blood and salivary samples (morning, after the administration of 1 mg dexamethasone, and after ACTH administration) in control subjects, patients with Cushing's syndrome causing adrenal adenomas, and in patients with nonhyperfunctioning adrenocortical adenomas indicated that 6β-hydroxycortisol in salivary and blood samples was a more sensitive marker of cortisol secretion than blood or salivary cortisol concentrations. The findings obtained from *in vitro* hormone secretion of cells isolated from normal human adrenals and from human adrenocortical adenomas, as well as the results of hormone measurements in blood from selective adrenal vein samples revealed that 6β-hydroxycortisol is not only a metabolite produced by the liver but also a product of normal and adenomatous adrenocortical cells. When analyzing the clinical and hormonal findings of 187 patients with primary aldosteronism, the postural test combined with furosemide administration, which is most commonly used for the diagnosis of primary aldosteronism and for distinguishing aldosterone-producing adenomas from idiopathic hyperaldosteroism, had a specificity of 92% and a a sensitivity of 69%. For the first time in Hungary, a DNA-based screening method was introduced for the detection of the chemeric aldosterone-synthase/11β-hydroxylase gene present in patients with familial hyperaldosteronism type I. A retrospective analysis of the results of adrenal venous sampling showed that false negative results can be avoided by calculating the aldosterone/cortisol ratios in suprarenal veins followed by a correction with the same ratios in vena cava inferior. This new method proved to be useful for the localization of unilateral adenomas when adrenal vein sampling was successful only in the nonadenomatous side.

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TAMÁS TEREBESSY (2006)

The intracellular signaling of the "paradoxical" stimulation of renin gene transcription caused by angiotensin II

Supervisors: László Rosivall, István Mucsi

Despite its more than 100 year history the renin angiotensin system (RAS) still stands in the focus of investigations. The biological importance and the regulation of the lately discovered tissue RAS and the growth factor like effects of Angiotensin II (AngII) are not yet clarified. It has recently been observed in animal models that contrary to its typical effect AngII may increase renin production in certain conditions. In our experiments the regulatory effects of AngII in gene transcription and the AngII-induced paradox renin stimulation has been studied using transient transfection and western blot methods in *in vitro* models. The effect of AngII on the transcriptional activity of c-fos and renin promoters and the intracellular regulatory mechanisms of this effect have been examined. Our results indicate that AngII stimulates c-*fos* promoter through the activation of the Extracellular Signal Regulated Kinase (ERK) cascade and that the Rac-1 smal G-protein is involved in the process. The paradox stimulation of the renin promoter due to AngII is performed through c-jun-N-terminal kinase (JNK) enzyme and tyrosin kinases. In our experimental model the JNK and the tyrosin kinases seem to be elements of the same signaling pathway, where tyrosin kinases contribute to the activation of the JNK enzyme. Our result suggests a possible mechanism for the maintenance of progressive tissue fibrosis with increased local RAS activity.

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ZHI-REN ZHANG (2008)

Probing the structure and function of cystic fibrosis transmembrane conductance regulator using chemical modification

Supervisor: László Rosivall

The cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel and the gene defective in cystic fibrosis encodes CFTR. We performed patch-clamp analyses in oocytes expressing CFTR mutants containing cysteines engineered at putative pore-lining positions (near the predicted outer vestibule of the CFTR pore) in transmembrane domain six to determine: 1) the minimal functional unit of CFTR and 2) to detect the conformational changes in the outer vestibule of the CFTR pore associated with ATP-dependent gating events at the nucleotide binding domains (NBDs). Wild-type CFTR exhibits distinct subconductance states, which could represent currents of separate pores or different conductance states of a single pore. In comparison, cysteine engineered mutant CFTR channels altered the duration and probability of occurrence of these sub-conductance states without altering their relative amplitudes. Covalent modification process of single R334C-CFTR channels by SH-modifying reagent, $MTSET^+$, monitored in real-time, resulted in simultaneous modification of all three conductance levels in a single step suggesting that functional CFTR channel confers a single pore. The modification process of R334C-CFTR upon rapid exposure to $MTSET^+$ in outside-out macropatches was described best with a single exponential function suggesting that functional CFTR channel is formed as a monomer by a single CFTR polypeptide. In contrast, modification process was much slower when channels activity was much higher, such as in the presence of additional non-hydrolyzable nucleotide, or when the R334C mutation was coupled to a second mutation, K1250A, which also enhances channel activity greatly; modification was faster in R334C/K464A-CFTR channels, which exhibit very low channel activity. In single R334C-CFTR channels studied in excised patches, modification by $MTSET^+$ occurred only during channel closed states. These data together suggest that (1) the functional CFTR channel confers a single-pore, and is formed as a monomer by a single CFTR polypeptide; (2) the chemical reactivity of the engineered cysteine in R334C-CFTR is state-dependent, providing evidence of changes in pore conformation in the outer vestibule coupled to ATP binding and hydrolysis at the NBDs.

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PROGRAM 1/5.

CLINICAL AND EXPERIMENTAL CARDIOLOGY AND ATHEROSCLEROSIS

Coordinators:
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Program overview

The aim of the Program is to carry out experimental and clinical studies on the pathological mechanisms responsible for the cardiac and vascular disorders leading the mortality statistics. Metabolic disturbances, as diabetes, is one of the most important risk factor in this process—therefore it is used as a model. The different approaches are given by the sub-programs.

Titles of research projects	***Supervisors***
Effect of nutrients on atherogenesis and on the formation of the "atherogenic" lipoproteins	Lajos Szollár
The role of "new" risk factors and metabolic syndrome in the pathogenesis of the atherosclerosis	Lajos Szollár
Lipidology (pathobiochemistry, pathophysiology and clinical investigation of lipid and lipoprotein disorders)	Lajos Szollár
Effect of endogenons cardiovascular mediators and drugs on electrophysiological properties of isolated heart preparation	Valéria Kecskeméti
Thrombotic aspects of coronary heart disease. Prothrombolic states and their treatment in the clinical practice. The role of endothelial receptors in the atherothrombosis	Róbert Kiss
Perilous thrombotic complications in ischaemic heart disease	Róbert Kiss
Regulation of circulation in metabolic disease	Ákos Koller
Cellular and molecular genomic alterations of the endothelial metabolis in ischaemic heart disease	István Préda
Endocardial and body surface mapping and their clinical application	István Préda
Electrocardiological diagnostics of ischaemic heart disease in obesity	Mihály Medvegy

Ph.D. students		***Supervisors***
Anna Buday	pt	Ákos Koller
Emese Ábrám	pt	András Végh
Béla Debreczeni	a	Ákos Koller
Petra Gulácsi-Bárdos	ft	István Préda
Katalin Horváth	pt	Lajos Szollár
Nóra Kiss	pt	István Préda

Ph. D. candidates		*Supervisors*
Bernát János Béres	ft	Róbert Gábor Kiss
Gábor Duray	i	István Préda
Balázs Imre Hauser	i	Lajos Szollár
Anita Rácz	ft	Ákos Koller
Gabriella Szabó	i	Róbert Gábor Kiss
Katarina Vargova	ft	István Préda

Ph. D. graduates	*Supervisors*
István Hizoh	István Préda
Gani Hetem Bajraktari	Ferenc Horkay
János Tóth	Ákos Koller

a, absolutorium; ft, full-time, pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006 and 2007

ISTVÁN HIZOH (2006)

Programmed cell death as a pathogenic factor for contrast nephropathy: radiocontrast-induced renal tubular cell apoptosis *in vitro*

Supervisor: István Préda

Background: Radiocontrast-induced nephropathy (RCIN) is a major complication of invasive cardiological procedures. DNA fragmentation (a hallmark feature of apoptosis) of renal tubular cells has been observed in RCIN and this was attributed to medullary hypoxia. We investigated the mechanism of this DNA fragmentation in an *in vitro* model. We showed that radiocontrast agents induce DNA breakdown of renal tubular cells even under normoxic conditions, which is related to the hypertonicity of contrast agents. Since a hyperosmolal extracellular environment induces oxidative stress via reactive oxygen species, we also tested the hypothesis that antioxidants decrease hypertonicity-induced apoptosis of renal epithelial cells.
Methods: Madin Darby Canine Kidney (MDCK) cell monolayers were incubated with isoiodine doses of the highly hyperosmolal, ionic radiocontrast agent diatrizoate or of the less hyperosmolal, nonionic substance iopamidol. Mannitol, urea and NaCl control media of corresponding hyperosmolality were used to evaluate the contribution of hypertonicity, hyperosmolality and ionic strength to radiocontrast toxicity. We also analyzed the effects of the antioxidants N-Acetylcysteine (NAC) and taurine on hypertonicity-induced apoptosis of MDCK cells. DNA fragmentation was assessed using flow cytometry, agarose gel electrophoresis and terminal deoxynucleotidyl transferase-mediated deoxyuridine nick end labeling (TUNEL); cell morphology was analyzed in Giemsa-stained cytospins.
Results: Diatrizoate induced concentration- and time-dependent DNA fragmentation of MDCK cells along with morphological signs of apoptosis. Iopamidol caused no detectable DNA breakdown. In contrast, hypertonic mannitol and sodium chloride, but not hyperos-

molal urea induced DNA fragmentation in MDCK cells, albeit less than diatrizoate. Taurine reduced DNA degradation in both diatrizoate- and NaCl-treated cells. In contrast, NAC failed to reduce the DNA breakdown in this model of hypertonicity-induced renal tubular cell apoptosis.

Conclusions: Diatrizoate induces DNA fragmentation of MDCK cells *in vitro* under normoxic conditions which is mainly due to its hypertonicity and ionic strength. The radiocontrast/hypertonicity-induced DNA fragmentation of MDCK cells is attenuated by taurine but not by NAC. Since both agents are antioxidants, the antioxidant property is not sufficient for the observed cytoprotective effect. Hence, the antiapoptotic effect of taurine has to be attributed to other, yet to be defined mechanisms. Our results suggest that pharmacological doses of taurine may be protective against RCIN.

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- *Hizoh I, Haller C (2002) Radiocontrast-induced renal tubular cell apoptosis: hypertonic versus oxidative stress. Invest Radiol 37: 428–434.*
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GANI HETEM BAJRAKTARI (2007)

Relationship between insulin resistance and left ventricular diastolic dysfunction

Supervisor: Ferenc Horkay

Aim: The aim of this study was to explore the relationship between insulin resistance (IR) and the left ventricular diastolic function in patients with type 2 diabetes and subjects with impaired glucose tolerance (IGT).

Methods: The study included 119 subjects who underwent oral glucose tolerance test (OGTT). Insulin resistance was assessed using Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI). Left ventricular diastolic function was assessed using trans-thoracic Doppler echocardiography.

Results: Based on the OGTT results, 29 subjects had normal glucose tolerance (NGT), 20 subjects had impaired glucose tolerance (IGT), and 70 patients had type 2 diabetes. There were significant differences among the patients in groups with NGT, IGT and diabetes regarding HOMA-IR (4.20±1.20 vs. 6.45±3.83 vs. 8.70±6.26; $p<0.001$) and QUICKI (0.54±0.11 vs. 0.49±0.08 vs. 0.47±0.08; $p<0.001$). In subjects with NGT, IGT and patients with diabetes the pulsed Doppler transmitral variables were: E wave (0.72±0.16 cm/s vs. 0.62±0.13 cm/s vs. 0.58±0.17 cm/s; $p<0.001$), A-wave (0.61±0.13 cm/s vs. 0.62±0.11 cm/s vs. 0.71±0.14 cm/s; $p=0.006$) and E/A ratio (1.22±0.33 vs. 1.02±0.24 vs. 0.85±0.26; $p<0.001$). The proportion of subjects with an E/A ratio <1 was 27.6% in the group with NGT, 55% in the group with IGT and 75.7% in the group with diabetes ($p<0.001$). The E/A ratio correlated with HOMA-IR ($r=-0.30$, $p=0.001$) and QUICKI ($r=0.37$, $p<0.001$). Multiple linear regression model showed that IR (assessed by QUICKI) was an independent correlate of diastolic dysfunction ($P=0.034$).

Conclusions: In subjects with impaired glucose tolerance and patients with type 2 diabetes, insulin resistance is associated with impaired diastolic function of the left ventricle.

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JÁNOS TÓTH (2007)

Connection between oxidative stress, nitric oxide and asymmetric dimethyl arginine in light of clinical and basic science

Supervisor: Ákos Koller

Regulation of tissue blood flow and peripheral resistance is an important function of microvessels, primarily those of skeletal muscles arterioles. One of the key local mechanisms regulates blood flow by sensing changes in wall shear stress during increases in blood flow. Inflammation plays an important pathophysiological role in the development and progression of atherosclerosis, hypertension, and other conditions associated with vascular damage. In addition, reactive oxygen species (ROS) act as signaling molecules modulating vascular tone and structural changes to the microcirculation as well as the development and progression of atherosclerosis.

It has been shown that L-arginine is the substrate of NO synthase and NOS can be stimulated by administration of L-arginine resulting in arteriolar dilation. Methylated L-arginine, N^{ω}-nitro-L-arginine (L-NNA), NG-monomethyl- L-arginine (L-NMMA) or N^{ω}-nitro-L-arginine-methyl-ester (L-NAME) has been shown to inhibit NO synthase with the consequent elimination of NO mediated dilations of vessels.

In several human diseases, such as hyperhomocysteinemia, diabetes mellitus and hypertension, there is an increase in the serum level of methylated L-arginines, such as asymmetric dymethylarginine (ADMA). Yet, the functional consequence of increased level of ADMA on the vasomotor function of resistance vessels has not been delineated. We hypothesized that elevated level of exogenous ADMA inhibits NO-mediation of flow/shear stress dependent dilation of isolated arterioles.

In the presence of indomethacin, isolated arterioles from rat gracilis muscle were incubated with ADMA, which eliminated the dilations to increases in intraluminal flow. ADMA did not affect dilations to nifedipine or 8-bromo cGMP. In addition, ADMA elicited significant constriction of arterioles which was prevented by prior incubation of arterioles with polyethylene-glycol-superoxide dismutase /(PEG)-SOD/. Correspondingly, ADMA increased

(PEG)-SOD reversible production of arterial superoxide assessed by lucigenin-enhanced chemiluminescence and ethidium bromide fluorescence of gracilis arterioles.

It has been shown that impaired cardiac function due to myocardial infarction is frequently accompanied by peripheral vascular dysfunction and a pro-inflammatory condition, which may be associated with each other and with elevated levels of angiotensin II. Thus, we hypothesized that the magnitude of flow mediated dilatation (FMD) of the brachial artery of post myocardial infarction patients will correlate with the serum levels of tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP), indicators of a pro-inflammatory condition. We also hypothesized that treatment with angiotensin converting enzyme inhibitors (ACEI) will increase FMD by reducing TNF-α and CRP.

Patients with reduced left ventricular ejection fraction were treated with low dose (10 mg/day) quinapril (Q) or enalapril (E) and their effects on FMD and inflammatory markers were evaluated after 8 and 12 weeks of treatment. Hemodynamic parameters did not change significantly in either group. Before treatment, in both groups FMD showed a low value, whereas TNF-α and CRP were elevated. In the Q group, but not in the E group FMD significantly increased, whereas TNF-α and CRP significantly decreased after 8 and 12 weeks of Q treatment. Moreover, the magnitude of FMD showed a strong inverse correlation with serum levels of TNF-α and CRP after Q treatment.

The long-term ACE inhibition treatment might increase ADMA degradation by upregulating the activity of dimethylarginine dimethylaminohydrolase via suppression of angiotensin II mediated endothelial superoxide production. Further study is indicated to evaluate whether ACE inhibition could modulate endothelial ADMA metabolism directly. Thus, the present study provides an additional rationale for using ACE inhibitors with high tissue affinity to improve endothelial function and reduce vascular inflammation and oxidative stress, both of which may delay the further development of peripheral vascular disease and heart failure in post myocardial infarction patients.

- *Toth J, Racz A, Kaminski P, Wolin M, Bagi Z, Koller A (2007) Asymmetric dimethylarginine (ADMA) inhibits flow/shear stress dependent dilation of isolated arterioles and increases basal tone via superoxide release. Hypertension 49: 563–568.*
- *Kovacs I, Toth J, Tarjan J, Koller A (2006) Correlation of flow-mediated dilation with inflammatory markers in patients with impaired cardiac function. Beneficial effects of inhibition of ACE. Eur J Heart Fail 5: 451–459.*

SCHOOL OF PH. D. STUDIES

2. CLINICAL MEDICINE

Chairman:
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General overview

The Clinical Medicine Doctoral School has the largest number of programmes among the eight Doctoral Schools of Semmelweis University. The training and research programs offer research projects in a large number of subdisciplines of clinical and applied medicine.

PROGRAM 2/1.

OXIDATIVE STRESS AND IMMUNOLOGICAL REACTION IN LIVER DISEASES

Coordinator
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Program overview

Evidence accumulates that natural (vitamins, flavonoid type molecules) and synthetic butylated hydroxytoluene, dihydro-quinolin-type molecules) antioxidants exert a preventive effect on local oxidative damage in several models *in vitro* and *in vivo*. Therefore, the aim of our Program is to investigate the role of oxidative stress and the shift in pro/antioxidant balance in the pathogenesis of several gastrointestinal and immunological diseases, metabolic disorders and drug side effects by direct and indirect methods. The ongoing experiments focus on steatosis, hepatitis, cirrhosis, hepatocellular carcinoma, gallstone formation, cholestasis, inflammatory bowel diseases, colon neoplasm and metabolic disorders (carbohydrate, lipid) as well as amiodarone toxicity.

Titles of research projects	*Supervisors*
Study of redox homeostasis	Anna Blázovics
Neurochem ical examination of neural elements innervating the gastrointestinal visceral organs	Erzsébet Fehér
Pathogenesis and therapy of non-alcoholic liver disease	János Fehér
Food intake, lifestyle and the liver diseases	János Fehér
Alcoholic liver disease	János Fehér
Up to date treatment in hepatobiliary diseases. The effect of ursodeoxycholic acid and interferon on viral hepatitis and the oxidative stress status	János Fehér
Up to date treatment in hepatobiliary diseases. The effect of ursodeoxycholic acid and interferon on viral hepatitis and the oxidative stress status	Gabriella Lengyel
Food intake, lifestyle and the liver diseases	Gabriella Lengyel
The link among carbohydrate and lipid metabolism and free radical reactions and their role in the development of arteriosclerosis	Anikó Somogyi
Effect of metal complexes on the liver pathobiochemistry	Klára Szentmihályi

Ph. D. students		*Supervisors*
Rózsa Fehér	pt	János Fehér
Zoltán Mihály	pt	Anna Blázovics
Géza Nagy	ft	Anikó Somogyi
Eszter Németh	ft	Gabriella Lengyel
Éva Bernadett Pongor	pt	Erzsébet Fehér
Erzsébet Réka Selyem	pt	János Fehér
Tímea Varga	ft	Anikó Somogyi
Alexandra Wimmer	pt	János Fehér

Ph. D. candidates		*Supervisors*
Márta Kovács	pt	János Fehér
Péter Pusztai	pt	Anikó Somogyi
László Váli	ft	Anna Blázovics

Ph. D. graduates		*Supervisors*
Dieter Clemens Bröring	it	János Fehér
Ádám Lelbach	ft	János Fehér
Balázs Nemes	i	János Fehér
Erika Rapavi	ft	Anna Blázovics
Edit Székely (Gáspárné)	pt	Anna Blázovics

pt, part-time; ft, full-time, i, individual; it, international

Abstracts of Ph. D. theses successfully defended in 2006 and 2007

DIETER CLEMENS BRÖRING (2006)

Living donor liver transplantation

Supervisor: János Fehér

During the last 14 years living donor liver transplantation (LDLT) has evolved to an indispensable surgical strategy to minimize mortality of adult and pediatric patients awaiting transplantation. The crucial prerequisite to performing this procedure is a minimal morbidity and mortality risk to the healthy living donor. Little is known about the learning curve involved with this type of surgery.

From January 1991 to August 2003 a total of 165 LDLTs were performed in our center. Of these, 135 were donations of the left-lateral lobe (LL, segments II and III), 3 were of the left lobe (L, segments II–IV), 3 were full-left lobes (FL, segments I to IV) and 24 were of the full-right lobe (FR, liver segments V–VIII). We divided the procedures into three periods: Period 1 comprised the years 1991 to 1995 (LL: n=49, L: n=2, FR: n=1), period 2 covers 1996–2000 (LL: n=47) and period 3 2001 to August 2003 (LL: n=39, FR: n=23, FL: n=3, L: n=1). Perioperative mortality and morbidity were assessed using a standardized classification. Length of stay on ICU, postoperative hospital stay, laboratory results (bilirubin, INR and LFTs) and morbidity, as well as the different types of grafts in the three different periods, were compared.

One early donor death was observed in period 1 (03/07/93, case 30) (total mortality: 0.61%). Since 1991 the perioperative morbidity has continually declined (53.8% vs. 23.4% vs. 9.2%). In period 1, 28 patients had 40 complications. In period 2, 11 patients had 12 complications and in period 3, 6 patients had 9 complications. Within the first period one donor underwent relaparotomy because of bile leakage. Postoperative hospital stay was 10 days, 7 days, and 6 days, respectively. Donation of the full right lobe, in comparison to that of the left lateral lobe, resulted in a significantly diminished liver function (bilirubin and INR) during the first five days after donation but did not increase morbidity. One donor from period 1 experienced late death due to amyotrophic lateral sclerosis.

In a single center, morbidity after living liver donation strongly correlates to center experience. Despite the additional risks associated with temporary reduction of liver function, this experience enabled the team to bypass part of the learning curve when starting right lobe donation. Specific training of the surgical team and coaching by an experienced center should be implemented for centers offering this procedure, in order to avoid the learning curve.

- *Broering DC, Wilms C, Lenk C, Schule J am Esch II, Schönherr S, Mueller L, Kim JS, Helmke K, Burdelski M, Rogiers X (2005) Technical refinements and results in full-right full-left splitting of the deceased donor liver. Ann Surg 242: 802–813.*
- *Broering DC, Kim JS, Mueller T, Fischer L, Ganschow R, Bicak T, Mueller L, Hillert C, Wilms C, Hinrichs B, Helmke K, Pothmann W, Burdelski M, Rogiers X (2004) One hundred thirty-two consecutive pediatric liver transplants without hospital mortality. Lessons learned and outlook for the future. Ann Surg 240: 1002–1012.*

• *Broering DC, Wilms C, Bok P, Fischer L, Mueller L, Hillert C, Lenk C, Sterneck M, Schulz KH, Krupski G, Nierhaus A, Ameis D, Burdelski M, Rogiers X (2004) Evolution of donor morbidity in living related liver transplantation. Ann Surg 240: 1013–1026.*

ÁDÁM LELBACH (2006)

Effects of acute phase reaction on the regulation of the IGF sytem in rat liver

Supervisor: János Fehér

Catabolism is associated with decreased serum concentrations of IGF-I and IGF binding protein-3 (IGFBP-3) associated with elevated IGFBP-3 protease activity and increased concentrations of IGFBP-1 and -4. Various lines of evidence support the role of acute phase cytokines such as interleukin (IL)-6, IL-1 and tumour necrosis factor α (TNFα) as putative mediators for many of the metabolic manifestations of catabolic conditions.
Our aims were: (1) Investigation of the effects of the acute phase mediators IL-6, IL-1β and TNFα on biosynthesis of IGF-I and IGFBPs in primary cultured rat liver cells, *in vitro*; (2) Determination the effect of intramuscular injection of turpentine oil on IGF-I and IGFBPs gene expression in an *in vivo* rat model.
In hepatocytes, Kupffer cells (KC) and co-cultures of hepatocytes with KC IL-6 reduced IGF-I biosynthesis dose-dependently. IL-6 stimulated mRNA expression and protein secretion of IGFBP-1 and -4 in hepatocytes and that of IGFBP-3 in KC, respectively. In co-cultures, biosynthesis of IGFBP-1, -3 and -4 were increased dose-dependently by IL-6, while the effects of IL-1β or TNFα were less prominent. At neutral pH, proteolytic activity against IGFBP-3 was not detected in media of co-cultures treated with IL-6.
IGF-I gene expression decreased in liver, spleen and a small decrease was also observed in muscle and heart but not in kidney, lung and intestine. The amount of IGFBP-1 transcripts increased in liver and kidney but not in lung, intestine, spleen, heart and muscle. IGFBP-3 mRNA increased in all organs studied except for the kidney where a decrease was observed. IGFBP-4 mRNA expression increased in liver, kidney, lung and intestine but not in spleen, heart and muscle. In liver IGFBP-1, -3 and -4 protein secretion was increased. In sera of treated animals, IGFBP-1, -3 and -4 were decreased with a short increase before reaching the control level again. At neutral pH, proteolytic activity against IGFBP-3 was not detected after treatment.
The alterations of IGF-I, IGFBP-1 and -4 observed in catabolism correlate with the effects of IL-6 on biosynthesis of these components in primary rat liver cells, while a neutral IGFBP-3 protease was not detectable.
The changes of IGF-I and IGFBPs biosynthesis observed in our *in vivo* model correlate with our *in vitro* studies, but organs other than the liver also play an important role in the regulation of the bioavailability of IGFBP-3, and -4 during acute phase response.

• *Lelbach A, Scharf J-G, Ramadori G (2001) Regulation of insulin-like growth factor-I and of insulin-like growth factor binding protein-1, -3 and -4 in co-cultures of rat hepatocytes and Kupffer cells by interleukin-6. J Hepatol 35: 558–567.*
• *Lelbach A, Műzes Gy, Fehér J (2004) Molecular mechanisms of cancer cachexia. Orv Hetil 46: 2313–2360.*

BALÁZS NEMES (2006)

Evaluation of certain factors, with an impact of the outcome of the Hungarian Liver Transplant Program, with special regards to the hepatitis C virus

Supervisor: János Fehér

The author retrospectively evaluates the outcome of the Hungarian Liver Transplant program, including the different implantation methods. Further a comparison is performed between the domestic and a respected European center. There is a separate analysis regarding the HCV positive recipients, as the biggest homogeneous group of indication. Analysis is made with respect to the fulminant recurrence of the HCV infection in the implanted graft. A presumed association is evaluated among the serum titer of the HCV RNA, the histological patterns, the clinical course and the expression of the endoplasmatic chaperones in HCV graft biopsies, that responsible for the endoplasmatic stress response. In the results it is declared that there is a four-fold increase in the yearly activity in the last 10 years. The 1, 3 and 5 years cumulative patient survival in time periods are 55%, 45% and 39% (1995–97), 72%, 64% and 61% (1998–2000), as well as 78%, 77% and 77% (2001–2004). Total mortality decreased from 53% to 31%, mortality within 60 days decreased from 24% to 5%. Cumulative survival for HCV positive patients was respectively 64%, 55% and 51%, versus all other chronic indications, that were 73%, 66% and 63%. Virus recurrence was 43% within 1 year, which increased from 11% to 51% till 2003. Factors with an impact on patient survival were computed with a Cox-regression multivariate analysis. They were the postoperative kidney failure, hepatic artery thrombosis, biliary necrosis, cholangitis pulmonal infection, abdominal infection, the amount of intraoperative colloid and transfusion. The result of the international comparison also revealed on the transfusion rate as significant difference and factor for survival. All chaperones (XBP1, ATF6, HSP70, GRP98, GP96, Calnexin and Calreticulin) were upregulated in the graft biopsies of HCV positive patients, irrespectively from the serum HCV-RNA titer. This upregulation statistically decreased after 1 year of interferon treatment, further the XBP1 became downregulated. In case of acute rejection the upregulation of all chaperones was significantly higher than in HCV recurrence. In summary, the HCV counts for more than a third of all transplant activity. There is an association between the serum HCV-RNA titer and the early recurrence. It is represented by an RNA cut off point above which the histology and clinical course shows bad prognosis, and also a worse survival. However this difference was not reflected in the upregulation of different chaperones and in the different induction of endoplasmatic reticulum stress response.

- *Nemes B, Fazakas J, Sárváry E, Gerlei Zs, Sótonyi P, Doros A, Gálffy Zs, Ther G, Járay J, Kóbori L (2005) Factors in association with sepsis after liver transplantation. The Hungarian experience. Transplant Proc 37: 2227–2228.*
- *Nemes B, Sárváry E, Kóbori L (2005) Serum hepatitis C virus-ribonucleotide acid monitoring after liver transplantation. The Hungarian experience. Dig Liver Dis 37: 68–69.*

ERIKA RAPAVI (2007)

Importance of herbal preparations in liver and bowel diseases

Supervisor: Anna Blázovics

The redox homeostasis of cells and tissues is vital for fundamental life processes, its disturbance may cause damage to organism. The goal of our investigations was to examine the effect of Hungarian and foreign herbal teas on blast transformation of human peripheral blood lymphocytes *in vitro*, and to study their impact on redoxi processes of the healthy mammal organism *in vivo*. Our further aim was to evaluate the effect of the therapeutic dosage of a diosmin-hesperidin-containing drug (Detralex®) on redox parameters of alimentary-induced fatty liver in rats. Some authors reported that dietary phenolics exhibit pro-oxidant and cytotoxic properties under certain conditions. Based on these observations we examined the effect of high dosages of this drug on the redox state of liver in healthy animals, as well as on redoxi processes of liver regeneration in rats with experimental hepatic injury induced by lipid-rich diet and thioacetamide. Biochemical, analytical, and histological methods were used.

The results show that the aqueous extracts of the herbal teas examined have antioxidant properties as a function of concentration and steeping time in non-enzymatic systems *in vitro*. The effect of the infusion of calyx of *Hibiscus sabdariffa* L. and Beiqishen herbal tea on the cell-mediated immune response suggests that these infusions have *in vitro* immunomodulating, cytostatic properties. Both infusions decreased mitogen-induced blastogenesis in normal subjects. These extracts did not affect, however, the level of spontaneous proliferation of human lymphocytes. Aqueous extracts of the herbal teas studied significantly influenced redox homeostasis of the liver and plasma in healthy rats. Treatment with a drug containing diosmin (450 mg) and hesperidin (50 mg), in therapeutic dosage, may improve the antioxidant defensive system and the redox state in alimentary-induced fatty liver disease. High dosages of the drug (425 mg/body weight/day and 315 mg/body weight/day, respectively) deteriorated or did not change the redox state of the liver during the recovery period.

In conclusion, it may be stated that high dosage of herbal drugs and preparations may have pro-oxidant effect in both healthy and sick organisms.

- *Rapavi E, Szentmihályi K, Lugasi A, Vági E, Bányai É, Balázs A, Szőke É, Blázovics A (2006) The influence of the steeping time on the antioxidant properties of a Chinese herbal tea. Acta Alim Hung 35: 213–222.*
- *Rapavi E, González-Cabello R, Szentmihályi K, Székely E, Blázovics A (2006) The effect of calyx infusion of Hibiscus sabdariffa L. on T-cells-mediated immune response in mitogen-induced blastogenesis of human lymphocytes in vitro. Acta Alim Hung 35: 281–288.*

EDIT SZÉKELY (GÁSPÁRNÉ) (2006)

Investigation of redox homeostasis and the effect of additional antioxidant treatment in porphyria cutanea tarda

Supervisor: Anna Blázovics

The role of oxidative stress was proven in the pathogenesis of several diseases. This is why a great importance is attributed lately to the antioxidant therapy, and lots of studies are dealing with this issue.
In PCT, the oxidative stress is caused by the excess iron, following liver damage. The redox homeostasis of the patients is modified, and the level of antioxidants is decreased. So, it is expectable that the antioxidant vitamin E and alpha-lipoic-acid treatment will have a beneficial effect during the treatment of PCT.
The purpose of my study was the analysis of the redox status of the liver, and a follow up study on the effects of additional antioxidant treatment in phlebotomized PCT patients.
We made biochemical, analytical and metal ion determinations for the study of the free radical-antioxidant balance. We studied the vitamin E and alpha-lipoic-acid effect on the indicators of the oxidative stress, urine porphyrin level, haemorrheological parameters and element ions homeostasis.
Unlike the results of other studies, the alpha-lipoic-acid did not proved to be effective in improving the patients redox-homeostasis.
According to the clinical data, phlebotomy proved to be an effective treatment in PCT, but it did not improved the leveles of element ions in the whole blood of the patients. Vitamin E treatment has an additional beneficial effect by directly scavenging free radicals.
The results of the haemorrheological studies are in correlation with the changes of the redox parameters.

- *Székely E, Szentmihályi K, Tasnádi G, Kurucz T, Pallai Zs, Somogyi A, Blázovics A (2006) Element status of total blood and redox homeostasis of phlebotomized sporadic porphyria cutanea tarda patients with diabetes mellitus and in heavy drinkers. Trace Elem Electrolyt 1: 43–49.*
- *Székely E, Bor M, Tasnádi Gy, Várnai K, Almási A, Blázovics A (2006) Hemorheological status and redox homeostasis of phlebotomised porphyria cutanea tarda patients with diabetes mellitus and in moderate alcohol consumer. Clin Hemorheol Microcirc 35(3): 387–396.*
- *Székely E, Vereckei A, Almási A, Rapavi E, Tasnádi Gy, Várnai K, Pallai Zs, Lugasi A, Blázovics A (2007) Effects of vitamin E administration on hemorheological status and redox homeostasis in patients with porphyria cutanea tarda treated with phlebotomy. Clin Hemorheol Microcirc 36: 13–23.*

PROGRAM 2/2.

FETAL AND NEONATAL MEDICINE

Coordinator:
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Program overview

The Ph.D. Program is designed for medical doctors who wish to specialize in prenatal genetics and fetal medicine. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of genetic and fetal medicine, to provide suitable environment for clinical or laboratory based research project, to enable students for the use of laboratory techniques such as PCR, F-PCR, RFLP, blotting techniques, etc. to train students in modern prenatal diagnostic methods, like color-Doppler ultrasound, intrauterine echocardiography, etc.

Titles of research projects	***Supervisors***
Fetal and neonatal developmental disorders of the heart	Júlia Hajdú
Clinical and embryological aspects of assisted reproduction	János Urbancsek

Ph.D. students		***Supervisors***
Ágnes Flóra Ballóp	pt	Zoltán Papp
Zsófia Róna	ft	Zoltán Papp
Tibor Várkonyi	ft	Bálint Nagy

Ph.D. graduates		***Supervisors***
Péter Fancsovits	i	János Urbancsek
Márta Gávai	i	Zoltán Papp
Ágnes Harmath	i	Zoltán Papp
Petronella Hupuczi	i	Zoltán Papp
József Gábor Joó	i	Zoltán Papp
Levente Lázár	ft	Zoltán Papp
Gyula Richárd Nagy	i	Zoltán Papp
Sándor Nagy	pt	Zoltán Papp
György Szendei	i	Zoltán Papp
Zsanett Judit Szigeti	i	Csaba Papp

pt, part-time; ft, full-time, i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

PÉTER FANCSOVITS (2006)

Assessment of oocyte, zygote and embryo morphology in *in vitro* fertilisation treatments

Supervisor: János Urbancsek

During *in vitro* fertilisation (IVF) treatments embryos are selected for transfer on the basis of their developmental stage and morphology. However, more and more data are published which demonstrate that morphological features of oocytes and zygotes can be related to the embryo viability. The aim of this study was to correlate morphological characteristics of oocytes and the timing of early embryonic development to the further embryo quality and the outcome of IVF treatments. During our retrospective study we analysed the data of IVF treatments performed between October 2001 and February 2005 in the Division of Assisted Reproduction, First Department of Obstetrics and Gynaecology, Semmelweis University School of Medicine, Budapest. Assessment of oocyte morphology included the characteristics of the first polar body, the size of the perivitelline space and occurrence of granules or vacuoles in the cytoplasm. During assessment of early embryonic development we recorded the timing of the pronuclear membrane breakdown and the first cleavage. On the basis of our results, we concluded that a fragmented first polar body, enlarged perivitelline space, minor granulation of cytoplasm and occurrence of insignificant vacuolisation have no detrimental effect on fertilisation and further embryo development. However, a large or immature polar body, refractile bodies, dense centrally located granularity, considerable vacuolisation or occurrence of a sacculus-like structure in the cytoplasm correlated to a lower chance for fertilisation and decreased embryo quality. Thus, we do not suggest transferring embryos developed from oocytes with these anomalies. We suggest performing the assessment of early pronuclear breakdown between 22–25 hours post-insemination. Those embryos which passed pronuclear breakdown by this period have faster development and better morphological quality. Transferring these early developing embryos resulted in a higher pregnancy and implantation rate than those which had intact pronuclei at the assessment of early embryonic development. On the basis of our result, we suggest that assessment of early pronuclear breakdown should be included in the scoring system to select the most viable embryos for transfer.

- *Fancsovits P, Urbancsek J, Papp Z (2002) In vitro fertilisatióval létrehozott praeembryók beágyazódási esélye a petesejt, a zigóta és a praeembryo morfológiai jellemzői alapján. Magyar Nőorvosok Lapja 65: 231–242.*
- *Fancsovits P, Toth L, Takacs FZ, Murber A, Papp Z, Urbancsek J (2005) Early pronuclear breakdown is a good indicator of embryo quality and viability. Fertility and Sterility 84: 881–887.*
- *Fancsovits P, Tóthné GZs, Murber Á, Takács FZ, Papp Z, Urbancsek J (2006) Correlation between first polar body morphology and further embryo development. Acta Biol Hung 57: 331–338.*

MÁRTA GÁVAI (2008)

Abdominal myomectomy. Reproductive outcome after surgery

Supervisor: Zoltán Papp

The most frequent benign tumour of the female genital tract are leiomyomas of the uterus. Many ways of therapy have become familiar and accepted. The reason I chose abdominal myomectomy for my dissertation was that only very few papers have been published on abdominal, conservative, surgical treatment of the uterus in Hungary in the last 15 years although there has been an increasing demand for preserving the uterus in our society.
My aim was to contribute to defining the place of abdominal myomectomy in the surgical treatment of fibroids by presenting our experience gained through systematic observations in the past one and a half decade.
Hospital charts were reviewed. Patients' age, indication for surgery, type, size and number of removed fibroids, entry into the uterine cavity during the procedure, perioperative complications, duration of hospital stay were recorded and analysed. I selected those patients whose age was above 48 years and compared to a matched selected patient group treated by hysterectomy. I studied whether abdominal myomectomy could be a choice for patients with symptomatic fibroids above the age of 48 years, who wish to get hormonal replacement therapy and desire to preserve their uterus. I analysed whether there is any difference in perioperative morbidity and the consequently required management between cases where the uterine cavity was opened or remained closed during abdominal myomectomy. I analyse the reproductive outcomes in these cases following abdominal myomectomy and try to determine the role of myomectomy in the treatment of infertility.
504 abdominal myomectomies were performed at the 1st Department of Obstetrics and Gynecology, Semmelweis University Faculty of Medicine, Budapest between January 1990 and December 2004 over the study period, the annual number of operations increased significantly. In cases of women of childbearing the aim of myomectomy was the immediate (26.7) or delayed restoration of the capacity of reproduction (73.3%). In the age group of 41–45 years the rate of women without a demand for reproduction in this age group was 72.6%. Above the age of 48, there were 9 (1.8%; 9/504) patients, and 6 (1.19%; 6/504) of them requested HRT after abdominal myomectomy. Submucosal fibroids occur at the highest rate in the age group over 40 (8/19, 42.1%). In the age group between 31 and 35, fibroids of $\geq$70 mm diameter are the most frequent (72/172; 41.48%). In cases of patients over 40, opening of uterus cavity can be expected more frequently. In cases of submucosal fibroids the uterine cavity was opened in a significantly higher percentage of the procedures (63.2% vs. 36.8%; $p<0.0001$). Analyzing the complications we found that in the opened uterine cavity group significantly more bleeding occurred during the surgical procedure and significantly more patients needed postoperative blood transfusion (23.9% vs. 6.7%; $p<0.0001$). There was no significant difference in febrile morbidity between the compared groups. There were no unintended surgical procedures in either group. The percentage of relaparotomies did not differ significantly between the compared groups. Our results also confirm that the pregnancy rate is not influenced by entering the uterine cavity.
In Hungary there is an increasing demand for uterus sparing surgery, so it is very important for gynaecologists to be well trained in this type of operations. Abdominal myomectomy is a very good treatment for uterinal smooth muscle fibroids in case of patients insisting

on uterus-sparing surgery, partly because they would like to have child or because they regard the uterus as a symbol of their feminity. Age should not prevent conservative surgery. Myomectomy can be an alternative treatment for hysterectomy in the peri- and postmenopausal period either for keeping female identity or for hormonal treatment. Opening the uterine cavity and suturing the endometrial layer has no impact on perioperative morbidity. Post-operative pregnancy, birth and spontaneous abortion rate is not significantly influenced by the fact whether the endometrium is opened or not during abdominal myomectomy. Growing of the myoma during of pregnancy can cause fetal ischemic disease and brain injury.

- *Gávai M, Berkes E, Takács ZF, Papp Z (2007) Can myomectomy be suggested for perimenopausal women before administering hormone replacement therapy? Maturitas 58: 107–10.*
- *Gávai M, Hargitai B, Váradi V, Belics Z, Csapó Zs, Hajdú J, Hauzman E, Berkes E, Papp Z (2007) Prenatally diagnosed fetal brain injuries with known antenatal etiologies. Fetal Diagn Ther 23: 18–22.*

ÁGNES ÉVA HARMATH (2007)

Consequences of prenatal diagnosis in perinatal management of congenital diaphragmatic hernia

Supervisor: Zoltán Papp

Congenital diaphragmatic hernia and thoracic malformations of non-cardiac origin may have severe consequences for fetal development and perinatal outcome. Early prenatal diagnosis and relevant supplementary tests allow for the confirmation of possible other developmental disorders and chromosome aberrations.

The main objective was to determine the distribution of organs herniated into the thorax, the distribution of associated malformations and to determine the expected survival rate based on data of a Hungarian busy centre.

From the database of the 1st Department of Obstetrics and Gynaecology, Semmelweis University Faculty of Medicine, cases with the diagnosis of congenital diaphragmatic hernia were collected in the period between 1 July 1990 and 30 June 2005. Data of Genetic Counselling Unit as well as perinatal and neonatal data, the data of surgery and autopsy were analyzed in the two halves of the observed period.

Data of 107 newborns/fetuses were retrospectively analyzed. From the 96 (44 and 52) cases diagnosed prenatally 7 (5 and 2) proved to be false positives postnatally. From the 100 cases of congenital diaphragmatic hernia analyzed in the dissertation, 89 were confirmed prae- and postnatally and 11 were diagnosed only postnatally. Associated malformations were found in 71% of the cases (71/100), most of which were cardiac malformations. In 29 patients (12 and 17 cases) the isolated form of diaphragmatic hernia was detected. 85% (39/46) of all cases and 67% (6/9) of isolated diaphragmatic hernia cases diagnosed before the 24th week ended with termination. There were 52 live births. After the perinatal period, 27% of the patients (14/52) were discharged. There were 11 surviving patients out of the 27 patients delivered by cesarean section while out only 3 patients survived of those 24 who were delivered vaginally. In case of liver herniation the survival rate was reduced to 25% of the overall survival rate, while in case of stomach herniation it became

slightly higher than 50%. In case of herniation of "rare herniated organs" no survival can be expected. The herniation rate of the small intestines was significantly higher than that of the colon. In the group of isolated malformations no right- or both-sided hernia was detected. There was no surviving case in the both-sided hernia group, while the survival rate was 17% (1/6) in cases of right-sided and 46% (10/22) in cases of left-sided malformations. Congenital diaphragmatic hernias can be diagnosed prenatally in 85–90% of cases. The rate of diagnosing the malformation before the 24th gestational week increased significantly during the past 15 years. Polyhydramnios was usually found together with associated anomalies. Considering the 44–54% association rate of cardiac developmental disorders in the prenatally diagnosed malformations, fetal echocardiography is required. The best prognosis can be expected in cases with a left-sided isolated hernia delivered by cesarean section after the 37th gestational week. Common use of HFOV (high-frequency oscillatory ventilation) respirator contributed to the increase in survival rates by preventing hyperventilation. Young maternal age had no influence on whether the anomaly was isolated or associated with other malformations.

- *Harmath Á, Hajdú J, Csaba Á, Hauzman E, Pete B, Görbe É, Beke A, Papp Z (2006) Associated malformations in congenital diaphragmatic hernia cases in the last 15 years in a tertiary referral institute. Am J Med Gen, A 140A: 2298–2304.*
- *Harmath Á, Hajdú J, Hauzman E, Pete B, Róna Zs, Papp Z (2007) Experiences in perinatal management of congenital diaphragmatic hernia in the last 15 years in a tertiary referral institute. Fetal Diagn Ther 22: 209–216.*

PETRONELLA HUPUCZI (2006)

Retrospective study of perioperative treatment in patients with HELLP syndrome

Supervisor: Zoltán Papp

HELLP syndrome (Haemolysis, Elevated Liver enzymes, Low platelet count) is a grave, life threatening form of preeclampsia, which was named by Weinstein in 1982, on the basis of characteristic changes in laboratory findings (haemolysis, elevated level of liver enzymes and thrombocytopenia). Its development is accompanied by a significant increase in maternal and foetal morbidity and mortality alike, therefore it is essential that obstetricians are familiar with the disease.

In the past ten years, 107 patients were treated for HELLP syndrome at the Intensive Care Unit (ICU) of the 1st Department of Obstetrics and Gynaecology, Semmelweis University. In my thesis I summed up my experience with the treatment of patients, with special regard to the typical symptoms of HELLP syndrome, the course of the disease, postpartum maternal complications and irreversible maternal morbidity developing in the years after childbirth.

The frequency of HELLP syndrome in live births was found to be 0.37% at our department. The incidence of HELLP syndrome has been on the rise over the past years. In our sample, among the liver enzymes, AST and LDH, the level of total bilirubin (indicating the degree of haemolysis), and repeated thrombocyte counts were suitable for following up the clinical course of HELLP syndrome. Among the patients who recovered from this disease, the AST, LDH and bilirubin levels returned to normal on the 4–5th days, 6–7th

days and 3rd day, respectively, while the thrombocyte count reached the critical level at 100 000/μl on the 3–4th days. The share of surgery under regional anaesthesia was 37% in our sample. As it could be observed, there were no anaesthesiological complications after spinal anaesthesia in the cases in which the immediate preoperative thrombocyte count was over 50 000/μl and if no signs of haemorrhage were found during the examination. In persistent or progressive cases in the postpartum period, the elimination (uterine curettage and lavage) of factors responsible for the persistence of the disease (toxic and vasoactive agents in the endometrium) resulted in the recovery of one third of the patients. Maternal thromboembolic complications developed in 11% of the patients, each of them was affected in Mississippi Group I, with the lowest thrombocyte count. Of the two changes contributing to the development of clotting disorders, only Factor V (Leiden mutation) could be associated with HELLP syndrome. After a pregnancy complicated with HELLP syndrome, there was no decrease in spontaneous conceptions. In the subsequent pregnancies, the risk of premature deliveries exceeded 40% in our patients. The combined incidence of the repetition of 'mild' and 'severe' preeclampsia was seen at 44%, while that of HELLP syndrome was found to be 14%. In the years after pregnancies complicated with HELLP syndrome, the frequency of hypertension and autoimmune diseases rose significantly. Cases of hypertension tripled, while allergic and autoimmune diseases rose by one and a half time, compared to figures before the delivery.

The immediate termination of a pregnancy in which HELLP syndrome emerges may save the patient's life. Intratracheal narcosis is not the only anaesthetic method in surgical deliveries in patients with HELLP syndrome. It is recommended to try and lift foci applying uterine curettage and lavage as the first step, if the mothers' condition persists or progresses after delivery. The development of HELLP syndrome may call attention to a congenital clotting disorder (Leiden mutation). In subsequent pregnancies after HELLP syndrome, both premature births and maternal morbidities are much more common than in the general population. The prenatal care of those pregnant women should be provided at centres with much experience in pathological pregnancies.

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GÁBOR JÓZSEF JOÓ (2006)

Retrospective study of the characteristics of craniospinal disorders diagnosed prenatally

Supervisor: Zoltán Papp

As far as their morphology and aetiology are concerned, craniospinal malformations present a heterogeneous group of congenital disorders.

In his study, the author has processed the data of 1689 craniospinal malformations that were diagnosed at the Department of Obstetrics and Gynecology, Medical University School of Debrecen and the Genetic Counselling Unit of the 1st Department of Obstetrics and Gynecology, Faculty of General Medicine of Semmelweis University, in 25 years (1976–2001).

Comparing the different disorders with high incidence the author has concluded that in cases of spina bifida + hydrocephalus or hydrocephalus alone, the maternal age over 30 was more common than in the other disorders.

In malformations with high incidence it could be observed that the number of female fetuses exceeded that of the male ones in each disorder.

In approximately a quarter (25%) of all of the cases there was a positive obstetrical-gynecological history while positive genetic history and positive general medical history were found in approximately 9% and 2.5%, respectively.

Anencephaly was typically diagnosed in the 17–20th gestational weeks, i.e. after ultrasonography, performed in the possession of the maternal AFP-findings, while the cases of hydrocephalus were recognized between the 16th and 32nd gestational weeks, the time of diagnoses showing an almost even distribution.

The author has found the association of corpus callosum cases with some disorders of the central nervous system in approximately 53%.

On investigating the occipitofrontal diameter (OFD), the incidence of values above 90 percentile was 51% compared to the given gestational age of pregnancies.

In 98% of the cases with neural tube closure defects—depending on the time of diagnosis—the pregnancies were terminated via induced abortion.

Induced abortions were performed in 24% of pregnancies with corpus callosum dysgenesis, while the pregnancies were carried further in 76%. The intelligence tests conducted at the Neonatology Follow-up Unit at our Department showed normal mental development in 48% of the children; in 20% mental retardation was found, while in the rest of the cases cerebral atrophy, periventricular leucomalacia and holoprosencephaly were found.

Examining five-year-periods in the cases of neural tube closure defects and combined spina bifida-hydrocephalus malformations, the author found a decrease in the risk of repetition, while in the case of hydrocephalus, the risk of repetition was more or less constant.

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LEVENTE LÁZÁR (2007)

Bidirectional transplacental DNA traffic between fetus and mother. New perspectives in non-invasive prenatal diagnosis

Supervisor: Zoltán Papp

The discovery of fetal cells and cell-free fetal DNA in the peripheral circulation of pregnant women opened new perspectives in non-invasive prenatal diagnosis. The importance of non-invasive prenatal diagnosis is justified by the fact that it enables to avoid the risk of fetal loss, which is present in the case of invasive sample taking methods. The possibilities of non-invasive diagnosis are further enhanced by the rapid development of molecular biological methods.

The aims of this study were to detect the transplacental, two-way DNA traffic between mother and fetus; to determine fetal gender and Rh(D) status using cell-free fetal DNA isolated from maternal plasma, with consideration to the gender of previous child(ren) and transfusion history; and, in the case of male fetuses, to determine the quantity of cell-free fetal DNA. Another aim of the study was to detect the presence of maternal origin DNA in newborns peripheral circulation 30–120 minutes after delivery. The usefulness of detection and measuremet of the quantity of cell free fetal DNA in case of ectopic pregnancy cases compared with intrauterine case was an other aim of the study. In the case of gender determination 89% of the results were informative. Non-invasive fetal gender determination from maternal plasma gave identical results with karyotype gender analysis in 97% of the cases. The quantity of total cell-free and cell-free fetal DNA rises with the gestation weeks. As for fetal Rh(D) satus determination, Rh(D) status determined from maternal plasma and Rh(D) status determined from amniotic fluid were the same in 80% of the cases. The maternal DNA was detectable in each of the 10 newborn cases examined as late as 30–120 minutes upon delivery and the quantity of DNA was found to grow with gestational age. Cell-free fetal DNA was detectable in ectopic pregnancy cases as well, but the quantity of DNA was ten times higher than in patients with normal, intrauterine gravidity. This quantity increased with gestational age, but there was no correlation with the HCG level measured at the same gestational age. The results of the studies allow us to draw the following conclusions:

Cell-free fetal DNA can be detected in maternal plasma between the 16th and 22nd weeks of gestation. The quantity of DNA is enough to determine fetal gender and Rh(D) status. The gender of previous child(ren) and previous transfusions do not affect the reliability of the method. Maternal DNA is present in fetal circulation and the quantity of DNA increases with gestational age. The persistence of maternal cells and DNA could be a possible explanation for the "grandmother effect" observed in Rh(D) negative nulligravid women. The measurement of cell-free fetal DNA level in ectopic pregnancy could also provide important information in the differential diagnosis of intrauterine and ectopic pregnancy cases.

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- *Lázár L, Bán Z, Szakács O, Nagy B, Beke A, Oroszné NJ, Rigó J Jr, Papp Z (2003) Fetal sex determination with real time PCR of fetal DNA in maternal plasma. Orv Hetil 49: 2405–2409.*

• *Lázár L, Nagy B, Bán Z, Nagy GR, Papp Z (2006) Presence of cell-free fetal DNA in plasma of women with ectopic pregnancies. Clin Chem 8: 1599–1601.*

GYULA RICHÁRD NAGY (2007)

First steps of future noninvasive diagnosis from fetal cells

Supervisor: Zoltán Papp

There is clear evidence that fetal cells enter the maternal circulation during pregnancy. Successful isolation and analysis of these cells provide noninvasive prenatal diagnosis. Nowadays the target-cell types of researchers are the fetal nucleated red blood cells, they are present in significant numbers in the blood of early fetuses, but are unlikely to circulate in the peripherial blood of a normal adult; they have a short lifespan which makes unlikely that a false diagnosis results from fetal cells which have persisted from a previous pregnancy; they can have a specific marker (embryonic hemoglobin) to prove fetal origin, and possible placental mosaicism does not have an effect on them. Because of the low number of fetal cells in the maternal peripherial blood, they have to be enriched prior analysis. Gradient centrifugation with Percoll, magnetic activated cell sorting with anti-CD71 magnetic microbead conjugated antibodies, micromanipulation and single cell PCR analysis of anti-hemoglobin-epsilon fluorescent antibody stained fetal cells seems to be a promising technique. The future of prenatal diagnosis is the use of microarrays combined with noninvasive techniques. Because of the low number of fetal cells in maternal peripheral blood samples, only after technological advances in the future will they provide suitable sample for whole-genome expression oligonucleotide microarray analysis. Owing to this, I used genetic amniocentesis, the safest invasive prenatal diagnostic method for sample collection, when examining the ability of global gene expression array analysis in everyday prenatal diagnosis. My study demonstrated that a routinely collected amount of amniotic fluid (as small as 6 ml) is enough to make whole-genome expression analysis with a microarray. In the forthcoming years we hope that noninvasive prenatal diagnosis through the analysis of fetal cells in maternal blood can be combined with this new technology.

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SÁNDOR NAGY (2006)

Clinical significance of subchorionic and retroplacental hematomas detected in the first trimester of pregnancy

Supervisor: Zoltán Papp

The author investigated the rate of early pregnancy failure and long-term clinical significance of intrauterine hematomas detected in the first trimester of pregnancy in a general obstetric population.

Objectives: To evaluate the clinical significance and characteristics of intrauterine hematomas detected in the first trimester of pregnancy followed by spontaneous abortion and adverse perinatal outcome. To analyse the significance of Doppler mapping of the intervillous circulation and spiral arteries in the detection of placental dysfunction.

Materials and methods: During the study period totally 230 patients with hematoma was diagnosed from 7862 screened patients. Prospective study was designed to compare the obstetrical outcome in 43 pregnant women with intrauterine hematoma, who spontaneously aborted to 187 controls, who delivered after the hematoma was diagnosed. The perinatal outcome in 187 pregnant women with intrauterine hematomas to 6488 controls in whom hematomas were not detected at first trimester by ultrasound examination was compared. Doppler assessment was performed in 30 patients with hematoma compared to 30 control patients.

Results: There was no significant difference with regard to maternal age, and medical history. The incidence of intrauterine hematoma in the first trimester in a general obstetric population was 3.1%. Retroplacental or subchorionic position of the hematoma was not significantly correlated with an increased risk for spontaneous abortion. However a retroplacental position of the hematoma was significantly correlated with an increased risk for adverse maternal and neonatal complications in the third trimester. The peak systolic velocity, pulsatility index and resistance index increased significantly in patients with hematoma.

Conclusions: The presence and the characteristic of an intrauterine hematoma during the first trimester may identify a population of patients at increased risk for adverse pregnancy outcome. Indeed, the ultimate goal of triaging patients into low-risk and high-risk populations is to more accurately target our available forms of surveillance and therapy.

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- *Nagy S, Bush MC, Berkowitz RL, Fishbein TM, Gomez-Lobo V (2003) Pregnancy outcome in liver transplant recipients. Obstet Gynecol 102: 121–128.*
- *Nagy S (2005) Terhesség alatti vérzések. In: Rigó J, Papp Z (szerk) A várandós nő gondozása. Medicina Kiadó, Budapest.*

GYÖRGY SZENDEI (2006)

The role of diagnostic and operative laparoscopy in the treatment of infertility

Supervisor: Zoltán Papp

The demographic data of the last few years indicate that the Hungarian population may decrease to nine million people in a few decades. The reason for this fact can be found on the one hand in the decline of the average life expectancy, the causes of which are the general aging and the worsening state of health of the population. On the other hand, birth rate has also been falling during the last decades due to unwillingness to have more than one or two children. Besides, we can notice an increase in the number of sterile couples, whose proportion may reach 15–20% these days. As a result of the statistically growing number of the infertility patients, the medical research on infertility has been in the limelight all over the civilized world. Laparoscopy, which has been widely used in the world since the 1970s, has opened new perspectives in the diagnostics of infertility. It has become a routine method in Hungary since the 1990s. The more and more frequent chronic pelvic alterations—with special regard to ones caused by endometriosis and different types of infectious diseases spreading by sexual intercourse—may play a role in infertility. Operative laparoscopy helps not only with defining the status of the pelvic organs but with the treatment of them, which may give the patient a chance of a spontaneous pregnancy. In the case of irreversibly damaged organs operative laparoscopy provides conditions for the use of modern assisted reproductive techniques. The author summarizes the results of his ten year work on the field of operative laparoscopy. He determines the frequency of the causes of chronic pelvic pain in Hungary. He compares the results of operative laparoscopy to that of other examination methods. He calls attention to the importance of operative laparoscopy as a new examination method in the treatment of sterility and chronic pelvic pain. The author first has drewn up a diagnostic protocol for patients suffering from infertility and/or chronic pelvic pain. In the protocol he included the indications for diagnostic and operative laparoscopy. In the case of infertility patients he compared the HSG findings with the later performed LSK findings. The more and more frequent chronic pelvic alterations—with special regard to ones caused by endometriosis and different types of infectious diseases spreading by sexual intercourse—may play an important role in infertility. Operative laparoscopy helps not only with defining the status of the pelvic organs, but with the treatment of them, so it may give patients a chance for a spontaneous conception. In the case of irreversibly damaged oviducts the operative laparoscopy increases the success rate of the modern assisted reproductive techniques. Laparoscopy or laparotomy was performed and the patients suffering from histological verified endometriosis received a 6 month GnRH analogous treatment. In 8–10 weeks after the medical treatment a second-look laparoscopy was suggested. The necessary surgical interventions (cystectomy, ovarian resection, endocoagulation, adhaesiolysis) were made during both operations. He is publishing the data of spontaneous or ART conceptions within 12 month by the infertility patients getting individualized ovulation-induction treatment. Due to CPP caused by endometriosis the patients received surgical—GnRH analogous—surgical treatment, after which similarly to infertility patients he used first in Hungary a non-conventionally administered, monophasic, oral contraceptive treatment containing third generation gestagen. He worked up the McGill pelvic pain scores given by the patients before the first surgical intervention, those of given before the second-look laparoscopy after the

medication, and those of given at the end of the 6th, 12th, 18th, and the 24th month after the control operative intervention, or after the and of stilling. The recurrence of CPP complaints decreased by half thanks to the non-conventionally administered, monophasic oral contraceptive therapy, which was indicated. The results of the 24-month check-up period show that with the help of this therapy the recidivism of PP and DM decreases by half, and that of DP decreases by third. The number of the cases needing radical surgical solution (hysterectomy + adn.lu.) is significantly lower in the group of patients successfully treated for infertility than in the group treated for only CPP.

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- *Szendei Gy, Máthé Zs, Hernádi Zs, Péter A (2004) Subileus caused by intestinal endometriosis. Experience from three cases. Int J Colorect Dis 19: 502–504.*
- *Fancsovits P, Tóth Lné, Murber Á, Szendei Gy, Papp Z, Urbancsek J (2005) Catheter type does not affect the outcome of intrauterine insemination treatment a prospective randomized study. Fertil Steril 83: 699–704.*

JUDIT ZSANETT SZIGETI (2007)

Perinatal autopsy as the quality control of prenatal diagnosis. Experiences with major fetal trisomies

Supervisor: Csaba Papp

I evaluate the correlation of prenatal ultrasound findings with the results of subsequent pathologic examinations in fetuses with major aneuploidies. Singleton fetuses, who underwent genetic testing following prenatal sonography during the second trimester in our institution, and had trisomy 21, trisomy 18 or trisomy 13 constituted the study-population. Findings of second trimester sonography and fetal autopsy were compared by organ system and their correlation was assigned to one of three categories. Altogether 611 separate major structural malformations were diagnosed during autopsy in fetuses with major trisomies. Full agreement was achieved between sonography and autopsy in 36% of the malformations, whereas additional findings at autopsy (64%) involved mainly two organ systems: face (including ears and eyes) and extremities (including hands and feet). Some ultrasound findings were not confirmed at autopsy (n=49). Concordance rates between sonography and autopsy findings regarding soft markers were considerably high in some markers (increased nuchal fold thickness, short femur/humerus). On the other hand, fetal autopsy had limited value regarding hyperechoic bowel and echogenic intracardiac foci. Perinatal autopsy is a valuable tool for measuring the quality of prenatal sonography. Pathologic examination provides additional information in many fetuses with aneuploidy and may indicate possible directions of sonographic screening for major chromosome aberrations. However, the two methods should be considered as complementary ways of increasing our knowledge about the possible symptoms of fetal aneuploidies.

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PROGRAM 2/3.

PREVENTION OF CHRONIC DISEASES IN CHILDHOOD

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Program overview

The present research and doctoral program consists various topics of paediatrics, prevention creates the common basis of the program. No reliable method is available to determine the beginning of a chronic disease. In Hungary the causes of the majority of the leading fatal diseases are to be found already in childhood, although without clinical signs. Their progression gradually leads to a permanent manifest disease with expressed clinical symptoms. A fundamental precondition of preventing the development of chronic diseases is to detect the possibly existing risk factors. Getting to know the cellular and subcellular mechanisms promoting the development of a disease may be of help not only in the prevention, but also in the successful therapy and in eliminating the complications, as well. The doctoral Program is dealing with research fields having outstanding significance in adult cardiovascular morbidity and mortality and where the identification and elimination of risk factors could prevent long-lasting impairments. In the pathogenesis of cardiovascular diseases sodium homeostasis and its cellular regulation are of utmost importance. Within the doctoral Program this question is dealt with in 3 sub-programs. The research work is aimed to study the altered activity, structural changes and genetic regulation of Na/K/ATP-ase enzyme in diseases accompanied by irregular sodium homeostasis. In insulin dependent diabetes mellitus the prevention of late complications: vascular alterations and hypertonia are of cardial question. Two sub-programs are devoted to study genetic, metabolic and haemodynamic risk factors in animal experiments and clinical physiological examinations. The sub-program dealing with the correlation between chronic renal diseases, cardiovascular alterations as well as uraemia and bone metabolism focuses on the regulatory role of the kidney as regards vascular alterations and bone structure deformities. The pre-term birth and treatments applied involve several late complications and hazardous situations. The harmful side effects of oxygenization can be due to the multiplication of oxidative radicals. This hypothesis has been considered as a possible pathogenetic factor in several other diseases, too. The study of this theory in pre-term

babies may provide explanations similarly valid in other systems, in a wider sense, as well. The investigation of postnatal body composition, the hydrodynamic changes accompanied by electrolyte movement may reveal several fundamental regularities. This sub-program offers a completely new approach by using multifrequency bioelectric impedance analysis. The additional four sub-programs seem to be heterogeneous, however, they have one common aspect, namely, all of them are aimed to detect risk factors thereby improving life's quality. The investigation of the connatal urinary tract malformations in neonates and pre-term babies, in addition to the discovery of basic facts, has directly practical significance: to determine the optimal time and technique of surgical intervention. The number of infantile atopic airway diseases shows an increase proportional with the environmental pollution pointing out the importance of getting to know the natural course and pathomechanism of the disease. The questions of paediatric gastroenterology deal with the immunologic correlation existing between food allergens and intestinal diseases. The deeper knowledge of the pathophysiology of childhood epilepsy syndromes and primary headache disorders may result in a better life quality in adulthood.

Titles of research projects	***Supervisors***
Pediatric gastroenterology	András Arató
Study of inflammatory mediators in pediatric migraine and epilepsy caused by limbic encephalitis	Viktor Farkas
The role of haemodinamic and genetic factors in the pathomechanism of acute and chronic allograft nephropathy	Andrea Fekete
Examination of genetic variability in type 1 diabetes mellitus. Detection of genetic linkage	Róbert Hermann
Effect of anesthesy and operative intervention to the immune system	István Kocsis
Role of seasonal anc circadian rhythmicity in the development of chronic complication and progression of diabetes mellitus	Anna Körner
Genetic, haemodynamic and metabolic risk factors and molecular pathogenesis of the development of diabetic nephropathy	László Madácsy
Diagnosis, prevention and treatment of infections following transplantation	György Reusz
Cardiovascular effects of renal failure and transplantation in childhood	György Reusz
Cardiovascular disorders and renal failure. Possibilites for the preventive options in renal hyoparathyreoidism and osteodystrophy in the early phase of renal failure	András Szabó
Studying of pathomechanism, genetic background an therapy of chronic allograft nephropathy	Attila Szabó
Use of hypothermy in the treatment of hypoxic-ischemic encephalopathy of neonates	Miklós Szabó
Pediatric liver diseases. Hereditary metabolic diseases	László Szőnyi
Examinations of factors influencing the morbidity and mortality of pediatric intensive carefocus on the carbohydrate metabolism	Péter Tóth-Heyn
Functional immunological studies in pediatric diseases	András Treszl
Functional immunological studies in pediatric diseases	Tivadar Tulassay
Significance of functional genomic examinations in the early and late complications of premature babies	Barna Vásárhelyi

Molecular biological examination of the ischemic injury of the kidney	Ádám Vannay
Role of adaptive immunity (T regulatory cells and lymphocyte markers) in different gastrointestinal disorders (inflammatory bowel disease, celiac disease, allergic colitis)	Gábor Veres

Ph. D. students		***Supervisors***
Márta Bangó	ft	Miklós Szabó
Nóra Fanni Bánki	ft	Andrea Fekete
Áron Cseh	ft	Barna Vásárhelyi
Orsolya Cseprekál	ft (a)	György Reusz
Krisztina Fischer	pt (a)	András Szabó
Ágnes Jermendy	ft	Róbert Hermann
Dorottya Kelen	pt	Miklós Szabó
Éva Kis	ft	György Reusz
Krisztina Lukács	ft	Róbert Hermann
Krisztina Pásti	ft	Attila Szabó
Ágnes Prókai	ft	Attila Szabó
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Erna Sziksz	ft	András Szabó

Ph. D. candidates		***Supervisors***
Ádám Balogh	ft	Barna Vásárhelyi
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Antal Dezsőfi	i	András Arató
Krisztina Rusai	ft	György Reusz
Alexandra Szabó	pt	Endre Cserháti
Marina Varga	i	György Reusz

Ph. D. graduates		***Supervisors***
Géza Miklós Bokodi	ft	Barna Vásárhelyi
László Derzbach	ft	Barna Vásárhelyi
Csaba Hermann	i	László Madácsy
Éva Károly	i	György Reusz
János Tibor Kis	i	András Treszl, Tivadar Tulassay
András Nobilis	i	Barna Vásárhelyi
Bea Pászthy	i	Tivadar Tulassay
Gábor Rudas	i	Tivadar Tulassay
Beáta Szebeni	ft	András Arató
László Szőnyi	i	András Arató

a, absolutorium; pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

GÉZA MIKLÓS BOKODI (2007)

Genetic polymorphism and the development of chronic lung damage of the newborn

Supervisor: Barna Vásárhelyi

Chronic lung damage of preterm infants is called bronchopulmonary dysplasia (BPD). The risk factors for BPD are prematurity, disturbed lung development, systemic and local inflammation as well as therapeutic interventions of the perinatal period such as mechanical ventilation and inadequate nutrition. Furthermore, recent research highlighted the potential implication of genetic polymorphisms, mainly single nucleotide polymorphisms (SNPs) in BPD and the majority of its risk factors.

In our study we investigated the association of SNPs with BPD and ventilation characteristics in low birth weight infants. We investigated TNFγ $G^{-308}A$, IL-1β $C^{3954}T$, IL-6 $G^{-174}C$ and IL-10 $G^{-1082}A$ SNPs and demonstrated that the carrier state of the TNFγ $G^{-308}A$ allele was associated with a 40-hour longer period of mechanical ventilation ($p=0.004$) and an additional 36 hours of oxygen supplementation on average ($p=0.0008$). The association was significant after its adjustment for perinatal risk factors for lung damage. Examining the IFNγ $T^{+874}A$ and IL-12 p40 promoter GC/CTCTAA polymorphisms we found that IFNγ^{+874}A allele is overrepresented in LBW infants. Carriers of IFNγ^{+874}T allele required mechanical ventilation and oxygen supplementation for a 41% and 35% shorter period of time, respectively, than those not carrying IFNγ^{+874}T allele. Stepwise logistic regression analysis revealed that carriers of IFNγ^{+874}T allele are protected against BPD (OR[95% CI]: 0.35 [0.12–0.99]) and patent ductus arteriosus (0.43 [0.19–0.97]), while carriers of IFNγ^{+874}A allele are at higher risk of severe hypotension (3.40 [1.01–11.52]) and respiratory distress syndrome (4.03 [1.30–12.50]). Some SNPs were associated with other perinatal complications with an impact on ventilation and BPD-risk. Carriers of IL-12 GC allele were protected against pneumonia (0.32 [0.14–0.75]). Carriers of IL-12 CTCTAA allele were at higher risk of developing necrotizing enterocolitis (2.37 [1.01–5.53]). Examining the ACE I/D and AT1R $A^{1166}C$ polymorphisms we did not detect any association between ACE and AT1R genotype and BPD or ventilation characteristics.

According to our results genetic factors may play a role in perinatal lung damage. Identification of genetic risk factors may establish the possibility of indentifying infants at high BPD risk and the targeted and individual prevenetion and treatment of the disease.

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LÁSZLÓ DERZBACH (2007)

Genetic polymorphism of selection proteins and estrogen-receptors in perinatal morbidities and in preeclampsia

Supervisor: Barna Vásárhelyi

During my Ph.D. work I investigated the association between the polymorphisms of the E-, P, L-selectin and estrogen receptor-α (ER-α) genes and perinatal morbidity and preeclampsia. We enrolled 125 low birth weight infants in our retrospective study and we determined the Ser128Arg polymorphism of the E-selectin, the Thr715Pro polymorphism of the P-selectin and the Pro213Ser polymorphism of the L-selectin genes from dried blood samples with PCR-RFLP method. We showed that carrier state of the mutant (213Ser) L-selectin allele increases the risk of prematurity and bronchopulmonary displasy. In women with preeclampsia we found that the presence of the 715Pro P-selectin allele was associated with an earlier onset of hypertension. In the background could stand, that this polymorphism decreases the soluble P-selectin level, which has anti-inflammatory properties.

We showed an association between the PvuII polymorphism of the ER-α gene and necrotising enterocolitis, ductus Botalli persistens, the lenght of oxygen supplementation and intraventricular hemorrhagia in low birth weight boys. In girls we found no associations. Theoretically, the PvuII polymorphism could be clinically significant only in male neonates, who have lower estrogen levels. This could be one of the explanation for the sex dependency of the perinatal complication.

We also investigated the role of the PvuII and XbaI polymorphisms of the ER-α gene in preeclampsia. The "PP"/"XX" genotype combination occured frequently in severe preeclamptic patients and the "xx" genotype was associated with lower risk for fetal growth restriction. In the background could stand that in case of the "P" allele, the binding site of the B-myb transcription factor is eliminated, therefore the expression of the ER-α decreases. The relative estrogen deficit increases the resistency of the uterine vessels, which is part of the patomechanism of preeclampsia.

The perinatal inflammatory process could be therapeutically influenced by immunomodulatory drugs. In my field of research I have collected preliminary data about the direct effect of phosphodiesterase enzyme inhibitor pentoxifylline and estradiol on the L-selectin, CD11b expression, phagocyte and burst function of neutrophile granulocytes and monocytes. These drugs had no effect on the investigated immune functions in our *in vitro* system.

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CSABA HERMANN (2008)

Investigation of associations between genetic polymorphisms and type 1 diabetes mellitus

Supervisor: László Madácsy

Type 1 diabetes (T1DM) is an autoimmune disease caused by multiple genes interacting with non-genetic factors. Coeliac disease (CD) is characterised by severe inflammation of the small intestine, which is triggered by gliadin. Prevalence of CD in type 1 diabetes mellitus children is higher than that in nondiabetic children. The environment of the ongoing diabetic autoimmunity may be a stimulant to the development of CD, a disease that possesses autoimmune features. The genes of the inflammatory proteins which contribute to the development of T1DM, coeliac disease or both may contain certain polymorphisms. These polymorphisms or combinations of polymorphisms might play an essential role in the pathogenenesis of T1DM, CD or both by influencing the quality or the quantity of the protein coded by the gene.

In our study we investigated the association between TNFα $G^{-308}A$, IL-1β $C^{3954}T$, IL-6 $C^{-174}G$, HSPA1B $A^{1267}G$ single nucleotide polymorphisms (SNP) and T1DM. We also investigated the association between TNFα $G^{-308}A$, TNFα $G^{-238}A$, CD14 $C^{-260}T$, TLR-4 $A^{896}G$ SNP-s, certain HLA-DQ haplotypes and coeliac disease in diabetic children.

We found an association between the joint presence of high TNFα ($^{-308}$AA and AG) and low HSP72 (^{1267}AG and GG) producer genotypes in one hand and the risk of T1DM on the other. Higher production of TNFα may contribute to the development/maintenance of destructive insulitis and lower level of HSP72 makes β-cells less resistant to the autoimmune process, and hereby might contribute to the development of T1DM. We found an association between IL-6 $^{-174}$G allele carrier state and older age-at-onset of T1DM, but only in the presence of high IL-1β (^{3954}T allele carrier state) and TNFα producer genotypes. The higher IL-6 production associated with the $^{-174}$G allele in Langerhans islets, might have a protective effect against the autoimmune process and might delay the destruction of the β-cells. We found a significantly higher rate of carriers of TNFα $^{-238}$A allele in the histology-proven CD group than in the non-CD group. We found that in children with T1DM the frequency of the high CD14 producer $^{-260}$TT genotype was decreased, but in children affected by both CD and T1DM the occurrence of the CD14 TT homozygous mutant genotype was not decreased. CD14 is an important factor of inflammation in coeliac disease, but may have some protective effect in the pathogenesis of T1DM.

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ÉVA KÁROLY (2008)

A study of pathogenetic factors relating to lesions of the renal parenchyma in children with urinary tract infection

Supervisor: György Reusz

The most common disease entities in pediatric nephrology practice are urinary tract infections (UTIs). It is well known that the risk of renal parenchymal lesions is increased in UTIs. Progressive renal parenchymal lesions can lead to hypertension, pre-eclampsia in pregnancy, renal insufficiency and end-stage nephropathy. Vesicoureteral reflux (VUR), UTI at early age, delay in the treatment of UTI, and recurrant UTIs are known risk factors in the pathogenesis of renal parenchymal lesions. The outcomes of pediatric UTIs and the development of renal scarring are probably greatly influenced by genetic background.

The clinical data on the children with UTIs revealed an association with VUR in 48.5% of cases, but other urinary tract anomalies also occurred significantly frequently. The frequency of renal parenchymal involvement was 38.8%, it was very high (76%) in those with high-grade VUR and in the patients with 3 or more episodes of pyelonephritis in the history (62.9%).

The genetic examinations demonstrated that the *HSP A1B*(1267)GG genotype and the carriage of the *HSP A1B*(1267)G allele led to a susceptibility to uropathogenic infections and predispose to renal parenchymal lesions.

Carriage of the *TLR4*(896)AG mutation and the *TLR4*(896)G allele is associated with an enhanced risk of UTIs, and primarily characteristic of UTI patients without VUR. Our data show that the carriers of this genetic variation are at high risk of urinary tract inflammations also without anatomical anomaly of the urinary tract.

The ID and DD genotypes in the I/D polymorphism of the *ACE* gene were characteristic of the high-grade VUR patients and those with renal parenchymal lesions.

Our clinical observations have provided Hungarian data confirming that the high-grade VUR and recurrent UTIs significantly increase the risk of renal scar development in children.

TLR4 has a significant role in the recognition of the pathogens and the early immune response, *HSP72* with its repairing functions and the *ACE* gene polymorhisms with their part in the renal fibrotic processes are probably also such predisposing factors of UTI and the complex processes of later renal scarring due to the UTIs.

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JÁNOS TIBOR KIS (2008)

The role of the T cells in autoimmune type 1 diabetes mellitus

Supervisors: András Treszl, Tivadar Tulassay

Type 1 diabetes mellitus (T1DM) affects 1 in 400–600 people; the incidence of the disease is rising worldwide. The T1DM is caused by the chronic autoimmune destruction of the insulin producing β-cells. The exact etiology and the primary autoantigen is not known. The autoimmune, chronic and progressive nature of the disease raised the possibility of intervention, which can slow down or stop the destruction of the β-cells preferably as early as possible in prediabetic stage. A lot of proofs were gathered about, that the T cells, mainly the regulatory T cells and the natural killer cells have fundamental role in regulating the autoimmune process.

We isolated human CD4+ T cells from healthy (H), from newly diagnosed T1DM (less than 4 months –ndT1DM), from long term T2DM (more than one year ltT2DM) patients. We studied the gene expression of the CD4+ T cells with affymetrix gene chip, then we confirmed the results with other genetic studies (PCR) and with checking it in protein level (Western blot and FACS analysis), and with functional assay. The iNKT cells can potentially regulate the all CD4+ T cell population. Thus we measured the frequency of these cells, we isolated (from H, ndT1DM, ltT1DM and ltT2DM patients) and cloned them. We furthermore characterized the different subgroups and their function.

The all CD4+ T cell population gene expression pattern showed significant differences, mainly in the regulatory genes of the immune signal and cell cycle. The ratio of the CD4+ iNKT cells significantly decreased among the ltT1DM patients compared to the H and ltT2DM patient groups. The cytokine production of the iNKT cells shifted to the harmful direction in ndT1DM and in ltT1DM groups. The differences in the all CD4+ T cell population and the CD4+ iNKT cell subgroup, which has more potent regulatory function and the changed cytokine production can be helpful to understand the immuno-pathogenesis of type 1 diabetes mellitus. The clinical studies, modulating the T cells as well as the iNKT cells, have already started. We hope that our results can help to these intervention trials.

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ANDRÁS NOBILIS (2008)

The associationg of genetic polymorphism of angiotensin converting enzyme (ACE I/D) and angiotensin II type 1 receptor (AT1R A1166C) with disturbed perinatal adaptation in preterm infants born with very low birth weight

Supervisor: Barna Vásárhelyi

Perinatal adaptation is a complex process with dramatic alterations in the function of organ systems and metabolic processes. Along with these alterations the activation of renin-angiotensin system also occurs. Disturbances of the activity of renin-angiotensin system may have a role in the development of complications during perinatal adaptation in the preterm neonate.

Genetic polymorphisms may have an impact on the functionality of renin-angiotensin system. Most data are available concerning the I/D polymorphism of angiotensin converting enzyme and A1166C polymorphism of angiotensin II type 1 receptor. The aim of our studies was to investigate whether there is any association between carrier state of these polymorphisms and risk of complications such as circulatory failure, ductus arteriosus Botalli and acute renal failure.

We have enrolled preterm neonates born with low birth weight (1500 gram or lower). Preterm neonates were grouped according to the presence of the investigated complications (i.e. infants with and without circulatory failure; infants with and without ductus arteriosus persistent on the 5th postnatal day; infants with and without acute renal failure). We determined the distribution of the investigated genetic polymorphisms in each group and adjusted the observed associations for the known risk factors of the complications.

We have demonstrated that carrier state of D allele of angiotensin converting gene polymorphism is protective against circulatory failure; ductus arteriosus is closed before the 5th postnatal day in each infant carrying 1166CC genotype of angiotensin II type 1 receptor. Carrier state of investigated polymorphisms was not associated with the risk of acute renal failure.

We concluded that functional polymorphisms may have an impact on perinatal adaptation and, therefore, some may enhance short term survival (although this hypothesis should be verified with a larger patient number). Those polymorphisms were found to be protective against perinatal complications that have been linked with increased cardiovascular morbidity and mortality in the adult. This finding raises the possibility that the observed increased susceptibility of adults born with low birth weight to cardiovascular disease may be the result, at least partly, of carrier state of some genetic polymorphisms.

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BEA PÁSZTHY (2007)

Psychosomatic characteristics of anorexia nervosa in childhood and adolescence

Supervisor: Tivadar Tulassay

Anorexia nervosa (AN) is a psychosomatic disease. According to the traditional diagnostic criteria it is characterized by weight loss or a halt in weight gain, intensive fear from weight gain, body image distortion and primary and secondary amenorrhea caused by malnutrition. In the last few years the disease has started more at an early age and its prevalence has increased.

In my doctoral work I tried to emphasize the complex, bio-psycho-social approach of this disease with the unit of seemingly distant areas, such as anthropometrics, immunology and family studies. I have studied three areas, which although use different research conceptions and methods, help in the early recognition of the disease with better interpretation of potential somatic complications and effective therapeutic strategies. A diagnostic threshold of AN is based on the severity of somatic changes such as the degree of weight loss. The most appropriate parameter to define the limit for pathologic emaciation by comparing body maturity and nutrition of AN adolescents with age-matched standards. The conclusion is that in children and adolescents with eating disorder we can define someone as being pathologically underweight more precisely utilizing the "difference from the ideal body mass index (BMI)" parameter with the age-adjusted BMI percentile values, as compared to the absolute BMI and difference from ideal weight parameters used at present.

Contrary to general belief, AN patients, despite being severely underweight, are less prone to infections except at the terminal stages of the disease. There have been no earlier studies on the role of regulatory T cells in the immunmodulation associated with AN. In this work we examined the ratio, function and cytokine producing ability of regulatory T cells and their cell network along with the activation kinetics of CD4+ cells after different *in vitro* stimulants and intracellular stains analyzed with the flow cytometric technique. According to the measurements in adolescent AN patients the number and function of regulatory T cells and the relative number of the subgroups of dendritic cells does not change, and the reaction of monocytes to LPS is normal.

Based on these results, there are probably other regulating mechanisms responsible for the immunologic changes observed in adolescent AN. However, we concluded that the IL-2 production of CD4+ cells is decreased in AN. We found in our study that the gamma interferon and IL-4 production of CD4+ cells in anorexia nervosa does not differ from the healthy control group. We were the first to study the excitability of lymphocytes in AN and the kinetics of calcium-flux after cell activation. We concluded, that the membrane potential of circulating lymphocytes is normal in AN, however after activation it takes twice as long to reach the maximal calcium level as compared to the healthy control group. There is an association between the changes in activation characteristics and differences in calcium-flux kinetics with the decreased IL-2 producing ability.

Development and persistence of eating disorders is a combined result of biological, psychological and social factors. Of these factors, there is an increased role of social norms regarding thinness, the behaviour and attitude of the parents in relation to body weight, the fear, anxiety and depression of parents regarding their child's eating disorder, family connections, friends' dietary habits and perceptions of body weight and body image.

Several studies proved the multifactorial origin of eating disorders, where the family environment plays an important role.
The familial interactions responsible for the development and persistence of AN were studied. We were looking to determine whether there is a connection between the parents' symptoms of anxiety and depression and their child's depressive symptoms along with the objective and subjective characteristics of the child's eating disorder. We also examined—considering circular causality as one of the major elements of family therapy—what the connection is between the child's eating disorder and the parents' mood and anxiety. We can expand the evidence for the presence of circular causality—like enmeshment, parental overprotection and increased anxiety level known from the literature to be present in families with eating disorder—playing a role in the development of AN with our family studies.
According to our conclusions, a decrease in the AN child's body mass index increases the anxiety of the parents and the depressive symptoms of the mother. The degree of the parents' anxiety has an effect on the child's eating disorder and also has an impact in the development of depressive symptoms. The child responds to the increased parental anxiety with further weight loss. There is a circular association between the depressive symptoms of the AN patient and her parents' reaction. It is proved that the family members have a mutual impact on each other's emotional state. We were able to identify with our study the vicious circles lying between the child's anorectic symptoms and the parents' depression and anxiety.

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GÁBOR RUDAS (2007)

The occurrence and the pathomechanism of cardiovascular autonomic dysfunction in childhood renal failure

Supervisor: Tivadar Tulassay

My goal was to find some new ultrasound diagnostic methods to answer a few questions (pathological and diagnostically) of some neonatal neurology diseases.
I divided my studies into two parts according the two major types of the neurosonography used: (1) the measurement of the cerebral blood flow (CBF) by doppler method and (2) the so-called 2D ultrasound examination of spine.
In case of the CBF examinations I used two kinds of the doppler spectrum analysis. At first I used the so-called Résistance Index to exam the effect of dopamine on cerebral blood flow in the sick preterm and term neonate. The background was, that dopamine has been the drug of choice to combat hypotension in critically ill neonates. However, the pathogenesis of intraventricular hemorrhage (IVH) and periventricular leucomalatia (PVL) is almost certainly related to changes in CBF. Because autoregulation of CBF in preterm neonates is impaired, enhanced responsiveness of these infants to the pressor

effects of dopamine may put them at risk for the development of IVH and/or PVL. The effect of the dopamine-induced homodynamic changes on CBF in the sick preterm infants has not been clarified. In addition, we have had a suspicion, that the dopamine had a direct, selective vasodilatory effect on the cerebral vasculature, too, and this can also lead to IVH or PVL.

The aims of our study were to know (1) what is the Effect of low-dose dopamine infusion on cerebral blood flow and autoregulation; (2) Does the dopamine has a direct, selective vasodilatory effect on the cerebral vasculature?

The changes of the CBF velocity in the Anterior Cerebri Artery (ACA) as well as the calculated RI were used to assess changes in CBF during the study by doppler method and by calculation of the Resistant Index. All of the examinations were so called autocontroll examination. Arterial blood gases, Htc, Heart rate, blood pressure (BP), and pO2 were monitored as part of routine clinical care. The preterm and term neonate has had a clinically indicated dopamine infusion (2–4 μg/kg/min).

Conclusions: (1) The administration of 2μg/kg/min dopamine did not induce significant changes in BP or RI at either term or preterm neonates. (2) In contrast, 4μg/kg/min significantly increased BP in both group. (3) We found an intact autoregulation of CBF at term neonates. (4) In the preterm neonates we could find a not quite effective autoregulation of CBF: the pressor effects of dopamine may put them at risk for the development of IVH and/or PVL. (5) We could not find a significant selective vasodilatory effect of dopamine on the cerebral vasculature.

During the next study (Changes of the CBF after asphyxia) I have used the so called Time Average Peak Flow Velocity (TAPFV) Doppler spectrum analysis. The advantage of this method, that the correlation between the Xe 133 clearence measurement of the CBF and the TAPFV is 0.82.

The diagnosis of the asphyxia is not easy: the clinical picture quite often not typical, in addition the patient often require ventilation and/or sedation. The 2D ultrasound and the CT, during the acute period, are not informative. The MR (T2, Diffusion WI, ADC and MR Spectroscopy) has a good sensitivity and specificity, but it is quite complicated and the so called therapeutic window is very short. Another problem, that from clinical picture we can not distinguish the acute (intrapartum) and the subacute (intrauterine) asphyxia.

I have worked out a new ultrasound diagnostic method, wich could detect much better the asphyxia and the changes of the CBF, than all of the other formerly methods. However, have been raised, that the asphyxia has a quite long process, that is why I have measured the changes of the CBF in the ACA and median cerebry artery (MCA) every 6 hours during the first 72 hours of life.

Our goals were: (1) With a new method to examine the changes of the CBF in the ACA and MCA at asphyxiated and healthy term newborns during the first 72 hours of life; (2) To examine whether the TAPFV a reliable method to make the diagnosis of the asphyxia, is it a good prognostic factor and can influence the therapy?

I could determine that the asphyxia is—presumably—not a uniform disease. We can find a diffuse hyperemia in the bigger parts of the seriously asphyxiated cases, but sometimes —in the quite seriously ill patients—the CBF can strongly decrease. The hyperemia is much longer as we believed formerly.

The measurement of the TAPFV can be a reliable diagnostic method, prognostic factor and leader of the therapy.

In case of the asphyxia, by using different diagnostic methods—measurement of the

TAPFV, amplitude integrated EEG and the MR—, presumably we could distinguish much better the different form and phases of the disease and the therapy could become much more effective.

The other group of the new neurosonographically methods belong to the 2D examinations. With these methods I have made the examinations of the spinal subarachnoidal (SSA) spaces in case of the posthaemorrhagic (PHH) and postinfectiosus hydrocephalus (PIH). I have had the first publication about the changes of the echogenecity of the SSA in case of PHH and PIH. In my opinion, the increased echogenecity of the SSA is a sign of the blood and/or the arachnoiditis in the SSA and it can play a role in the development of the progressive ventricular dilatation (PVD).

The next was a prospective, controlled study to evaluate the frequency and clinical significance of echogenecity in SSA at risk for PVD.

My conclusions were: (1) I could find echogenic SSA space after IVH III–IV (ca. 70–80%) and after meningitis; (2) Echogenic SSA space could explain the discrepancy often observed between the biological and chemical constitution of CSF from ventricular and lumbar punctures and it has a strong influence on the next diagnostic and therapeutic activity; (3) This change after the IVH—presumably—has a "filter" function, but in case of the meningitis it has an "obstruction" result; (4) This change can play a role in the developing of the PHH or PIH, because in patients with echogenic SSA space I could find 90% PVD after the bleeding or meningitis, and in contrast the patients without echogenic SSA space I could find PVD; (5) Further studies are required to determine the role of the increased echogenecity of the SSA space in the developing of the PHH or PIH.

Because of the last question I have decided to make a new study where I can follow the spread of blood from IVH until the SSA space and a follow-up until the PVD can disappear or shunt surgery is required.

The aims of the next study were to study the frequency and rate of spread of blood into the SSA space and to evaluate the relationship of this findings and PHH.

Serial cranial and spinal sonography were made during the first day of life—before the IVH—and 12–24 hours after the bleedings. During the next 8 weeks I have made a control examination weekly. The control group has not had IVH.

That was the first publication of the visualization of spread of blood from ventricular system into subarachnoid space after IVH. More than half of the cases within 12 hours I could find the blood in the SSA space. I have had 7–9 weeks long fellow up of the increased echogenecity of the SSA space. My results suggest that there is a strong correlation between the echogenecity of the SSA space and post-hemorrhagic ventricular dilatation.

At last the so called Intracranial Compliance (ICC) examination, which was introduced in Hungary by me. With ICC we can distinguish the PHH with a slit increased ICP from the atrophia cerebri.

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BEÁTA SZEBENI (2008)

Role of Toll-like receptors in the development of immunpathogenetic gastrointestinal diseases

Supervisor: András Arató

Besides the central role of the adaptive immunity, innate immune responses may also play an important role in the development of coeliac disease (CD), inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC). Toll-like receptors (TLRs), CD14 and caspase recruitment domain (CARD) 15 are key regulators of the innate immune system. The aim of this study was to characterise the expression of TLR2, TLR3 and TLR4 in duodenal or colonic biopsy samples taken from children with CD or with IBD and controls. Moreover, my aim was to evaluate whether single nucleotide polymorphisms (SNPs) of CD14, TLR4 and CARD15 genes are associated with the risk of NEC in very low birth weight (VLBW) infants.

We found higher TLR2 and TLR4 mRNA expression and protein levels in the duodenal mucosa of children with treated CD and untreated CD than in controls. TLR2 and TLR4 mRNA expression and protein levels were even higher in the duodenal mucosa of children with treated CD than in untreated CD. TLR2 and TLR4 mRNA expression and protein levels were higher in the inflammed colonic mucosa of children with freshly diagnosed (fd) IBD and relapsed (r) IBD compared to controls. In the non inflammed colonic mucosa of children with fdIBD and rIBD, TLR2 and TLR4 mRNA expression and protein levels were similar to controls. TLR2 and TLR4 mRNA expression and protein levels in either inflammed or non inflammed colonic mucosa were similar in children with fdIBD and rIBD. No significant differences were found in the prevalence of CD14 $^{-260}$T, TLR4 $^{+896}$G and $^{+1196}$T and CARD15 $^{+2722}$C, $^{+2104}$T and 3020insC alleles between WLBW infants—with and without NEC—and healthy term newborns. Furthermore I did not found any association between genotype and prematurity or sepsis, which are important risk factors of NEC.

In summary, my results of increased expression of TLR2 and TLR4 even in treated CD may indicate the primary role of these pattern recognition receptors (PRRs) in the pathogenesis of CD. However, the non elevated expression of these PRRs in the non inflammed colonic mucosa does not support their primary role in the development of IBD. Carrier state of the tested CD14, TLR4 and CARD15 SNPs is not associated with the risk of NEC and other perinatal complications in VLBW infants.

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LÁSZLÓ SZŐNYI (2006)

Alpha-1-antitrypsin deficiency in paediatric liver and immunological diseases

Supervisor: András Arató

The alpha-1-antitrypsin deficiency is associated with premature development of pulmonary emphysema and in some cases chronic liver disease. The frequency of PiM allele, PiZ and PiS alleles are 97.2%, 0.95% and 1.65%, respectively. Therefore, the hypothetical prevalence of PiZZ and PiSS homozygous alleles are 1:10526 and 1:6060, respectively. We estimated the prevalence of mutant allelic forms in children with chronic liver disease and their relation to clinical signs. The results showed that the alpha-1-antitrypsin deficiency is a common inherited disease in Hungary. Its clinical picture is similar to that described in the literature. A correct diagnosis requires knowledge of the serum level and the phenotype of AAT, especially in the event of neonatal cholestasis and an elevated transferase activity. We determined the AAT phenotype in cystic fibrosis patients with different types of hepatobiliary involvement and establish whether the phenotype has any influence on the severity of the liver disease in CF. The mutant alleles have no effect on the development of the liver disease in CF patients.
AAT phenotype is not associated with the risk of primary IgA nephropathy (IgAN), but might have an impact on disease outcome as well as on the risk of secondary IgAN.
The recipient's Pi phenotype can change after liver transplantation. We observed a very strange "Pi MZS" phenotype in a transplanted child. The alpha-1-antitrypsin is a glycoprotein, therefore in congenital disorder of glycosylation (CDG) the picture of the isoelectric focusing can change. This can be the first step to the proper diagnosis in CDG. Very rare Pi variant can cause typical AAT deficiency with fatal liver disease and haemorrhage cerebri in infancy.

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PROGRAM 2/4.

GASTROENTEROLOGY

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Program overview

The importance of gastroenterological diseases is increasing, and because of their frequency and complications, they are considered as one of the most menacing group of diseases. This fact is confirmed by the out-patient and in-patient numeric data, as well as morbidity and mortality indices. Gastroenterology has developed such a discipline that combines the knowledge of different specialities. It includes the pathophysiological data resulted from basic research, the results of clinical research and observations. The research of the gastrointestinal diseases can be done by different approaches and different methodological possibilities. This interdisciplinar topic offers unprecedented opportunities for scientific research. The achievements of last one and half decade resulted relevant changes in several aspects of gastroenterology, and the conventional understanding of development, progression and treatment of diseases had been changed. Despite of the undoubted results several questions need to be answered and new questions are appearing. Gastroenterological diseases can be the topic of wide-spread researches that fit to the scientific basis of public health priorities.

Titles of research projects	*Supervisors*
Intensive care in postoperative and septic conditions associated with gastrointestinal disorders	Katalin Darvas
Examination of proteolytic enzyme systems and cell kinetic parameters in gastrointestinal tumors and digestive diseases	László Herszényi
Novel factors in the development of digestive tract mucosal lesions	László Kopper
Pathogenesis of the *Helicobacter pylori* related diseases	László Kopper
Pancreas transplantation	Róbert Langer
Examination of peripheral blood mRNA expression markers in patients with colorectal cancer	Béla Molnár
Link among exercise, glucose and lipid metabolism, exercise therapy in diabetes and obesity	Csaba Nyakas
Effect of exercise and food intake on the normal and pathologic brain aging cell physiological processes	Csaba Nyakas
Pathophysiology of the alimentary system	László Rosivall
The role of pattern recognition receptors in non-alcoholic fatty liver disease	Gyöngyi Szabó
Mechanisms of innate immune alterations in HCV infection and its modulation by alcohol use	Gyöngyi Szabó

Toll-like receptor 4-mediated signaling in alcoholic liver disease	Gyöngyi Szabó
Hypoxia-inducible factor in alcoholic liver disease	Gyöngyi Szabó
Extrahepatic complications of chronic liver diseases: hepatic osteodystrophy, autonomic neuropathy. Wilson disease gene mutations, chronic C virus hepatitis and the liver disease associated	Ferenc Szalay
Inflammatory ibowel diseases and the osteopenia	Miklós Szathmáry
Application of video-endoscopic surgical methods in gastroenterology	Tibor Tihanyi
Study of molecular factors deteremining the evolution of bone metastasis	József Tímár
Inflammatory intestine illnesses and osteopenia	Zsolt Tulassay
New factors the digestive system mucosa in the development of laesio	Zsolt Tulassay

Ph. D. students		***Supervisors***
Gabriella Bekő	pt (a)	Ferenc Szalay
Katalin Leiszter	ft	Béla Molnár
Béla Lombay	pt	Ferenc Szalay
János Osztovits	ft (a)	Ferenc Szalay
Mária Pádár	pt	Ferenc Perner
Rita Stangl	pt	Katalin Darvas
Sándor Szántó	pt (a)	István Pulay
Júlia Luca Szőcs	pt	Péter Andréka
Rita Temesi	pt (a)	Tibor Tihanyi
Kinga Tóth	ft	Béla Molnár
Gábor Valcz	ft	Béla Molnár
Zsolt Visnyei	pt	Ferenc Szalay

Ph. D. candidates		***Supervisors***
Gábor Bognári	i	Lajos Flautner
Tímea Csák	ft	Ferenc Szalay
Levente Ficsór	ft	Béla Molnár
Anikó Folhoffer (Kuczyné)	ft	Ferenc Szalay
Bernadett Lévay	pt	Lajos Flautner
István Pregun	i	László Herszényi
Sándor Spisák	ft	Béla Molnár
Dominika Szőke	ft	Zsolt Tulassay
Viktor Sebestyén Varga	i	Béla Molnár

Ph. D. graduates		***Supervisors***
György Miklós Buzás	pt	László Herszényi
Zsolt Tibor Csapó	i	Róbert Langer
Orsolya Galamb (Udvardyné)	ft	Béla Molnár
András Győrffy	ft	Zsolt Tulassay, Balázs Győrffy
István Hritz	ft	László Herszényi
István Kisfalvi	pt	Gábor Varga
András Ladányi	i	Béla Molnár
Pál Miheller	i	László Herszényi

Zoltán Mihály Péter	i	Zsolt Tulassay
Ferenc Sipos	ft	Béla Molnár
Viola Weszelits	i	Ferenc Perner
Zsolt Zéman	pt	Tibor Tihanyi

a, absolutorium; pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

GYÖRGY MIKLÓS BUZÁS (2007)

Eradication of *Helicobacter pylori* infection in outpatient care

Supervisor: László Herszényi

The doctoral thesis investigates the possibilities of *Helicobacter pylori* eradication in outpatient practice. The subject is grouped in three topics.
My aims were: (1) First-, second- and third-line eradication of *Helicobacter pylori* infection in duodenal ulcer cases in prospective, controlled studies; (2) Meta-analysis of the Hungarian and European results of eradication regimens; (3) Assessment of the quality of life in functional dyspepsia.
Patients and methods: (1) 686 duodenal ulcer patients received first line, proton pump inhibitor/ranitidine bismuth citrate + clarithromycin + either amoxicillin or a nitroimidazole for 7–14 days; 321 cases were enrolled in prospective, controlled, parallel-group studies, 365 patients were selected from the routine outpatient practice. Further, 134 cases with persistent infection after the primary treatment received second-line triple regimens in a crossover manner. Forty-one cases with resistant infection to the two previous regimens were treated with quadruple combinations. (2) The results of the eradication studies published in Hungary between 1993–2002 and that of European congress abstracts (1997–2002/2004) were meta-analysed. The pooled eradication rates and odds ratios of the different therapeutic regimens was assessed. (3) The quality of life was determined using a disease specific questionnaire translated and validated in Hungarian in 123 healthy persons, 101 *Helicobacter* positive and 98 negative functional dyspepsia patients.
Results: (1) The primary eradication was successful in 75–80% of the cases, while the efficacy of the second-line regimens was 62% and that of the third-line therapy in 58%. The rate of eradication of the consecutive regimens was decreasing. (2) Meta-analysis of the Hungarian and European publications showed that proton pump inhibitor/ranitidine bismuth citrate-based triple combinations achieve eradication in over 80% of the patients, confirming the validity of the national and European consensus. (3) The quality of life is impaired in functional dyspepsia. Eradication of the infection or cisapride treatment lead to an improvement of the quality of life during the one-year follow-up period.
Conclusions: In outpatient care, a *Helicobacter pylori* infection could the eradicated with first-line regimens in 80% of the cases. However, persistant infections still constitute a therapeutic problem. Meta-analysis of the Hungarian and European studies showed that the efficacy of the main regimens are similar, confirming the validity of the consensus

recommendations. Assessment of the quality of life is a useful method for the study of natural course of functional dyspepsia as well in observing therapeutic effects over time.

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- *Buzás GyM, Józan J (2006) First-line eradication of Helicobacter pylori infection in Europe: a meta-analysis based on congress abstracts, 1997–2005. World J Gastroenterol 12: 5311–19.*
- *Buzás GyM (2006) Quality of life in patients with functional dyspepsia: effect of a Helicobacter pylori eradication with pantoprazole, amoxicillin and clarithromycin or cisapride. A prospective, parallel group, controlled study. Curr Ther Res 67: 305–307.*

ZSOLT TIBOR CSAPÓ (2008)

Innovations in kidney transplantation

Supervisor: Róbert Langer

The author reviews his own clinical experiences and the international literature how to expand the number of used kidneys for transplantation, to achieve longer functioning graft and patient survival, and experimentally use topical for avoiding drug side effects. In the case of an extended criteria donor dual kidney transplantation seems to be a good solution. According to our 5 patients, 2 years graft and patient survival and graft functions are good, especially for older recipients without major surgical complication. In the case of donors under the age of 5 we used the kidneys separately as single transplants instead of the most widely used *en bloc* transplants. We compared 38 pediatric kidney transplants to 121 "ideal adult donor" (18–45 years of age) transplants. Without surgical complications the pediatric kidney function was equivalent to that of "ideal" adult kidney transplants not showing deterioration over time. There were no graft losses due to technical reasons among pediatric transplants. Smaller pool of potential kidneys for transplant are influenced with progressive arterial diseases—such as fibromuscular dysplasia—and questioned to be suitable for transplants. Observing our 7 patients with FMD even count with the only case we reported we think that the overall risk to the potential donor remains unknown, the risk to the recipient is low compared with the potential benefits of transplantation.

The author observed new immunosuppressant combinations to prevent the transplanted organs in prematurely high risk ethnic groups. A concentration-controlled sirolimus-cyclosporine-prednisone regimen in 470 patients reduced the incidence of acute rejection episodes and increased 6-year graft survivals without an augmented toxicity profile. The author was one of the first clinicians who used Campath-1H, if the rejection was refracter to the usual therapy. Campath-1H is accepted in leukemia therapy and for induction due to its broad immunosuppressive act. We used it as rescue therapy in 5 patients became well tolerated, effective and relatively free of adverse events.

In an experimental study the author could prove significant wound healing impairment effect of sirolimus in mice using daily 8 mg/kg sirolimus orally. He could document enhanced wound healing with the use of nucleotide topical especially in the first 2–6 days in the same experimental settings.

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ORSOLYA GALAMB (UDVARDYNÉ) (2008)

mRNA expression analysis and classification of colonic biopsy samples using oligonucleotide and cDNA microarray techniques

Supervisor: Béla Molnár

Despite tremendous progress in the past few decades, certain important aspects regarding the diagnosis, therapy, and follow-up of CRC still remain unsolved. The high incidence and not completely known pathogenetic background, origin, molecular biology of CRC necessitate further research of this disease. In my Ph. D. thesis I searched for biomarkers of the development of colorectal carcinoma, and performed gene expression analysis for colorectal disease classification using whole genomic oligonucleotide and cDNA microarray technology and colonic biopsy samples. I have established that the oligonucleotide whole genomic microarray analyses of biopsy samples wholly fulfill the Affymetrix quality requirements, are highly standard and reproducible. I have established that the Taqman Microfluidic Card System which offers an opportunity for the analysis of the expression levels of 96 genes on 10–100 samples, is particularly suitable for high-throughput, quick and cost efficient RT-PCR validation of gene expression changes detected by microarrays. I have shown that the sequential overexpression of osteopontin and osteonectin mRNAs and proteins significantly correlates with the progression of the colorectal adenoma-dysplasia-carcinoma sequence. I have identified and validated by RT-PCR ten novel tissue markers which show continuously increasing mRNA expression in line with the colorectal adenoma-dysplasia-carcinoma transition. These are the following: tissue inhibitor of metalloproteinases-1 and -3, von Willebrand factor, interleukin 8, melanoma cell adhesion molecule, thrombospondin 2, collagen 4A1, matrix Gla protein, interleukin 1 receptor antagonist and calumenin. I have established that the prostaglandin D2 receptor and the amnionless homolog are novel, validated sequentially downregulated markers of the colorectal adenoma-dysplasia-carcinoma sequence. During my analyses, I have identified the top 27, 13 and 10 genes associated with adenoma, CRC, and IBD, respectively. Using whole genomic microarrays I have also determined the top100 most differentially expressed discriminatory genes which are suitable for molecular-based discrimination of early (Dukes A and B stage) and advanced (Dukes C and D) colorectal cancer.

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- *Galamb O, Györffy B, Sipos F, Spisák S, Németh AM, Miheller P, Dinya E, Molnár B, Tulassay Z (2007) Vastagbél-adenoma, vastagbélrák és IBD-specifikus génexpressziós mintázatok meghatározása teljes genomszintű oligonukleotid microarray-rendszerrel. Orv Hetil 148: 2067–2079.*

ANDRÁS GYŐRFFY (2008)

Systems biology: gene expression signatures and the regulation of transcription

Supervisors: Zsolt Tulassay, Balázs Győrffy

Microarray technologies provide tremendous information about the transcription activity in normal and diseased cells and tissues. The resulting databanks can be mined by appropriate statistical methods to get insight into the expression changes of single genes. However, our knowledge regarding the common controlling networks for all of these genes as a biological complex is relatively scarce.

During my Ph.D. studies I focused on these regulatory systems using in silico systems biology tools. I have investigated both cis and trans transcription regulation. Among the trans transcription regulatory elements I have investigated the role of transcription factors. Their role was investigated in correlation with doxorubicin and 5-fluorouracil resistance using previously published chemotherapy-resistance associated gene lists. The E47 transcription factor was identified as a common transcription factor responsible for co-regulation of gene expression signatures associated with doxorubicin resistance in breast cancer. The modulation of the apoptotic machinery was identified as a key mechanism of 5-fluoruracil resitance.

The cis regulation was investigated focusing on antisense transcription. The antisense expression was measured in 1182 mouse genes. Here, 43% genome-wide frequency of antisense transcription was measured. Chromosomes 14 and 1 have a relatively high level of antisense transcription compared to the other chromosomes. At high expression levels an inverste sense-antisense gene expression correlation was observed. These results support the regulatory characteristics of antisense transcription in mouse genomes.

During these studies I have also executed a technological development for the diagnostical measurement of microarray results. Here, a positioning system and an automated pipetting system was developed. The positioning system has a 99% accuracy for spotting oligo drops on a microscopic glass slide. The achieved results are briefly summarized in the resuls section and are also included in two submitted patent applications.

- *Gyorffy A, Surowiak P, Tulassay Z, Gyorffy B (2007) Highly expressed genes are associated with inverse antisense transcription in mouse. J Genet 86: 103–109.*
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- *Gyorffy A, Vasarhelyi B, Szoke D, Dietel M, Tulassay T, Gyorffy B (2006) Comparative promoter analysis of doxorubicin resistance-associated genes suggests E47 as a key regulatory element. Anticancer Res 26: 2971–2976.*

ISTVÁN HRITZ (2008)

Immunohistochemical analysis of cell kinetics of the gastric- and esophageal epithelium in response to various stimuli

Supervisor: László Herszényi

Maintaining cell turnover is a key feature in organs with high metabolism such as gastric mucosa and esophageal epithelium. Increased cell turnover may lead to tumor development while the suppressed state results in mucosal damage and ulcer formation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used groups of drugs. Although these compounds represent a very effective class of medicines, their use is associated with a broad spectrum of untoward reactions, among the gastrointestinal especially gastroduodenal complications are the most significant. Proton pump inhibitor (PPI) co-therapy is considered as the best strategy in preventing gastrointestinal complications during NSAID treatment but there is limited information available on its effect on gastric mucosal cell kinetics. To evaluate the effect of PPI co-therapy on gastric mucosa we investigated epithelial cell proliferation, apoptosis, epithelial growth factor receptor (EGFR)- and p53 expression in patients on chronic non-selective NSAID (ns-NSAID) or cyclooxygenase-2 selective inhibitor (COX-2) treatment. While p53 expression is increased and EGFR expression decreased, there is a trend towards increase of cell proliferation and apoptosis in the gastric mucosa after chronic ns-NSAID treatment but the difference does not reach statistical significance. Chronic administration of selective COX-2 inhibitors is associated with increased cell proliferation and decreased EGFR expression of the gastric epithelium but is not accompanied by increased p53 expression, while the apoptosis shows a trend towards increase. These suggest that prevalence of NSAID gastropathy is likely not due to an effect on gastric cell turnover, however PPI co-therapy normalizes the disturbed cell kinetics irrespective of NSAID treatment used. Furthermore, six-month PPI treatment alone does not significantly increase gastric epithelial cell proliferation and EGFR expression and has no effect on apoptosis and p53 expression. Alterations can be found only in the localization of immunohistochemical staining density during chronic PPI administration confirming the safety features of these drugs.

Barrett's esophagus (BE) is a condition in which the normal stratified squamous epithelium is replaced by metaplastic columnar epithelium that predisposes to the development of 7 esophageal adenocarcinoma. Neoplastic progression in BE occurs by a multistep process associated with early molecular and morphological changes. To evaluate the process of malignant transformation of the esophagus we analyzed cell proliferation and p53 expression and expression of glutathione S-transferase (GST) and matrix metalloproteinase-9 (MMP-9) in the development and progression of normal epithelium, reflux esophagitis, BE, dysplasia and adenocarcinoma sequence of the esophagus. Overexpression of p53 is found to be typical in the malignant transformation of BE and increases with histological progression. Cell proliferation of Barrett's epithelium increases with progressive grades of dysplasia and is linearly correlated with p53 expression. GST is downregulated, while MMP-9 is upregulated in reflux esophagitis-BE-dysplasiaadenocarcinoma sequence of the esophagus. These demonstrate that activation of cell proliferation and p53 protein accumulation with simultaneous downregulation of GST and upregulation of MMP-9 may play a crucial role in the multistep esophageal carcinogenesis. Secretory leukocyte protease inhibitor (SLPI) represents a multifunctional protein of the gastrointestinal mucosa

exerting antimicrobial and anti-inflammatory effects. SLPI expression is generally induced during inflammation; however *H. pylori*-mediated gastritis is associated with significantly decreased antral SLPI levels. We have investigated whether SLPI downregulation of the gastric mucosa represents a specific phenomenon of *H. pylori* infection or is rather generally linked to gastric inflammation. In *H. pylori*-associated and lymphocytic gastritis the SLPI immunostaining of the mucosa is strongly reduced and frequently almost not detectable, while epithelial SLPI expression is not affected in NSAID-induced or autoimmune gastritis, suggesting that SLPI downregulation is specifically linked to *H. pylori* infection and does not just represent a general feature of gastric inflammation.

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- *Wex T, Treiber G, Venerito M, Leodolter A, Peitz U, Kuester D, Hritz I, Krueger S, Roessner A, Malfertheiner P (2006) Helicobacter pylori-induced downregulation of the secretory leukocyte protease inhibitor (SLPI) in gastric epithelial cell lines and its functional relevance for H. pylori-mediated diseases. Biol Chem 387: 893–901.*
- *Hritz I, Kuester D, Vieth M, Herszenyi L, Stolte M, Roessner A, Tulassay Z, Wex T, Malfertheiner P (2006) Secretory leukocyte protease inhibitor expression in various types of gastritis: a specific role of Helicobacter pylori infection. Eur J Gastroenterol Hepatol 18: 277–282.*

ISTVÁN KISFALVI (2007)

Effect of galanin and its analogues, M15, M35 and C7 and somatostatin immunoneutralization on the gastrointestinal tract in human and animal models

Supervisor: Gábor Varga

The complex mechanism of digestive, secretory, motility and excretory functions in the gastrointestinal tract requires a very elaborated regulation involving neuropeptides, the central nervous system and enteric neuronal system. So far over 50 gastrointestinal peptides have been described, some of them are well characterized, like somatostatin. In case of some recently discovered neuropetides, like galanin, we are still in the process of collecting data about their effects and importance. By using modern techniques I studied the effect of galanin and somatostatin in details on gastric acid and pancreatic enzyme secretion *in vivo*. I described the effect of galanin and its newly developed potent antagonists on gastrointestinal motility (in the stomach and small intestine). My data provide evidence, for the first time, about the presence and effect of galanin in the human jejunum.

Galanin is a 29-amino-acid neuropeptide subsequently found to be widely distributed in the central nervous system and most parts of the gastrointestinal tract. Galanin has been suggested to have a wide range of biological functions in the central nervous system (CNS) and in the gastrointestinal tract. The importance of galanin in food intake, cognition, pain perception, aging and Alzheimer-disease stimulated a broad array of investigation focusing on the brain and the central nervous system, but little is known about its effect on the regulation of gastrointestinal functions. The most potent galanin antagonists in the CNS are the chimeric peptide analogues M15, M35 and C7. I was the first to study and publish the effect of these antagonists on physiological gastrointestinal actions.

I studied the effect of galanin, the putative analogues and somatostatin on the following gastrointestinal functions: gastric acid and panreatic amylase secretion *in vivo* in rats, gastric emptying in conscious rats, contractions of isolated rat and human jejunal smooth muscle, I showed the presence of galanin immunoreactive nerve fibers in the human jejunum, and finally, using somatostatin immunoneutralization I studied the involvement of endogenous somatostatin in the regulation of basal and stimulated gastric acid and pancreatic enzyme secretion *in vivo*, in anaesthetized rats.

Results and conclusion: Galanin inhibited stimulated gastric acid and pancreatic amyse secretion in a dose-dependent and reversible fashion. Galanin may have a physiological role in the regulation of gastric acid and pancreatic enzyme secretion. M15, M35 and C7 analogues functioned as agonists, indicating that the peripheral galanin receptors involved in the regulation of gastrointestinal secretory functions are distinct from those originally found in the brain and spinal cord.

In conscious rats neither galanin nor its chimeric analogues affected the gastric emptying of non-caloric liquids, indicating that galanin does not play a role in the regulation of gastric emptying.

Galanin and acetylcholine were equally effective in stimulated the contractions of the isolated rat and human jejunal muscle suggesting that galanin is a potent physiological regulator of jejunal muscle contractions both in rats and humans. M15 and M35 acted as galanin agonists.

The stimulatory effect of galanin on isolated jejunal smooth muscle contractions was not inhibited by either atropine or tetrodotoxin indicating a direct effect on the smooth muscle cells.

Our morphological studies (immunohistology) served as the first demonstration of the presence of galanin immunoreactive neurons in the human jejunum. Galanin immunoreactive nerve fibers were found in all layers of the small intestine. The widely distributed galanin immunoreactivity in the human jejunum wall suggests the involvement of galanin in the regulation of human intestinal functions (including motility)

The opposite effects of the galanin analogues in the gastrointestinal tract and in the central nervous system (agonist versus antagonist effects) requires more attention and careful administration of pharmacological agents affecting galanin responses in the future.

Immunoneutralization of somatostatin with a monoclonal antibody resulted in a considerable increase in basal acid secretion independ on the type of anesthetics. Somatostatin immunoneutralization, however, did not affect basal pancreatic amylase secretion but significantly increased the previously stimulated (CCK-8 induced) amylase secretion. Endogenous somatostatin mediates suppression of basal gastric acid secretion but not of basal pancreatic amylase secretion. However, the stimulatory effect of somatostatin immunoneutralization on CCK-stimulated pancreatic enzyme secretion suggests that endogenous somatostatin may be a physiological regulator of stimulated pancreas secretion.

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- *Kisfalvi I, Burghardt B, Bálint A, Zelles T, Vizi ES, Varga G (2000) Antisecretory effects of galanin and its putative antagonists M15, M35 and C7 in the rat stomach. Physiol (Paris) 94: 37–42.*

ANDRÁS LADÁNYI (2008)

Detection of circulating tumor cells in the peripheral blood of solid tumor patients

Supervisor: Béla Molnár

A considerable body of evidence indicates that tumor cells are shed from a primary tumor mass at the earliest stages of malignant progression. Some of these cells will travel via the peripheral blood to sites anatomically distant from the primary tumor and form metastases. As individual disseminated tumor cells present in low numbers, they can be occult to standard methods of investigation. However, these circulating tumor cells (CTCs) are understood to be a source of eventual lethal metastases, the major cause of treatment failure in cancer patients.

Many studies have concluded that the presence of CTCs provides important prognostic information predictive of disease-free and overall survival in various malignancies. By contrast, other reports have found no statistically significant relationship between CTC detection and prognosis. This discrepancy is most likely because even in metastatic patients, the frequency of CTCs is extremely low (estimated to be in the range of 1–10 CTCs/ml of PB). We hypothesize that the issue of varying conclusions regarding the prognostic significance of CTCs may be attributed to the technical difficulties associated with the reliable detection of such rare events.

The main goal of our work was to develop and improve approaches for CTC detection in the PB of cancer patients and to use this technology in patient studies to investigate the prevalence and characteristics of CTCs. The aims were to build methods that provide improved sensitivity and specificity of CTC detection while meeting the requirements of a practical clinical CTC assay. Both cell-based and molecular methods of CTC detection were investigated for areas of improvement. In addition, steps of pre-analytical and analytical procedures were dissected in order to identify potential sources of inaccuracy and irreprocubility. The developed instrumentations and preparation methods were used to investigate the prevalence of various rare cells, such as CTCs and Cytomegalovirus (CMV)-infected leukocytes. Furthermore, together with four different European laboratories a pilot study was conducted to assess the variation and inconsistencies of CTC detection and to formulate the basis of a standardized, quantitative CTC detection protocol.

Results from our investigations provided novel insights into the molecular and cell-based detection and quantitation of rare cells. Through the application of the procedures and models described in this thesis a potentially more accurate assessment of level signals may be obtained. This would facilitate the earlier diagnosis and more accurate therapy monitoring of infectious and cancerous diseases.

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- *Ladanyi A, Sher AC, Herlitz A, Bergsrud DE, Kraeft SK, Kepros J, McDaid G, Ferguson D, Landry ML, Chen LB (2004) Automated detection of immunofluorescently labeled cytomegalovirus-infected cells in isolated peripheral blood leukocytes using decision tree analysis. Cytometry A 58: 147–156.*
- *Soong R, Ladanyi A (2003) Improved indicators for assessing the reliability of detection and quantification by kinetic PCR. Clin Chem 49: 973–976.*

PÁL MIHELLER (2008)

Pathophysiological background of bone metabolism in inflammatory bowel disease

Supervisor: László Herszényi

Elevated serum tumor necrosis factor alpha (TNFα) concentrations, as a common pathological pathway has a major role in the pathogenesis of inflammatory bowel disease (IBD) associated osteoporosis. Bone formation and bone resorption is coupled in physiological circumstances, while these two mechanisms are uncoupled in IBD, especially in Crohn's disease (CD) (1). Current state of bone remodelling can be characterised by the ratio of serum levels of bone resorption (beta crosslaps—bCL) and bone formation (osteocalcin—OC) markers. Serum concentrations of receptor activator nuclear factor kappaB ligand (sRANKL) and osteoprotegerin (OPG) are informative concerning the regulation of the bone cells. Changes of these parameters are proved in IBD. Based on our data we conclude that in CD elevated OPG can reflect a contra-regulatory response to factors such as inflammatory cytokines or may indicate T-cell activation (2). Infliximab (IFX) has a beneficial effect to the bone homeostasis in inflammatory and fistulizing type of CD as well. This effect seems to be due to modification of OPG/RANK/RANKL system. IFX can influence the effects of cytokines produced by the activated immune cells in the gut locally. Our results show that there should be some other systemic effect of IFX to the bone homeostasis in fistulizing CD (3). Beneficial changes of bone homeostasis after anti-TNFα therapy were more characteristic in patients responded to the therapy than in non-responders. Direct relationship between anti-TNF therapy and OPG/RANK/RANKL system can be supposed based on this fact.

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ZOLTÁN MIHÁLY PÉTER (2006)

Candida colonization and candidiasis of the esophagus in alcoholic liver disease patients

Supervisor: Zsolt Tulassay

The incidence of disseminated *Candida* infection has dramatically increased in the last few years. The colonizing fungal flora of the gastrointestinal tract is considered as the primary source of infection, disruptions of the integrity of gastrointestinal mucosa are portal of entry of the organisms. Parallel to this, thanks to the alternative medicine as well, the interest in *Candida* infection of the gastrointestinal tract increased.

The most frequent manifestation of *Candida* infections of the gastrointestinal tract is esophagitis. Since most *Candida* infections are considered to arise from the endogenous colonizing flora, my goal was to study the hardly ever examined esophageal fungal colonization. As this was not investigated so far in alcoholic liver disease patients, while at the same time alcoholic liver disease is a predisposing factor for fungal esophagitis, I performed my research in this respective population. Due to the coherence of the colonization and fungal infection, I also studied the barely examined questions of fungal esophagitis in alcoholic liver disease, such as the complaints of the patients and the single-dose treatment.
Through our prospective study we found fungal esophagitis in 19.3% and fungal colonisation in 22.5 % of the alcoholic liver disease patients. Compared to the control groups, the rate of fungal infection was significantly higher, while there was no significant difference in the rate of colonization. Fungal colonization and fungal esophagitis were significantly more frequent in patients with fungi in their ororpharyngeal scrapings. Based on our results this was the consequence of a common predisposing factor and it was not due to the fungi drifted down from the pharynx.
Applying a more precise method than so far put in the practice, we concluded that the colony number of the sample taken from the esophagus is not suitable for identifying colonization.
Through our partially retrospective, partially prospective study we evaluated the complaints of alcoholic liver disease patients with fungal esophagitis. We did not notice any patient with odynophagia or dysphagia, symptoms which could draw the attention on esophageal disease. In the lack of esophageal complaints the other symptoms do not help in the setup of the diagnosis, neither in the raise of any suspicion. The proportion of symptom free patients (13.7%) was meaningful.
Examining prospectively the single-dose fluconazole therapy we found it to be equally effective as the 7-day treatment.

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FERENC SIPOS (2007)

Assay of changes in colonic epithelial cell kinetics in inflammatory bowel diseases allowing for the histological activity of inflammation

Supervisor: Béla Molnár

The initial step of the pathogenesis of inflammatory bowel diseases (ulcerative colitis and Crohn's disease) is the loss of epithelial barrier function, which allows immunogenic factors to penetrate into the deeper layers of the mucosa where they sustain a chronic inflammatory process accompanied by active flares. Beside the specific symptoms of active inflammation this longstanding inflammatory process subserves the development of malignant epithelial changes. I studied the changes developing in paralell with the histological activity of inflammation of such basic cell kinetical parameters (apoptosis, proliferation, tumor sup-

pression, regenerative mechanisms, cell aging) that are responsible for the altered epithelial barrier function. I showed that apoptosis and proliferation of colonic epithelial cells is independent from the type of the disease, only increase in line with the degree of inflammation. The inreased apoptosis indicates the vulnerability of the inflamed mucosa, while the elevated proliferative activity is a regenerative response to active inflammation. The elevated proliferation is already present in mild inflammation as well as the expression of regenerative signals (growth factor receptors: EGFR, IGFR, HGFR) and anti-aging factor telomerase. The function-lost p53 tumor suppressor protein expression is also increasing in line with the inflammatory activity. The proliferative zone of the colonic epithelium collects genetic harms of p53 in the active phase of inflammation, then in the long standing mild phase of the disease the genetically impaired epithelial cells becoming apoptosis resistant, surviving and imortalized starts pathological proliferation. With these characteristics these cells make the opportnity of a later malignant transformation.

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VIOLA WESZELITS (2008)

The role of 3D CT-angiography in the evaluation of patients before and after cadaver liver transplantation

Supervisor: Ferenc Perner

The goal of the study was to utilize the three-dimensional CT-angiography to assess the hepatic vascular anatomy of patients being considered for liver translantation and to determine the usefulness in patients who have undergone cadaver liver transplantation. The 3D CT-angiography is an accurate and reliable method for assessing the hepatic arterial anatomy of the recipients, the stenosis of the celiac trunc, the splenic artery anurysms, the portal vein patency and the degree of the portal hypertension. A statistically sigificant difference was noted between the basic types of hepatic arterial blood supply determined by 323 recipients examinations and the types found by Michels dissection of 200 cadavers. Technically successful 3D CT-angiography, as a non-invasive imaging modality can replace invasive digital angiography in patients being considered for liver transplantation. The indications of 3D CT-angiography in liver transplanted patients always based on a colour Doppler ultrasound examination. The diagnosis of the arterial complications was more accurate by 3D CT-angiography. In the diagnosis of venous complications color Doppler ultrasound was as accurate as 3D CT-angiography. In patients with decreased RI values, 87.5% of 3D CT-angiography was positive for arterial vascular complications. In

patients with normal RI values, the 61.9% of 3D CT-angiography was normal. A 3D CT-angiogram can be viewed from several projections and has greater diagnostic possibilities in the planning of interventional procedures. 3D CT-angiography is the next diagnostic modality in the detection of vascular complications in patients after liver transplantation following color Doppler US examination.

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ZSOLT ZÉMAN (2007)

Outcome of antireflux surgery based on quality of life assessment

Supervisor: Tibor Tihanyi

Background: Evaluation of quality of life (QoL) data and patient satisfaction to estimate the outcome of laparoscopic fundoplication (LF) is nowadays an important issue. **Aims:** (1) To develop a QoL questionnaire for patients with gastroesophageal reflux disease (GERD) who have undergone LF; (2) To report the mid-term results of the surgical management of GERD by LF and to evaluate surgical outcome, including QoL and patient satisfaction. **Methods:** *Development of the QOLARS questionnaire.* We undertook a retrospective analysis of 116 patients underwent LF for GERD in the 1st Department of Surgery, Semmelweis University between April 1994 and April 2002. The questionnaire consisted of 50 questions (including the Visick score, EORTC-QLQ-C30, and a modified GERD-HRQL). *Outcome of antireflux surgery.* We evaluated the outcome of QoL data of 41 patients who underwent laparoscopic Nissen (n=30) or Toupet (n=11) fundoplication at our department of surgery between April 2002 and May 2003. QoL was measured by using the QOLARS. Patients completed the questionnaire before surgery, 6 weeks, 1 year and 3 years after surgery. **Results:** *Development of the QOLARS questionnaire.* Internal-consistency reliability was high (alpha value overall 0.95, dimensions 0.74–0.96). Using convergent and divergent validity, construct validity was evaluated by examining Pearson correlation coefficients between items and scales. Construct validity was demonstrated based on observed correlations. Known-groups validity was upheld, as patients who experienced more symptoms and patients who have higher Visick-scores reported worse QoL than those with less symptoms or less Visick-scores. *Outcome of antireflux surgery.* The general quality of life score, the heartburn score and satisfaction with current status improved significantly 6 weeks after and showed further improvement by the end of the 1st postoperative year and it remained stable 3 years after surgery. **Conclusions:** Our questionnaire is a short and user-friendly instrument with excellent psychometric properties. It has been found to be valid and reliable. QOLARS is a sensitive tool to assess surgical outcome after LF. Quality of life response closely follows the clinical outcome of surgical treatment reflecting its side-effects as well. Laparoscopic fundoplication provides effective and durable relief of reflux in patients with GERD.

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PROGRAM 2/5.

DENTAL RESEARCH

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Program overview

The aim of this Ph. D. program is to select open minded, self-supporting applicants who are able to acquire the knowledge of complex understanding of theoretical and clinical state of the art in the field, and are able to use this knowledge in education and research. Therefore, the Program prepares for the two different but not unconnected directions: it focuses on the better understanding of physiological and pathological processes in the oral cavity, as well as on the development and application of new therapeutic methods in all branches of dentistry.

Titles of research projects	***Supervisors***
Preventive program monitoring, risk factor evaluation using laboratory and clinical epidemiological methods	Jolán Bánóczy
Examination of oral cavity manifestations using clinical and laboratory methods	Jolán Bánóczy
Biological and clinical study and development of maxillofacial soft and hard tissue surgical techniques in rehabilitation	József Barabás
Comparison of imaging techniques	Csaba Dobó Nagy
Effects of psychological-, electromagnetic- and heat stimulation on the expression and secretion of Hsp70 type stress proteins in salivary glands	Tibor Károly Fábián
Role of vascular endothelium in the regulation of the oral circulation	Árpád Fazekas
Role of sensory and autonomic nerve fibers in the development of inflammation in the parodontium and the pulp	Árpád Fazekas
Driven parodontal tissue regeneration	István Gera

The importance of screening of the oral human papillomavirus (HPV), and the role of the extracellular matrix and the tight junction structure in the pathogenesis of tumours in the oral cavity	Péter Hermann
Diagnostic and therapeutic aspects in detistry supporting the prevention and rehabilitation in diseases of the craniofacial region	Melinda Madléna
Assessment of xerostomia with associated sicca symptoms and evaluation of the minor salivary gland flow rates in different conditions and diseases	Krisztina Márton
Stomatological aspects of immunpathologic disorders	Gábor Nagy
Study of the clinical progression and examination of extracellular matrix (ECM) components in oral squamous epithelial cancers and in conditions preceding oral cancers	Zsuzsanna Suba
Histological integration of bone replacement and implanted materials in the oral region	Zsuzsanna Suba
Permanent teeth injuries and care	Ildikó Tarján
Study of the molecular regulation of epithelial ion and fluid secretion	Gábor Varga
A human salivary gland model to study the molecular mechanisms of epithelial differentiation and for the development of gene implantation techniques	Gábor Varga
Investigation of pediatric disease related orofacial disorders	András Végh
Salivary gland research	Tivadar Zelles

Ph. D. students		***Supervisors***
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Örs Lehel Tollas	pt	Tivadar Zelles
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Gabriella Óvári	ft	István Gera
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Ákos Szűcs	pt	Gábor Varga

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Gábor Fábián	i	Pál Fejérdy
Umberto Garagiola	i	György Szabó
Szabolcs Gyulai-Gaál	i	Zsuzsanna Suba
Zsolt Németh	i	Zsuzsanna Suba
Márta Radnai	i	István Gera
Csongor Suba	pt	György Szabó
Norbert Velich	ft	György Szabó

a, absolutorium; pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

ADRIENN BARTA (2007)

Examination of amylase secretion in rat parotid gland

Supervisor: Tivadar Zelles

The present study investigated the secretion of salivary amylase under physiological, pharmacological and pathological conditions, *in vivo* and *in vitro*. The concentration of amylase secreted mainly by the parotid is an important diagnostic factor in certain salivary diseases. The aim of our *in vivo* study was to investigate the relationship between parotid isoamylase concentration in serum and parotid tissue in response to various stimuli. Wistar rats were fed with standard laboratory chow and water was supplied *ad libitum*. In the first experiment, after a 16-h fasting rats received (s.c.) 5 mg/kg pilocarpine or saline (control group). In the second study, half of the rats were fed for 1 h and the other received no food, after fasting. In the third experiment half of the rats were fed during the experiment (2 h after the beginning of the dark period) and the other fasted in the last 2 h. Pancreatic and parotid isoamylase levels in serum were separated by gel electrophoresis utilizing their different physical-chemical properties. Amylase concentration was determined by using starch as a substrate. Our data show that such stimulation as pilocarpine or feeding in the fasted state, as well as the spontaneous food intake during the nonfasted state result in a decrease in parotid tissue amylase activity and a proportional increase in serum levels of parotid isoamylase.

In conclusion, parotid isoamylase in the serum is also regulated by spontaneous food intake. Amylase is an important digestive enzyme and diagnostic factor. It has antibacterial effects among salivary proteins (e.g. mucin). In our *in vitro* study, the actions of endo-

toxin on amylase secreting cell activity have been evaluated. Endotoxin (*Escherichia coli* lipopolysaccharide; 3 mg/kg, i.v., 5 h) evoked nitric oxide synthase 2 (NOS2) induction in the rat whole parotid tissue (assessed by Western blot and the citrulline assay) and in rat isolated parotid acinar cells (assessed by Western blot and immunhistochemistry). On the other hand it reduced basal and acetylcholine-stimulated amylase secretion from these isolated cells. NG-nitro-L-arginine methyl ester (LNAME) (0.1 mg/ml, 4 days in drinking water) did not affect amylase release under basal or acethylcholine-stimulated conditions, either in control or endotoxin treated acinar cells. In conclusion, basal, acethylcholin-evoked or endotoxin-treated amylase secretion from rat parotid acinar cells does not appear to be modulated by endogenous nitric oxide.

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FERENC DŐRI (2008)

Effect of combined therapeutical methods on healing of intra-bony defects in regenerative periodontal surgery

Supervisor: István Gera

Several methods are available to enhance the healing and regeneration of periodontal tissues after surgical therapy of intrabony defects. The main indications for the use of combined regenerative procedures are the extent and morphology of the osseous lesions.

The six studies of the present dissertation focused on the clinical effect of different barrier techniques, bone substitutes, enamel matrix derivatives and one growth factors containing adjuvant used in various combinations on the healing of severe periodontal intrabony impairments. Synthetic, xenogeneic and autologous materials were used in this randomized clinical studies. Mechanical barriers (polytetrafluoroethylene and collagen membranes) for GTR, biological barriers/enamel matrix proteins (EMD), synthetic (β-TCP) and xenogeneic (NBM) bone grafts and autologous platelet-rich plasma (PRP) were combined in the test and control groups of the trials. Main clinical variable was the clinical attachment level (CAL) and subsidiary the probing pocket depth (PPD), estimated at baseline and after one year.

The summation of the results after the statistical analysis takes cognizance of the following: (1) each of the eleven regenerative methods evaluated (ten combined procedures) leads to significant CAL gain and PPD decrease; (2) using β-TCP or NBM with EMD or with PRP + GTR and GTR's, the differences between the parameters of the test and control groups were not statistically significant; (3) in four studies was confirmed that the addition of PRP to graft materials has not increased significantly the positive outcomes independent from type of barrier or graft; (4) adding platelet-rich plasma to natural bone mineral no benefit was observed from the point of view of the clinical variables; (5) the polypeptide proteins of the platelet-rich plasma does not enhance the clinical regenerative effect of enamel matrix proteins.

In conclusion, the option of the periodontal surgeon between these methods depends mainly on the defect morphology, the patient's approach to the different types of materials, the medical concept of the physician, technical possibilities and the clinical experience of the periodontist.

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GÁBOR FÁBIÁN (2008)

Reasons, prevention and treatment possibilities of dental fear with special regards to the orthodontic and pedodontic practice

Supervisor: Pál Fejérdy

Our results indicated increased dental fear scores of adults and children in- and outside the borders of Hungary compared to West European and North American values. Furthermore in the case of adults the structure of dental fear seems to be similar to that of odontophobics. Gender, age, level of anxiety, and marital status (adults only) significantly influenced the dental fear scores of both adults and children. Beside these, the dental fear levels of the surrounding people, the contact with parents (especially with the mother), and the level of acceptance, affection and love towards the children in their families are also highly important factors related to dental fear. Quality of the dentist, perfect communication, pain-free treatment, and avoidance of traumatising, rough, fearful and defective dental treatments are also important influencing factors. The decrease of time pressure of dental treatment are needed to optimise the above treatment-related factors. In some cases there are deeper psychological factors as a cause of dental fear, such as regressive phenomena and the conflict of transitoriness of humans.

Prevention should be based on the elimination of the predisposing factors, and optimising the dental treatment based on the above. Maintenance of oral health, screening and recognition and special care of risk patients are also crucial. For screening several scales and surveys translated into Hungarian (DAS, DFS), introduced (background) and investigated (DBS) in our studies are useful instruments. For special care of risk patients hypnotherapy is a possibly good tool. The most important target populations are the ones below 16 years, because their attitude toward dentistry is still under maturation. But other populations should also be taken into consideration.

In the therapy the detailed diagnosis is crucial. For this purposes the instruments mentioned above (DAS, DFS, background and DBS) are also highly useful. Based on appropriate diagnosis, hypnotherapy is a good tool for treatment also in case of high dental fear values. But the deeper psychological relations of dental fear should be taken into consideration during treatment. Because of this, dentists need not only practical knowledge of

hypnotherapy but a high level of related psychological knowledge and consultation with other professionals as well.

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UMBERTO GARAGIOLA (2007)

Biointegration of bone grafting materials and osseointegrated implants in oral and maxillofacial surgery

Supervisor: György Szabó

Two different studies have been performed. The objective of the former was to determine whether donor site morbidity could be avoided by using pure-phase β-tricalcium phosphate (β-TCP). Bilateral sinus grafting was performed on 20 selected patients; β-TCP was used on the experimental side, and autogenous bone was used on the control side. In each patient, one side was randomly designated the experimental side. In 10 of the 20 patients, the maxilla reconstruction included sinus grafting and onlay bone grafting. Implants were placed 6 months after the procedure. Eighty bone biopsy specimens were taken at the time of implant placement. Histologically and histomorphometrically, there was no significant difference ($p=0.25$) between the experimental and control grafts in terms of the quantity and rate of ossification. Comparisons with other studies reveal that β-TCP is a satisfactory graft material, even without autogenous bone. The objective of the latter was to verify if it is possible to use osseointegrated implants in Ectodermal Dysplasia Syndrome (EDS) patients. Dental and surgical-implantological treatment for EDS patients may be very complicated. Guided Bone Regeneration (GBR) membrane technique associated to bone grafting was used to facilitate placement of osseointegrated implants in a guided prosthetically position. Two groups with the same bony anatomical features were assessed. The first consisted of 13 Ectodermal Dysplasia patients, where 66 implants together bone grafts and membranes were inserted. In the second control group 120 implants with GBR were placed in 20 patients. The implants were controlled at second stage surgery, and at a follow-up of a 1 year, 2 and 3 years functional loading period. The results showed no statistically significant difference in the osseointegration rate between the two groups. Despite anatomical defects associated with decreased occlusal vertical dimension and the diminished edentulous alveolar ridges, both in height and in width, osseointegrated implants, together with GBR and bone grafts may be successfully used in EDS.

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SZABOLCS GYULAI-GAÁL (2008)

Dento-alveolar surgical treatment of dental disorders and bone defects

Supervisor: Zsuzsanna Suba

Disorders of the teeth and jaws may cause many problems in the practical dentistry. The aim of our work was to examine the therapeutic possibilities of dentition anomalies (hypodontia, hyperodontia, retention) and jaw disorders. Clinical examination of bone regeneration stimulated by graft insertion was performed on experimental animals (Beagle dogs). The qualitative and quantitative changes were evaluated by histologic and histomorphometric methods.

2750 patients were included into the study with variable dentition anomalies of which impacted wisdom and retention of the upper canine were the most frequent. In 365 cases jaw cysts were surgically treated. The most frequent surgery was cystectomy, whereas marsupialisation and cystostomy were rare. Small sized odontogenic tumors (predominantly odontomas) occurred in 33 cases. In 27 odontoma cases tooth eruption anomalies were the complications. In edentulous patients elevation of the maxillary sinus base was performed in 31 cases by Cerasorb and BioOss graft insertion. In the majority of cases one stage technique was applied. However in several patients the bone replacement and the implant insertion was performed in two steps. In the second step at the place of implantation there was a possibility to gain a bone cylinder for histological examination.

In Beagle dogs the effect of Cerasorb and combined Platelet Rich Plasma (PRP) + Cerasorb mixture was experimentally examined during the healing of extraction wounds. Histological and histomorphometrical results supported that in the early phase (6 weeks) the PRP promoted a quicker bone regeneration. However, later the effect of the two types of treatment equalized.

Our results support that dental surgery has a very important role in the cosmetical and functional reconstruction of dental and jaw defects.

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ZSOLT NÉMETH (2006)

The evaluation of the effectiveness of chemoterapy in oral cancer patients

Supervisor: Zsuzsanna Suba

The mortality rates of oral cancer of Hungarian men and women are by far the worst in Europe. Apart from their self-destructive way of life, seeing a doctor at a late stage also plays a part in this outstandingly high mortality rate.
After the initial diagnosis—in planning the treatment—one can mainly rely on clinical tumor parameters (TNM). Treatment based on UICC staging, however, is not tailored to the individual thus some patients are often under-treated whereas others are over-treated. Thus there has been a demand to find independent prognostic and predictive factors which would enable the clinician to set a more precise course for treatment, more specific for the type of tumor the patient suffers from.
As a result of research it is known already that the decisive factor is not the number, position or size of metastatic lymph nodes, but the invasion of the capsule. Similarly it can be considered as an established fact that the vertical dimension, the depth of invasion of the primary tumor is a lot more important than its size on the surface. Apart from classical factors (TNM, localisation, grade, stage) other factors like capillary density, DNA content of tumor cells, the expression of certain extracellular matrix proteins and a number of other molecular pathological properties have an important role in setting up a prognosis in international practice.
In the present investigations patients were evaluated partly in randomized prospective, partly in retrospective studies.
It has been proven that the survival of patients responding with significant tumour regression to chemotherapy significantly exceeded that of patients only partially responding to therapy.
After analysing the data of a large number of patients who underwent combined anticancer therapy at our Department it has been noted that the best results are obtained with the combined treatment of operable tumors, that is, in cases where the operation is followed by chemotherapy or radiotherapy.
Upon investigating ECM proteins that play an important role in tumor progression and metastasis formation it has been found that there was a positive correlation between the expression of syndecan-1 and laminin-5 and tumor specific survival. Thus, these two factors are of a prognostic value. The changes in the expression of these two proteins during neoadjuvant chemotherapy were also studied. It was found that if their expression increased during chemotherapy that had a favourable effect on survival, thus it is an important predictive factor.
The present studies have found that the decrease in the expression of *surface* laminin-5 and syndecan-1 suggests a bad prognosis. It has also been proved in the present studies that an increase in *stromal* syndecan-1 expression is associated with quite bad survival rates. High levels of the proteinases (MMPs) that have a role in dismantling the stroma surrounding the tumor meant a bad prognosis in the present studies as well. It was also investigated whether the level of these proteins changed as a result of chemotherapy and whether this changed expression had any effect on survival or not. It was seen that in the case of MMP-2 the low levels measured before chemotherapy had a significant positive

effect on survival. This, however, could not be observed in the case of MMP-9. At the same time decreasing MMP-9 levels during chemotherapy were associated with a significantly better survival.
Finally, the ploidy of the tumor, the S-phase fraction and the ratio of the polyploid fraction were determined before and after neoadjuvant chemotherapy. In the present studies only the decrease of the S-phase fraction of all these parameters proved to have a significant positive correlation with better survival.
Upon reviewing the results of the present studies the conclusion can be drawn that predictive factors are more important in treatment planning and setting up a prognosis than "traditional" prognostic factors.

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MÁRTA RADNAI (2006)

Periodontitis and preterm birth. Oral health status of pregnant women in South-East Hungary

Supervisor: István Gera

A case control study was undertaken to detect if initial localized chronic periodontitis could be a risk factor for preterm birth and fetal growth restriction. The aim of the study was also to assess the overall health status, including caries and periodontal status, and microbial flora of gingival crevice, of pregnant women in South-East Hungary, and to reveal the effect of demographic and socioeconomic status on this state.
Material and methods: Healthy, otherwise non-selected postpartum women were included into the study. A preterm birth (PB) case was defined if a patient had a threatening premature event during pregnancy, preterm premature rupture of membranes, or spontaneous preterm delivery, before the 37th week of pregnancy, and/or the weight of the newborn was less than 2500 g. Into the case group, 77 women were allocated, while 84 went to the control group, all of whom had delivery after the 37th gestational week and with a newborn weighing 2500 g or more. Demographic, socioeconomic status and known risk factors, like smoking, alcohol consumption were recorded. Dental examinations were performed in accordance with the WHO guidelines in the Department of Obstetrics and Gynecology, University Szeged. Microbiological samples were taken from the three deepest pockets, and culturing according to appropriate protocol.
Results: A significant association was found between PB and initial localized chronic periodontitis, the criteria being bleeding at 50% or more of the examined sites and having at least at one site at a probing depth of 4 mm or more ($p<0.001$). The adjusted odds ratio

for initial localized chronic periodontitis was 3.3157, 95% CI: 1.6418–6.6963. The average weight of newborns of mothers with periodontitis was significantly less than in the women without periodontitis (p=0.002). The DMFT index for the women examined was 12.45. Parameters of the periodontal status were the follows: mean plaque index 0.67, frequency of calculus 21.07%, mean probing depth 1.67 mm and frequency of bleeding on probing 37.80%. Caries and periodontal status were influenced by demographic and socioeconomic status. Microbial findings showed a significant difference between the case and control group.

Conclusions: The results support the hypothesis that initial localized chronic periodontitis of pregnant women could lead to adverse pregnancy outcome and birth weight reduction.

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CSONGOR SUBA (2007)

Corrosion and surface analytical studies of anodically and thermically surface treated Ti/TiO_2 osteosynthesis plates

Supervisor: György Szabó

For the fixation of mandibular fractures, at the Department of Oral Surgery and Dentistry at Semmelweis University, anodically and thermically treated Ti/TiO_2 osteosynthesis plates are used.

The aim of the work was the study of the electrochemical reactions which may occur on the surface of the anodically and thermically treated Ti/TiO_2 osteosynthesis plates under the aggressive condition of the body.

The surface structures of the Ti/TiO_2 implants were analyzed by X-Ray diffraction, optical microscopy and SEM methods. Electrochemical studies were performed on the samples of anodically and thermally treated Ti/TiO_2 implants that were manufactured or removed from patients after various periods of use. These studies involved examination of the Ti discs and Ti/TiO_2 implants, by means of corrosion potential measurements, cyclic voltammetric measurements and electrochemical impedance spectroscopy. The electrochemical results were statistically analyzed with Origin 6.1 program (1991–2000 OriginLab Corporation).

The examination showed that the Ti/TiO_2 implant is polycrystalline material made of the anatase polymorph. The anodically and thermically treated Ti/TiO_2 implants are corrosion resistant, electrochemically stable and behave as inert electrodes. The cyclic voltammetric measurements demonstrated that the TiO_2 layer forms a highly protective barrier on the surface of Ti.

The author worked out a new method for tracking the minor local deteriorations in the oxide layer which may appear on the surface of the osteosynthesis plates.

Cyclic voltammetric and electrochemical impedance measurements point towards that local deterioration are more probably due to the mechanical damages caused during the surgeries. These sites were characterized by a continuous reformation and healing during cyclization. On the SEM images precipitations are visible on the surface of the Ti/TiO_2 implants. This demonstrates the spontaneous formation of a phosphate and hydro-phosphate (apatite) layer in the living organism and the strong connection between the implant and the body. The surface layer is covered with discrete precipitates and this layer can cover or fill up the pores opened up in body fluid therefore ensuring an excellent protective ability to the implant.

It was found that the results of the statistical estimation of the differently treated implants were relevant.

The reported techniques appear to comprise a reliable method of study for an evaluation of the long-term corrosion behaviour of implants.

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- *Suba C, Velich N, Turi C, Szabo G (2004) Surface analysis methods of biomaterials used in oral surgery. Literature review. J Craniofac Surg 15: 1–6.*
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NORBERT VELICH (2006)

Surface modification of titanium osteosynthesis plates by anodic and thermal oxidation and its significance in implant-organism interaction

Supervisor: György Szabó

Undesirable interactions with material transport may occur between an organism and the titanium osteosynthesis plates used for the fixation of bone fractures.

The author studied the changes in this interaction between the body and implants caused by the surface modification of the plates by anodic oxidation. Clinically detectable tissue damage and clinical signs caused by the implants were investigated by a retrospective clinical study, the roughness of the surface layer of implants was studied with cross-sectional microscopy and the surface structure and material composition and the changes thereof were studied with methods of surface analysis.

On the basis of the retrospective clinical study it was concluded that plates with a surface treated by anodic and thermal oxidation did not cause clinically detectable tissue damage (metallosis) and these plates were less frequently removed than other osteosynthesis plates as compared to international literary data.

The microscopic investigation proved that the surface provided by anodic and thermal oxidation covers the implant as a continuous layer.

Based on studies using methods of surface analysis it was concluded that the oxide layer produced by anodic oxidation is in the form of TiO_2 and it does not change during interaction with the organism. The ratios of pollutants in the superficial oxide layer were

determined and the changes of the same during interaction with the organism. The amount of possible allergens in the superficial oxide layer was found to be significantly less than on the surface of the basic metal. It was established that the original oxide layer is 120–150 nm thick, but the changes in the thickness could not be measured with the extremely sensitive methods of surface analysis.
Finally it was concluded that surface modification by anodic and thermal oxidation significantly reduces the undesirable interactions between implant and the human organism.

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PROGRAM 2/6.

CLINICAL HAEMATOLOGY

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Program overview

Studies about the prognostic factors and complex therapies of haematologic disorders can contribute to more efficient treatment of this patient group. The role of infectious agents and environmental factors in the etiology of malignant lymphomas is a very interesting and up to date field of research (e.g. post-transplantation. We will study the diagnostic use of the methods of modern molecular biology and its efficiency in the follow-up control of our patients, too. The pathogenesis of the thrombosis in malignancies and the frequency of the cytokine gene-polymorphism and p53 mutations and their therapeutic importance. The pathophysiological bases of the plasma cell-dyscrasies and their therapeutic implications are also part of our research. The bone marrow transplantation was a breakthrough in the therapy of the malignant diseases. The hemopoietic stem cell transplantation can be a model of the immunotherapy, and it may be studied well. The connection of the specific immunological state after transplantation with the complications of the transplantation, with the relapse of the disease and with the survival is still subject of investigation even nowadays. The course of the development of the immunological tolerance after allogen transplantation is still unknown either. The investigation of the haematologic disorders can be the subject of the research, which belongs to the scientific bases of the public health priorities because of the high frequency of oncohaematological disorders.

Titles of research projects	*Supervisors*
Role of cytokine DNA polymorphisms in the pathogenesis of malignant haematological diseases	Judit Demeter
Myeloprolferative disorders and their familial aspects	Judit Demeter
Immunohaematological aspects of malignant lymphomas	Judit Demeter
Role of growth factors and their receptors in the regulation of the development and destruction of lymphoma cells	László Kopper
Possibilities of individualized therapy in pediatric malignancies	Gábor Kovács
The possible role of neutrophil granulocytes in thrombosis associated to neoplastic diseases	Raymund Machovich
Study of liver injury following allogenic and autologous bone marrow transplantation: Role of toxic and immunological factors	Tamás Masszi
Human stem cell-membrane transport proteins and their alterations during cell differentiation	Balázs Sarkadi
Study of structure-function linkage in human ABC membrane transport proteins	Balázs Sarkadi
Role of growth factors and their receptors in the regulation of the development and destruction of lymphoma cells	Anna Sebestyén
Role of infectious agents and environmental factors in malignant lymphoid tumors	Lídia Sréter
Role mesenchymal stem cells in the regulation of immune processes	Ferenc Uher
Mesenchymal stem cells and regenerative medicine-stem cell therapy in type 1 diabetes	Ferenc Uher

Ph. D. students		*Supervisors*
Karolina Nemes	ft	Mónika Csóka
Márta Zubreczki-Hegyi	ft	Gábor Kovács
Adrienn Mohl	ft	Tamás Masszi

Ph. D. candidates		*Supervisors*
Valéria Dudics	pt	Ferenc Uher
Zoltán Gasztonyi	pt	Lídia Sréter
Krisztina Heltai	i	István Vályi Nagy
Veronika Urbán	pt	Ferenc Uher

Ph. D. graduate		*Supervisor*
Virág Vas	ft	Katalin Pálóczi

pt, part-time; ft, full-time; na, i, individual

Abstract of Ph. D. thesis successfully defended in 2006

VIRÁG VAS (2006)

Apoptosis and a characteristic marker (CD 138) in multiple myeloma

Supervisor: Katalin Pálóczi

Stem cells reside in customizing microenvironments (niches) that contribute to their unique ability to divide asymmetrically to give rise to an identical and to a daughter cell with distinct properties. We examined two potential stem cell fate regulator factors: the Notch ligand Jagged-1 and an animal lectin, the Galectin-1.

We found that recombinant Jagged-1 immobilization on stromal layer or on Sepharose-4B beads are required for the induction of self-renewing divisions of early haematopoietic stem cells, tested *in vitro* in cobblestone area-forming cell assay and *in vivo* in competitive repopulation assay. The free, soluble Jagged-1, however, has a dominant negative effect on self-renewal in the stem cell compartment. In contrast, free as well as immobilized Jagged-1 promote growth factor-induced colony formation of committed haematopoietic progenitor cells. Therefore, we propose that differences in Jagged-1 presentation and developmental stage of the Notch receptor-bearing cells influence Notch ligand binding results towards activation or inhibition of downstream signaling. Moreover, these results suggest potential clinical use of recombinant Notch ligands for expanding human haematopoietic stem cell populations *in vitro*.

The presence of gal-1 in the bone marrow has been detected but its role in the regulation of haematopoiesis is unknown. In the present study, we have shown that low amount of Galectin-1 increases the formation of granulocyte-macrophage and erythroid colonies on a lactose-inhibitable fashion. In contrast, high amount of gal-1 dramatically reduces the growth of the committed blood-forming progenitor cells as well as the much younger Lin- cells and induces apoptosis, in a fashion that is independent of the lectin property. The apoptotic effect of gal-1, however, depends on the maturation states of the cells, because the most differentiated cells are the most sensitive and the stem cells are the less sensitive for the growth inhibitory activity of the lectin. The gal-1 acts in a biphasic manner on the bone marrow cells depending on its concentration.

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PROGRAM 2/7.

CLINICAL ENDOCRINOLOGY AND ITS EXPERIMENTAL ASPECT

Coordinator:
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Program overview

This doctoral program is aimed at the investigation of various disorders of the endocrine system, further the role of hormones in cardiac diseases and in the pathogenesis of osteoporosis. Ph. D. students interested in these subjects are welcome to this programme, where clinical investigation as well as basic science research is possible.

Ph. D. graduates		*Supervisors*
Orsolya Dohán	i	Miklós Tóth
Szilvia Mészáros	ft	Csaba Horváth
Ildikó Pósa	i	Miklós Tóth
Balázs Sármán	ft	Miklós Tóth
Réka Skoumal	ft	Miklós Tóth

ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006 and 2007

ORSOLYA DOHÁN (2007)

Molecular characterization of an iodine transport defect (ITD) caused by a Na^+/I^- symporter (NIS) mutation (G395R), and immunohistochemical analysis of NIS protein expression in thyroid cancer

Supervisor: Miklós Tóth

The Na^+/I^- symporter (NIS) is a plasma membrane glycoprotein that mediates active iodide uptake in the thyroid—the essential first step in thyroid hormone biosynthesis—and in other tissues, such as stomach, salivary and lactating mammary glands. NIS plays key roles in thyroid pathophysiology as the route by which I^- reaches the gland for thyroid hormone biosynthesis, and as a means for diagnostic imaging and for radioiodide therapy in thyroid cancer.

We investigated at the molecular level two conditions with absent or diminished thyroidal I^- uptake: (1) a NIS mutation (G395R) causing congenital iodide transport defect resulting hypothyroidism; (2) differentiated thyroid cancer.

(1) Several NIS mutations have been shown to cause I^- transport defect (ITD), a condition that, if untreated, can lead to congenital hypothyroidism and ultimately cretinism.
The study of ITD-causing NIS mutations provides valuable insights into the structure-function and mechanistic properties of NIS. Here we report the thorough analysis of the G395R NIS mutation. We observed no I^- uptake activity at saturating or even supersaturating external I^- concentrations in COS cells transiently transfected with G395R NIS cDNA, even though we demonstrated normal expression of G395R NIS and proper targeting to the plasma membrane. Several amino acid substitutions at position 395 showed that the presence of an uncharged amino acid residue with a small side-chain at position 395 is required for NIS function, suggesting that glycine 395 is located in a tightly packed region of NIS. Substitutions of large amino acid residues at position 395 resulted in lower Vmax without affecting Km values for I^- and Na^+, suggesting that these residues hamper the Na^+/I- coupling reaction.
(2) We analyzed the Na^+/I^- symporter (NIS) protein expression in 57 thyroid cancer samples by immunohistochemistry with high-affinity anti-NIS Abs. As many as 70% of these samples exhibited increased NIS expression with respect to the normal surrounding thyroid tissue. Most significantly, NIS was located in these samples either in both the plasma membrane and intracellular compartments simultaneously, or exclusively in intracellular compartments. This suggests that NIS is clearly expressed or even overexpressed in most thyroid cancer cells, but malignant transformation in some of these 5 cells interferes either with the proper targeting of NIS to the plasma membrane, or with the mechanisms that retain NIS in the plasma membrane after it has been targeted. The results further indicate that, in addition to inducing NIS expression in cases where it is absent (~30%), improvements in 131I radioablation therapy might result from promoting targeting of NIS to the plasma membrane in the majority (~70%) of thyroid cancers.

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SZILVIA MÉSZÁROS (2007)

Comparison of quantitative bone ultrasound and osteodensitometry in different metabolic osteopathies

Supervisor: Csaba Horváth

The research has targeted the assessment of bone quality. Our work focused on bone quantitative ultrasound. Methodical researches and clinical evaluations and assays were done. The thesis analyzes the data of almost 5900 patients. Our results allowed to conclude several important statements, which could affect the everyday practice. For example diagnostic thresholds were assigned, based on fracture risk, which enabled the use of several quantitative ultrasound devices. The methodical researches support the usefulness of the

method following patients. Knowing the coefficient of variations the least significant changes were defined and calculated for each device.
We assessed that quantitative bone ultrasound is able to discriminate between subjects with normal, decreased and extremely decreased bone density, therefore the method is appropriate to diagnose osteoporosis according to the WHO classification, based on bone mineral density. We confirmed among Hungarian patients that quantitative bone ultrasound at the heel is able to discriminate between fractured (at the limb) and non-fractured osteoporotic patients, independently from bone mineral density.
Our results suggest that different pathological changes are developed in bone tissue in secondary osteoporosis and involutionry osteoporosis. Not only bone loss, but also deteriorate quality of bone tissue dominate the picture in the latter mentioned diseases. However, in the previously mentioned disorders bone ultrasound have not shown apparent results. In our researches we have examined the attenuation and speed of ultrasound in different metabolic bone disorders. Our results confirm the hypothesis that BUA primarily associates with bone density, and speed of ultrasound reflects bone strength, which is an important parameter determining bone fragility. No other methods are available on measuring bone strength *in vivo*, therefore our observations have clinical consequences.

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ILDIKÓ PÓSA (2006)

Alterations in the physiology of the circulation and in cardiac action in experimental diabetes

Supervisor: Miklós Tóth

Diabetes is a disease with high morbidity/mortality in these days. Main cause of death is some of the late complications developing in the cardiovascular system in many cases. Diabetes itself can also induce considerable morphological/functional alterations. The severity of these changes may further be influenced by the compounds used for the treatment of the disease. The aim of the present study was therefore to investigate potential cardiovascular side-effects of sulphonylurea compounds—applied in the treatment of type 2 diabetes—in experimental diabetes, in different animal models. The objective of further examinations was the enhanced arrhythmogenity characterizing diabetes: arrhythmia-provoking effect of endothelin-1 was analysed in diabetic and locally hyperglycaemic dog hearts. Our results draw attention to the differences existing between individual sulphonylureas and between their two generations concerning haemodynamic and

metabolic effects, their arrhythmogenity, and to the variations in drug effects that should necessarily be considered when medication is selected. Regarding their haemodynamic effects, low daily dose compounds show similar influence, appearing more moderately in case of glipizide. With respect to pyruvate/lactate metabolism—characterizing myocardial nutrient and oxygen-supply—, glibenclamide was the one showing effects becoming more beneficial in diabetes. Thus medication of diabetics should be determined individually in view of their metabolic and cardiovascular state. If simultaneous digitalis treatment is required, application of low daily dose sulphonylureas is advisable in blood glucose control. During ischaemia application of glibenclamide, during reperfusion that of glimepiride is recommended. In medication of diabetics with cardiopathic complications use of high daily dose compounds should be avoided as far as possible; their incidental use requires great caution. Investigation of the effects of endothelin-1 refer to the fact that in the course of treatment counteracting not only hyperglycaemia, but also that of hyperinsulinaemia—and those of other metabolic alterations—also should be attempted to moderate the elevated constrictive and arrhythmogenic tendency of the cardiovascular system. Finally, the differences observed in our experiments between the effects of the same compound in metabolically healthy and in diabetic or locally hyperglycaemic state accentuate the necessity of the accomplishment of studies concerning diabetes treatment also in diabetic state.

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BALÁZS SÁRMÁN (2007)

Role of transcription factors in the pathomechanism of cardiac hypertrophy

Supervisor: Miklós Tóth

The present work underlines important roles of nuclear transcription factors in the remodeling process *in vitro* and *in vivo*. In detail, the findings of our research are summarized as follows.

The transcription factor nuclear factor-κB (NF-κB) has been implicated in cardiomyocyte hypertrophy *in vitro* as well as *in vivo*. However, it is unknown if activation of NF-κB plays a mandatory role in the hypertrophic process. Here we characterized the importance of NF-κB signaling in moderate and severe left ventricular (LV) hypertrophy in rats with chronic pressure overload induced by angiotensin II (Ang II) infusion.

Administration of high dose of Ang II resulted significant increase in NF-κB activation. PDTC, a specific inhibitor of NF-κB, diminished Ang II induced LVW/BW ratio and growth of cardiomyocyte cross-sectional area, without compromising LV systolic function. Moreover, PDTC abolished Ang II-induced cardiomyocyte apoptosis and interstitial fibrosis. Lower dose of Ang II caused significant, but moderate LVH, however it did not accompanied by increased NF-κB /DNA binding activity.

In summary, our work demonstrates that NF-κB signaling is affected in the cardiac remodeling process *in vivo*, however, it is not involved in all stages of progression of maladaptive LVH. While NF-κB activation may play a role in the advanced phase of remodeling, development of moderate LVH is not dependent on NF-κB signaling. Moreover, these data support the hypothesis that reducing LVH does not necessarily compromise the capability of the heart to adapt to an increase in demands.

The signaling cascades that activate transcription factors during cardiac hypertrophy are largely unknown. Our aim was to examine the role of mitogen-activated protein kinases (p38 kinase, extracellular signal-regulated protein kinase, and c-Jun N-terminal protein kinase) in the left ventricular wall stress-induced activation of GATA-4 DNA binding in adult heart.

Isolated perfused rat hearts were exposed to elevated wall stretch by inflating a left ventricular balloon. Gel mobility shift assays were used to analyze the transacting factors that interact with the GATA-4 motifs of the B-type natriuretic peptide (BNP) promoter. Direct wall stretch produced rapid, significant increased the levels of phosphorylated p38 kinase, extracellular signal-regulated protein kinase, and c-Jun N-terminal protein kinase. The wall stress-induced GATA-4 activation in the left ventricle and atria was abolished both p38 and ERK1/2 upstream kinase MEK1/2 inhibitor. In contrast, the inhibition of c-Jun N-terminal protein kinase had no effect on the baseline or stretch-induced GATA-4 DNA binding.

In conclusion the present study demonstrates that both p38 and extracellular signal regulated protein kinase are required for the stretch induced GATA-4 binding in intact heart. Finally, it seems that the activation of GATA-4 DNA binding by increased wall stretch is dependent on Rho kinase but not protein kinase C.

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RÉKA SKOUMAL (2007)

Role of vasoactive factors in animal models of pressure and volume overload induced by hypertrophy

Supervisor: Miklós Tóth

Clinical studies have shown that the presence of left ventricular hypertrophy is the most powerful predictor for the development of heart failure, therefore exploring the signaling mechanisms that stimulate myocytes growth is crucial to reduce the incidence of heart diseases. Several lines of evidence have suggested that mechanical stress and neurohumoral factors promote cardiac hypertrophy in concert. However, it is uncertain if the hypertrophic effects of norepinephrine (NE) and angiotensin II (Ang II) are direct or require additional factors. Endogenous ouabain-like compound (OLC)—a Na^+/K^+-ATPase inhibitor—has been shown to trigger hypertrophic growth in cell culture; however, the significance in the hypertrophic process *in vivo* is unknown. Here we characterized the in-

volvement of OLC in left ventricular (LV) hypertrophy induced by NE and Ang II infusions in rats. Administration of NE or Ang II resulted in an increase in left ventricular weight to body weight (LVW/BW) ratio and in ANP gene expression. Adrenalectomy reduced both basal and norepinephrine-induced increase in plasma OLC levels. LVW/BW ratio was not modulated by adrenalectomy; however, ANP expression was blunted. Administration of exogenous ouabain further enhanced ANP gene expression induced by phenylephrine in cultured neonatal rat ventricular myocytes. These data show that OLC as an adrenal-derived factor may be required for the induction of left ventricular ANP gene expression during the hypertrophic process.

The prevalence of cardiovascular diseases related to obesity and dyslipidemia has reached epidemic levels in industrialized countries. Despite the magnitude of the problem, the pathogenesis of myocardial dysfunction in obesity is not well understood. Long-term dietary fatty acid intake alters the development of LV hypertrophy, but the signaling processes linking cardiac hypertrophy and hyperlipidemia are obscure. We studied here the role and the underlying signaling mechanisms of dietary fat intake in the early phase of hypertrophic process. Our results show that dietary fat type modulates the early activation of hypertrophic genes in pressure overloaded myocardium involving distinct activation of activator protein-1 as well as mitogen activated protein kinase signal transduction pathways. Since the intake of saturated fats and plant seed n-6 polyunsaturated fatty acids has been increased in Western-type diets, our results suggest that attenuation of the progression of associated cardiac hypertrophy is likely to require the alteration of dietary fat profile.

- *Skoumal R, Szokodi I, Aro J, Földes G, Göőz M, Seres L, Sármán B, Lakó-Futó Z, Papp L, Vuolteenaho O, Leppaluoto J, deChâtel R, Ruskoaho H Tóth M (2007) Involvement of endogenous ouabain-like compound in the cardiac hypertrophic process in vivo. Life Sci 80: 1303–1310.*
- *Foldes G, Vajda S, Lako-Futo Z, Sarman B, Skoumal R, Ilves M, deChatel R, Karadi I, Toth M, Ruskoaho H, Lepran I (2006) Distinct modulation of angiotensin II-induced early left ventricular hypertrophic gene programming by dietary fat type. J Lipid Res 47: 1219–1226.*

PROGRAM 2/8.

PHYSIOLOGY AND PATHOLOGY OF THE MUSCULO-SKELETAL SYSTEM

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Program overview

The Ph. D. Program is designed for medical doctors who wish to be specialized in basic science and clinical research of muscoloskeletal medicine, orthopaedics, trauma surgery and rheumatology. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of orthopaedics and trauma surgery, and rheumatology, and surgery of the bone and soft tissue tumors, to provide suitable environment for clinical or biomechanical laboratory based research projects, to enable students for the use of laboratory techniques such as classical histology, immunohistochemistry, collagen typisation and to train students in modern biomechanical laboratory techniques, as gait analysis.

Titles of research projects	*Supervisors*
Articular sport injuries of the knee	István Berkes
Development of assistive products for persons with mobility impairments	Péter Cserháti
Measurement in rehabilitation medicine	Zoltán Dénes
Application of high technology in rehabilitation	Gábor Fazekas
Role of psychosocial factors in the rehabilitation of patients with bone fracture due to osteoporosis	Gábor Fazekas
Clinical and experimental examination of treatment options in injuries of the articular loaded surface	László Hangody
Effect of orthopedic abnormalities and injuries on movement. Orthopedic movement analysis	Rita Kis
Importance of secondary conditions in the physical and rehabilitation medicine process	Lajos Kullmann
Infection and autoimmunity in inflammatory diseases of the joints	Gyula Poór
Examination of synovial sarcoma SYT-SSX fusion gene products in tissue cultures and xenografts	Zoltán Sápi
Clinical oncology in bone and soft tissue tumors	Miklós Szendrői
Non-surgical treatments of juvenile and aneurysmal bone cysts: Sclerotization, cyst modelling and examination of cyst remodelling	Miklós Szendrői
Quality of life after the complex therapy of bone tumors	Miklós Szendrői
Occurrence and treatment (minimal surgical interventions) of bone metastases	Miklós Szendrői
Recidival tumor forming ability, malignization in borderline bone tumors	Miklós Szendrői

Examination of prognostic factors in certain bone tumors	Miklós Szendrői
Replacement options in extensive bone defects, comparative study of massive osteochondral homografts, autografts, endoporthesis	Miklós Szendrői
Biomechanical effect of percutaneous vertebroplasty in osteoporotic vertebral fractures on the neighbouring vertebrae: clinical and laboratory investigations	István Szikora
Histological and kinetic alterations in diseases and developmental disorders of the locomotor apparatus	György Szőke

Ph. D. students		***Supervisors***
Kristóf Andrónyi	ft	Gábor Krakovits
István Baráth	pt (a)	Tibor Mózes
Szabolcs Benis	pt (a)	Tamás Farkas
Sándor Berki	pt	Tibor Vízkelety
István Béla Bors	pt	Rita M. Kiss
Dóra Mihola (Dombayné)	pt	Gyula Poór
Gyula Domos	ft	György Szőke
Zsuzsa Fekete	pt	Gyula Poór
Ágnes Finczicki	ft	Géza Bálint
László Fónyad	pt	Zoltán Sápi
Dénes Handl	pt	György Szőke
László Rudolf Hangody	ft	György Szőke
Lőrinc Ijjas	ft	György Szőke
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Zsolt Mihály Kulcsár	pt (a)	István Szikora
Zsuzsa Márialigeti	pt	Géza Bálint
Dorottya Judit Nagy	ft	Gyula Poór
Lívia Nagy	pt	Lajos Kullmann
Zoltán Oláh	pt	Miklós Szendrői
János Rupnik	pt	Antal Renner
György Tibay	pt	László Hangody
György Pál Végh	pt	János Hamar
Szilvia Zsilák	pt (a)	Gyula Poór

Ph. D. candidates		***Supervisors***
Lajos Bartha	pt	László Hangody
Zoltán Bejek	pt	Miklós Szendrői
János Bihari	ft	János Hamar
István Flóris	pt	Tamás Farkas
István József Kádas	pt	Zoltán Magyari
Ákos Péter Kynsburg	ft	Tivadar Tulassay
Béla Matthias Lieb	pt	János Hamar
András Melly	pt	Tamás Farkas
Károly Pap	ft	György Szőke
Borbála Pazár	pt	Gyula Poór
Judit Ildikó Pulai	i	Gyula Poór
Zsolt Vendégh	pt	János Hamar

Ph. D. graduates		*Supervisors*
Gayane Badalianvery	i	Miklós Szendrői
Zoltán Dénes	i	Lajos Kullmann
Gábor Fazekas	i	Lajos Kullmann
Anett Hruska	pt	Zoltán Sápi
Géza Kordás	i	László Hangody
Tamás Shisha	ft	György Szőke

a, absolutorium; pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

GAYANE BADALIANVERY (2008)

Phenotype/genotype of malignant tumor sin bone metastasis

Supervisor: Miklós Szendrői

Gene expression profile of metastatic lesions (bone metastasis) vs. primary lesions was the goal of our investigations. EGFR and VEGFR-2 protein expressions as well as K-RAS mutations were studied in samples derived from bone metastatic lesions parallel to their primary tumours, which were derived from RCC, BRC and NSCLC.

EGFR and VEGFR2 protein expressions are hallmarks of clear cell renal cancer (RCC) with questionable prognostic impact. Our studies demonstrated that the EGFR protein scores were significantly reduced in bone metastasis of RCC due to reduction of EGFR protein expression. The VEGFR2 protein-positive phenotype of clear cell RCC was relatively frequent but it was lost in small group (35%) of bone metastatic patients.

The genotype of breast cancer (BRC) is considered to be relatively stable during tumor progression, accordingly, determination of estrogen receptor and HER-2/neu status is currently based on the primary tumors. However, recent data including ours suggest that, the gene expression profile of metastatic lesions could be different compared to that of the primary BRC. The frequency of HER-2/neu in primary tumors was double as much as the metastatic ones practically half of BRC cases with HER-2 positivity lost their genotype in bone metastases whereas the HER-2/neu negative status was maintained during progression to the bones. Comparison of corresponding primary and metastatic NSCLC tissues indicated that down-regulation of EGFR was a rare event (<20%) compared to up-regulation in bone metastases. On the other hand, K-RAS mutation status of the primary tumors does not predict the status of bone metastatic tissue in NSCLC as we could detect all the possible existing models including up-regulation, down-regulation and maintenance of K-RAS.

Collectively our studies on the metastatic progression of major human cancer types suggests that determination of the phenol/genotype of the individual cancer must be repeated if matastatic tissues are available since the expression of the common molecular targets of therapies may differ form the one found in the primary tumors.

• *Badalina G, Barbai T, Raso E, Derecskei K, Szendroi M, Timar J (2007) Phenotype of bone metastases of non small cell lung cancer: Epidermal growth factor receptor expression and K-RAS mutation status. Pathol Oncology Res 13: 99–104.*

• *Lorincz T, Toth J, Badalian G, Timar J, Szendroi M (2006) HER-2/neu genotype of breast cancer may change in bone metastasis. Pathol Oncol Res 12: 149–152.*

ZOLTÁN DÉNES (2007)

Prevention, treatment and outcome of secondary complications during the rehabilitation of patients with severe brain injury

Supervisor: Lajos Kullmann

Recovery from brain injury is not only determined by the primary injury but a very important element is the development of secondary complications which have a major role determining the possibility of the achievement of available maximal functional abilities and the quality of life of the patients and their family after rehabilitation. This is why during medical treatment the prevention of secondary complications at least as important as the prevention of primary injury. Following the acute care of patients with severe brain injury most of the secondary complications are recognised and treated during rehabilitation.
After the acute care rehabilitation of patients with severe brain injury should be performed in specialised centers with multidisciplinary team for different functional deficits (physical-, cognitive-, communicational-, psycho-social impairments). Early and direct admission from neurointensive unit to the rehabilitation centrum seems to be optimal for best patient outcome (early intensive rehabilitation), because this lowers the chance for the development of secondary complications.
The most frequent complications in patients with severe brain injury at admission in our rehabilitation unit were: contractures (47%), pressure sores (35%), respiratory (14%) and urinary tract infections (11%), malnutrition (20%) and immobilization. The functional outcome was worse in the cases arriving with secondary complications during the same rehabilitation period. The length of stay in the rehabilitation unit was much longer in these cases. We strongly suggest that actions to prevent secondary complications have to start at the acute care unit. Consultation between acute care and rehabilitation professionals is necessary in order to discuss the treatment options and develop new methods to reach maximal functional recovery of patients with brain injury.

• *Dénes Z (2005) Baleset következtében súlyos agykárosodást szenvedett betegek rehabilitációjának eredményei. Orv Hetil 146: 2403–2406.*
• *Dénes Z (2004) The influence of severe malnutrition on rehabilitation in patients with severe head injury. Disability and Rehabilitation 26: 1163–1165.*
• *Dénes Z, Till A (1997) Rehabilitation of patients after hip disarticulation. Arch Orthop and Trauma Surg 116: 498–499.*

GÁBOR FAZEKAS (2008)

Application of robots at patients with paresis of the upper limb as a consequence of central motor neuron lesion for supporting physiotherapy during rehabilitation

Supervisor: Lajos Kullmann

Background-purpose: Stroke, traumatic brain injury and some other diseases often lead to spasticity, which is velocity and acceleration dependent. Exercises executing with a slow and constant velocity can be beneficial in reducing muscle hypertonia. This kind of exercises, first of all in high repetition number, can be more accurately managed by a robot than by a human. For this reason—in cooperation with engineer partners—a robot mediated physiotherapy system was developed for supporting the shoulder-elbow exercises of patients with spastic hemiparesis. **Patients and method:** An initial clinical trial was executed to gain experiences with the system. According to the experiences of this pilot study some parts of the system were modified. A controlled trial was executed using the modified system, so as to investigate whether this kind of therapy is beneficial for the patients. 30 patients with spastic hemiparesis were randomly divided into two groups, both including 15 persons. Members of both groups received the same amount of traditional physiotherapy on 20 consecutive workdays, while the experimental group received thirty-minute-long robot mediated sessions on the same days, in addition. Six motor impairment scales and two functional scales were assessed by a blinded physiotherapist. The difference in the scores between the assessments were statistically evaluated in both groups. Variables that showed a statistically significant change in both groups were also compared between the two groups. **Results:** The initial investigation proved that the robots execute the therapeutic programme safely and accurately. In the second trial the modified Ashworth score of shoulder adductors and elbow flexors showed a statistically significant improvement only in the robotic group. The improvement of the shoulder-elbow subsection of Fugl-Meyer score was significantly higher in the robotic group. As for the other parameters there was no statistically significant difference between the two groups. Subjects received altogether 150 hours of robot mediated therapy. No adverse event occurred. **Conclusion:** Supplementation of the traditional methods with this kind of robot mediated therapy can be beneficial in decreasing spasticity and improving motor impairments of the upper limb, and making rehabilitation more effective.

- *Fehér M, Stefanik Gy, Boros Zs, Tóth A (2004) Robotok alkalmazása féloldali bénultak felső végtagi gyógytornásztatásában. Orv Hetil 145: 1327–1331.*
- *Fazekas G, Horvath M, Toth A (2006) A novel robot training system designed to supplementary upper limb physiotherapy of patients with spastic hemiparesis. Int J Rehabil Res 29: 251–254.*
- *Fazekas G, Horvath M, Troznai T, Toth A (2007) Robot mediated upper limb physiotherapy for patients with spastic hemiparesis—a preliminary study. J Rehabil Med 39: 580–582.*

ANETT HRUSKA (2007)

DNA ploidy and molecular pathological examination of peripheral nerve sheath tumors and synovial sarcomas with special regard to differential diagnostic problems and prognostic factors

Supervisor: Zoltán Sápi

Nowadays it is accepted that two main groups of soft tissue sarcomas can be distinguished depending on the complexity of their molecular alterations. In this regard there is a group of sarcomas, whose cytogenetic alterations are relatively simple, usually balanced translocation resulting in formation of fusion genes, which are supposed to be implicated in the pathogenesis of these tumors. In this group the sarcomas (i.e. synovial sarcoma, myxoid liposarcoma, etc.) arise *de novo*, and the fusion genes are probably an initial and necessary event in their genesis. The second group of sarcomas (i.e. MPNST, leiomyosarcoma, etc.) is characterized by complex karyotypes and lack of fusion genes. Clear signs of chromosomal and genomic instability are present in this type of sarcomas. It seems that these tumors may arise *de novo* but sometimes a "dysplastic-precursor" condition does exist. In this work both type is represented; in case of peripheral nerve sheath tumors (PNST) we were focusing on the dysplastic-precursor route while in case of synovial sarcomas prognostic factors were highlighted. 44 PNST (27 schwannomas, 9 neurofibromas and 8 malignant peripheral nerve sheath tumors [MPNST]) were analyzed to determine DNA ploidy pattern and to clarify the conflicting data in the literature concerning this topic (whether benign PNSTs are aneuploid or not). For further insight we analyzed 6 schwannomas, one atypical neurofibroma and five MPNSTs by fluorescence *in situ* hybridization (FISH) technique using centromeric chromosome probes (7, 17 and 18) and automatic image analysis station, Metafer 4. Benign schwannomas could be characterized by euploid polyploidisation and by their 4c peak height value which was usually more than 10% of total cell number measured. These characters were not found among neurofibromas and MPNSTs. FISH analysis revealed and confirmed that the "normal" euploid-polyploid cells are mainly eusomic-polysomic containing two, four, eight or sixteen signals for each chromosome examined, but in a small proportion aneusomy was found among tumor cells of benign schwannomas (average: 2.58; range 1.33–3.44). In contrast, the atypical neurofibroma displayed marked aneusomy (18.44%) but it contained normal eusomic and polysomic cells, too. Two diploid MPNSTs proved to be clearly aneusomic with trisomy of chromosome 17 and monosomy of chromosome 18. All these data suggest that ploidy pattern determination combined with FISH analysis may be a very useful supplementary tool for making a right diagnosis (to differentiate benign versus malignant schwannomas in problematic variants) and to understand better the malignant transformation in PNSTs.

The treatment options for synovial sarcoma (SS) are very limited, though this type of sarcoma seems to be more heterogeneous than it has been traditionally considered. We investigated the Her-2 oncogene status of 20 cases of SS, to determine whether Her-2 amplification can be considered as a prognostic factor. Her-2 oncogene amplification was determined on smears using fluorescence *in situ* hybridization technique (dual color FISH with centromeric probe for chromosome 17 and specific probe for Her-2 oncogene). Moreover, protein expression was assessed by immunohistochemistry, and DNA ploidy status was measured using image analysis. We had 5 biphasic and 15 monophasic SSs,

patients' age ranged from 13 to 68 years (mean, 39.8 years). Tumor size was larger than 5 cm in each case. Follow-up time ranged from 6 to 78 months (mean, 38.5 months). For statistical analysis the chi-square test was used. Her-2 oncogene amplification was found in three cases (15.0%) of 20 SSs. These cases proved to be 2+ positive by immunohistochemistry, but massive amplification, characteristic of a subset of breast carcinomas, was not observed. Her-2 oncogene amplification was significantly associated with a lower risk of developing metastasis ($p<0.05$) (none of the 3 amplified cases had metastases), while no association was found with recurrence. Six cases proved to be aneuploid and 14 were diploid, but no association was found between Her-2 amplification status and ploidy, and between ploidy status and metastasis or recurrence. Our results emphasize and confirm that Her-2 oncogene amplification is a rare event in SS, but the small subset of SS with Her-2 amplification has a better overall prognosis. Furthermore, this may open a theoretically new treatment possibility with Trastuzumab for Her-2-amplified cases of SS.

- *Hruska A, Bollmann R, Kovács RB, Bollmann M, Bodó M, Sápi Z (2004) DNA ploidy and chromosome (FISH) patern analysis of peripheral nerve sheath tumors. Cell Oncol 26: 335–345.*
- *Sapi Z, Papai Z, Hruska A, Antal I, Bodo M, Orosz Z (2005) Her-2 oncogene amplification, chromosome 17 and DNA ploidy status in synovial sarcoma. Pathol Oncol Res 11: 133–138.*
- *Sapi Z, Hruska A (2005) DNA ploidy and chromosome (FISH) pattern analysis of peripheral nerve sheath tumors. Cell Oncol 27: 361 (Letter).*

GÉZA KORDÁS (2008)

Primary stability of osteochondral grafts in mosaicplasty

Supervisor: László Hangody

Focal chondropathies of weight-bearing joints may result in osteoarthritis in the long term. One of the many treatment methods for this condition is mosaicplasty. This technique involves transplantation of osteochondral grafts from the less weight-bearing areas of the knee into the defect. It is paramount for the success of the technique that grafts form a congruent surface and this congruency is maintained until bony union occurs. Primary stability of the grafts is secured by the press-fit mechanism between the bone plug and the recipient hole. It is not know to date if this press-fit stability strong enough to commence immediate full weight-bearing postoperatively. Improved primary stability would allow more vigorous physiotherapy and shorten or eliminate the non weight-bearing period. Primary stability is determined by graft diameter, dilation length, graft and drill hole length and multiple grafting. The aim of our studies was to quantify the effect of these parameters on primary stability after mosaicplasty. Mosaicplasty was performed on porcine distal femurs and push-in forces were measured. Our result showed that 6.5 mm grafts resist 1.3 times more compressive force than 4.5 mm grafts and dilation length of 15 vs. 20 mm resulted in significant difference at 3 mm below cartilage level only. Drill hole length has major impact on primary stability. 15 mm grafts inserted into 12 mm drill holes resulted in significantly higher level push-in forces, while matched graft and drill hole length provided better primary stability without increased push-in forces. Multiple grafting resulted in decreased primary stability compared to single grafting. Based on our results we recommend the use of relatively smaller diameter grafts, matched graft and

drill-hole length, especially in multiple grafting. The values for push-in forces in our experiment were close to the physiological stresses in the knee. Therefore, if better primary stability can be achieved by improved operative technique reliably, full weight-bearing after mosaicplasty could be allowed in the future.

- *Kordás G, Szabó J, Hangody L (2003) Mozaikplasztika: az osteochondralis graftok primer stabilitása. Magyar Traumatológia, Ortopédia, Kézsebészet, Plasztikai Sebészet 46: 147–153.*
- *Kordás G, Szabó JS, Hangody L (2005) The effect of drill-hole length on the primary stability of osteochondral grafts in mosaicplasty. Orthopedics 28: 401–404.*
- *Kordás G, Szabó JS, Hangody L (2006) Primary stability of osteochondral grafts used in mosaicplasty. Arthroscopy 22: 414–421.*

TAMÁS SHISHA (2008)

The response of muscles to limb lengthening

Supervisor: György Szőke

We analysed the effects of limb lengthening on the muscle tissue. Our objective was to determine the pathological background of the severe muscle related complications and also to find ways of preventing them. We lengthened the tibia of young and adult rabbits with the use of an external fixator, at different distraction rates. We used a bromodeoxyuridine technique to observe the proliferative activity of myogenic cells in the muscle belly and at the myotendinous junction. We measured the change of muscle mass and also determined the histopathological reactions behind weight change. The proliferation of myogenic cells and consequently, the genesis of muscle fibres occurs at the myotendinous junction, in physiological circumstances. The proliferative activity observed in the young animals was superior to that observed in the adult. More importantly, the distribution of the proliferating myogenic cells changes at high distraction rates; their number may increase to pathological levels in the muscle belly. The assessment of the proliferative activity myogenic cells may help to decide when myogenic stem-cell transplantation should be used in the future; in order to prevent muscle related complications. The muscles that tolerate well the distraction reacted with a loss of bulk, while the muscles that do not tolerate well the lengthening, increased their mass. This can be explained by the relative inactivity and consequent atrophy of the operated leg and by the pathological tissue reactions, such as fibrosis, bleeding and oedema, which act to increase mass. The change of muscle mass is an early predicting factor of complications. In clinical practice, mass could be replaced with other correlating parameters, such as volume or circumference. Distraction rate could be adapted during the lengthening procedure, to prevent severe complications. Identifying the best parameter and the potential implementation in human practice needs further investigations.

- *Shisha T, Kiss S, Pap K, Simpson H, Szőke G (2006) Relative ability of young and mature muscles to respond to limb lengthening. J Bone Joint Surg [Br] 88B: 1666–1669.*
- *Pap K, Berki S, Kiss S, Shisha T, Szalay K, Szőke G (2008) Structural changes in the lengthened rabbit muscle. Int Orthop 33(2): 561–566.*
- *Shisha T, Kiss S, Varga PP, Pap K, Szoke G (2006) Osteochondritis (Calvé's disease) of a vertebral body—a rare form of vertebra plana. Eur Spine J 15: 377–383.*

PROGRAM 2/9.

PULMONOLOGY

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Program overview

The subject of pulmonology comprises diseases of major public health importance, i. e. chronic obstructive lung disease (affecting 3–5% of the adult population), bronchial asthma (200,000 patients in Hungary), lung cancer and tuberculosis. This Program offers research opportunities and advanced training for physicians interested in pulmonological science. The 8 subprograms cover the major areas of respiratory research and students will acquire specialized clinical skills, physiology, microbiology, biochemistry and molecular biology laboratory experience. In addition, the Program offers comprehensive courses in clinical pulmonology and basic science.

Titles of research projects	***Supervisors***
Non-invasive investigation of airway inflammation in pulmonary diseases	Balázs Antus
The role of TNFX promoter polymorphism and anti Hsp 70 antibody in the development and prognosis of lung cancer	Zoltán Bártfai
Molecular immunologic characterization of BAL monocytes	András Falus
Animal experimental modelling of emphysema	Zoltán Hantos
Study of pulmonary mechanics	Zoltán Hantos
Pharmacology and role of inhalational drugs in the treatment of diseases of airway inflammatory diseases	Gábor Horváth
Non-invasive investigation of airway inflammation in pulmonary diseases	Ildikó Horváth
The activity, role and the interaction of enzyme systems in allergic bronchospasm	Márk Kollai
Lung tumors	László Kopper
Natural killer (NK) T lymphocytes in airway inflammation	György Losonczy
Examination of novel tyrosine kinase inhibitory molecules with selective antitumoral activity, modelling of relations between structure and biological action	György Mészáros
Lower respiratory tract infections	Ferenc Rozgonyi
Mechanism and clinical significance of angiogenesis and lymphangiogenesis in lung cancer	Balázs Döme
Application of molecular epidemiological methods in the clinical practice of tubercolosis	Ákos Somoskövi
Diagnosis and therapy of endobronchial diseases. Interventional pulmonology	János Strausz

Ph. D. students		***Supervisors***
Krisztina Gál	pt	Veronika Müller
Zsófia Lázár	ft	Ildikó Horváth
Tamás Tompos	pt	György Losonczy

Ph. D. candidates		***Supervisors***
Krisztina Czebe	i	Balázs Antus
András Lorx	pt	Zoltán Hantos
Judit Lukács	ft	Ákos Somoskövi
Ferenc Rényi-Vámos	i	Balázs Döme

Ph. D. graduates		***Supervisors***
Csaba Böcskei	i	Ildikó Horváth
Zsolt István Komlósi	ft	György Losonczy
Tamás Kullmann	ft	Ildikó Horváth
Gabriella Muraközy	i	Pál Magyar
Gyula Ostoros	i	Balázs Döme
Zoltán Süttő	i	Gábor Horváth
Zsuzsanna Szabó	i	Ildikó Horváth
Lilla Tamási	i	György Losonczy
Géza Vass	ft	Ildikó Horváth

pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

CSABA BÖCSKEI (2007)

Special questions of treating bronchial asthma (relative therapeutic index between inhaled formoterol and salbutamol in asthma patients, bronchial asthma and the gastroesophageal reflux)

Supervisor: Ildikó Horváth

The purpose of our work is to contribute to the more perfect provision, better control of the asthmatic patients. We are looking for answering the question, if in the treatment of asthma, can inhaled formoterol, that also has SABA characteristic, used effectively and safely as a rescue drug, compared to the traditionally applied short-acting salbutamol, and in what extent does the optimal treatment of the co-disease, gastroesophageal reflux, contribute to the decrease of reflux induced coughing, to the improvement of symptoms and respiratory function and, to the decrease of asthmatic drug requirement. To this, on one hand we determined the relative therapeutic index between inhaled formoterol and salbutamol, that we defined as the ratio between the relative local dose effect (max. FEV1) and the relative systemic dose effect (min SeK+); on the other hand, we proved the existence of reflux in patients, who were coughing despite the appropriate asthma treatment.

We analyzed the 24-hour oesophageal pH measures, with emphasize on the connection in time between coughing and reflux. We examined the effect of esomeprazole in dose of 40 mg/day for 3 months, beside antiasthmatic therapy. We concluded, that the relative therapeutic index is 2.5 for the formoterol, so the ratio of local bronchodilator, attack ceasing effect and systemic side effect is more favorable than atsalbutamol. The treatment of asthma is more effective, if the co-disease, that plays role in inducing the symptoms, is appropriately treated. We confirmed that in 75% of asthmatic patients coughing chronically, treated with anti-asthmatic drugs, there was reflux in the background of the symptoms. We found significant coincidence in time between reflux and coughing events. In 91% of the cases, reflux induced coughing, so we proved that this is "reflux coughing" which is a special group of the chronic coughing. Proton-pump inhibiting therapy, beside the improvement of respiratory function parameters, decreased the requirement for asthmatic drugs. The short-acting β2 receptor agonist requirement, in the two-week observational period after a 3-month treatment, significantly decreased in the GERD asthmatic group, and 41.5% of these patients did not require rescue drugs at all. During the three-month esomeprazole treatment following this, beside decreased dose ICS treatment, from this 39 patients, 38 patients' condition remained stable, well-controlled. This proves that GERD treatment is clinically significant in the effective treatment of asthmatic patients.

- *Rosenborg J, Larsson P, Rott Z, Böcskei Cs, Póczi M, Juhász G (2002) Relative therapeutic index between inhaled formoterol and salbutamol in asthma patients. Respir Med 96: 412–417.*
- *Rosenborg J, Larsson P, Rott Zs, Böcskei Cs, Póczi M, Juhász G (2002) Assessment of a relative therapeutic index between inhaled formoterol and salbuterol in asthma patients. Eur J Clin Pharmacol 58: S61–S67.*
- *Böcskei Cs, Viczián M, Böcskei R, Horváth I (2005) The influence of gastroesophageal reflux disease and its treatment on asthmatic cough. Lung 183: 53–62.*

ZSOLT ISTVÁN KOMLÓSI (2008)

Factors influencing severity and treatability of allergic airway inflammation

Supervisor: György Losonczy

Severity and treatability of allergic airway inflammation persisting in the background of asthma may be influenced by the innate immunity. Exposure to lipopolysaccharide (Lps) —or other natural immune stimulatory agents—as well as pregnancy [1] can induce the activation of innate immune processes. To further clarify the role of these factors the following examinations were performed:

In an experimental investigation [2], we have developed a model of eosinophil airway inflammation and airway hyper-responsiveness (AHR), which was induced by Lps and allergy together. Allergen challenge, preceded by Lps-priming resulted in more severe eosinophil inflammation and higher nitrite formation in sensitized BALB/c mice, than allergen provocation alone. After Lps priming, AHR and concentrations of Th2 cytokines in bronchoalveolar lavage fluid were decreased, but still remained significantly higher than in controls. Probably, some accessory—non-Th2—immune-mechanisms might have been activated by Lps, during the development of allergic inflammation. Eosinophil inflammation was partially, while nitrite production and AHR were observed to be largely

dexamethasone resistant in Lps-primed allergized animals. Thus, an animal model of steroid-resistant asthma was established in mice. The role of inducible nitric oxide synthetase (iNOS) in AHR was also assessed. Our results demonstrated that 1400W—a selective inhibitor of iNOS—effectively and rapidly (within 2 hours) reversed AHR in allergized mice. This effect of 1400W was, however, absent after Lps priming. In conclusion, Lps inhalation may exaggerate eosinophil inflammation and reduce responsiveness to anti-inflammatory treatment in allergic airway inflammation.

In our human study [3], the immunological interferences between asthma and pregnancy were examined. The IFN-γ^+ and IL-4^+ T cell counts—determined by flow cytometry—were markedly increased in peripheral blood of asthmatic pregnant women. Significant negative correlations were revealed between the sizes of these cell populations and maternal peak expiratory flow, as well as birth weight of their newborns. Thus, the culminating proliferation of IFN-γ^+ and IL-4^+ T lymphocytes may potentially impair fetal development as well as maternal airway symptoms.

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TAMÁS KULLMANN (2008)

Measurement of exhaled breath condensate pH

Supervisor: Ildikó Horváth

Background: Exhaled breath condensate analysis is a promising method for investigating airway pathology. However, exhaled biomarkers show great variability. The first aim of this study was to identify a reliably detectable biomarker. The pH is considered to be the most robust biomarker of the exhaled breath condensate. Current pH measurements do not take into account the effect of CO_2. My aim was to determine the effect of condensate CO_2 partial pressure on pH and to provide a more precise mode of condensate pH determination. The second aim of this study was to test the capacity of condensate analysis (pH, LTB4, cys-LT, IFNγ, TNFα, endothelin-1 content) in distinguishing between healthy individuals and patients with different pulmonary pathologies.

Methods: Condensate pH and CO_2 partial pressure were measured from 12 healthy volunteers and 12 asthmatics by blood gas analyser in neat, argon deaerated and CO_2 loaded samples. Regression analysis was used (1) to test the relation between pH and CO_2, (2) to calculate pH at 5.33 kPa CO_2 level, the physiological alveolar CO_2 partial pressure. Reproducibility of different pH readings was compared by Bland-Altman test. Leukotrienes and cytokines were measured with immunoassays.

Results: Condensate CO_2 concentration was variable either in neat or argon deaerated samples. There was a close negative logarithmic relation between CO_2 and pH ($r2>0.99$,

$p<0.01$). Calculation of pH at 5.33 kPa CO_2 level provided approximately 6 times better reproducibility than the currently used measurements. Leukotrienes and cytokines were undetectable in either healthy individuals or patients.

Conclusions: Condensate CO_2 partial pressure influences pH measurements. Determination of pH at a standard CO_2 level provides the most reproducible condensate pH values and the most reliable biomarker determination in the exhaled breath condensate to date. Although pH reading is not capable of detecting a difference between healthy individuals and treated asthmatics, it may be a useful method to clarify some methodological questions regarding condensate collection.

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GABRIELLA MURAKÖZY (2006)

Gene polymorphism of the interleukin-10, angiotensin-converting enzyme and transforming growth factor-β_1 genes in granulomatous disorders, sarcoidosis and Wegener's granulomatosis

Supervisor: Pál Magyar

Sarcoidosis and Wegener's granulomatosis (WG) are a granulomatous and vasculitic disease of unknown origin. Gene polymorphisms (PMs) are known to influence phenotypes of numerous diseases and PMs within the angiotensin-converting enzyme (ACE), transforming growth factor-β_1 (TGF-β_1) and interleukin-10 (IL-10) genes are suspected to modify the course of granulomatous disorders. Therefore we were interested whether the genotype frequencies of the named PMs differ in sarcoidosis and its three distinct phenotypes or in WG in comparison with healthy controls.

In 51 sarcoidosis patients and in 72 healthy blood donors genotyping for the a biallelic PM in codon 25 of the TGF-β_1 gene and a biallelic PM at position –1082 of the IL-10 gene was performed. Patients were retrospectively classified according to their course of disease, namely spontaneous remission, under therapy regressive or chronic-progressive.

In 39 patients with WG the genotypes of a deletion/insertion PM in intron 16 of the ACE gene, a biallelic PM in codon 25 of the TGF-β_1 gene and a biallelic PM at position –1082 of the IL-10 gene were determined and compared with healthy blood donors.

For TGF-β_1 and IL-10 PMs our statistical comparisons of the allele and genotype frequencies between the clinical defined sarcoidosis groups and the healthy blood donors revealed no significant differences. The results indicate that none the investigated TGF-β_1 and IL-10 gene PM associated with a susceptibility for sarcoidosis or play a central regulatory role in the pathogenesis of sarcoidosis.

For the ACE-PM no significant differences could be detected neither in the allele frequencies nor in the genotype frequencies in WG compared to controls.

For TGF-β_1 a trend ($p=0.058$) to genotype CG could be observed in WG.

Most interestingly a significant shift (p=0.021) to genotype AA of the IL-10 PM in WG was observed. IL-10 and TGF-β_1, immunoregulatory cytokines capable of down-regulating T-helper cell type 1 response, show a significant shift or a trend respectively towards genotypes associated with reduced cytokine release leading to the hypothesis that different immunoregulatory cytokine patterns dependent on gene PMs might be involved in the pathogenesis of WG.

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GYULA OSTOROS (2007)

Analysis of the effectiveness of first-line gemcitabin-cisplatin cytotoxic chemotherapy and second- and third-line use of the molecular targeting drug gefitinib in treating non small cell lung cancer based on Hungarian experiences

Supervisor: Balázs Döme

Lung cancer causes more deaths around the world than any other malignant disease. Unfortunately, Hungary is the world leader in lung cancer fatality among men, and progress in developing successful therapies has been slow. The goal of our study was to determine the efficacy of gemcitabin-cisplatin combination chemotherapy in Hungarian patients with either locally advanced or advanced cases of NSCLC. An analysis of the data from 120 patients undergoing gemcitabin-cisplatin therapy showed a therapeutic response rate of 40% and a disease stabilization rate of 38%. The time to progression was 28.1 weeks, and average survival was 54.9 weeks. We demonstrated a positive correlation between therapeutic response, time to progression, and overall survival. In addition, patients tolerated treatment with gemcitabin-cisplatin, with grade 3–4 toxicity occurring only occasionally during the study. Our study supports international observations that gemcitabin-cisplatin combination therapy can safely and effectively be given to patients in advanced and locally advanced stages of non-small cell lung cancer, even in an outpatient setting. Our findings also show that four cycles are sufficient to achieve the necessary therapeutic effect. Furthermore, doses slightly lower (70 mg/m^2) than internationally recommended doses of cisplatin are equally effective and increase patients' tolerance of the treatment.

Biological response modifiers represent a new possibility in the use of molecular chemotherapy in cases of NSCLC. Of the EGFR-TK inhibitors, erlotinib and gefitinib have proven effective. As part of the international Expanded Access Program, we analyzed the data from 50 Hungarian patients with progressive, advanced or locally advanced NSCLC who had undergone one or two previous protocol treatments with cytotoxic chemotherapy before receiving per os gefitinib therapy. The response rate was 10%, with 46% of patients exhibiting a stabilization of the disease. The average survival was 35 weeks. Patients with adenocarcinoma had a significantly greater rate of survival than those with squamous cell carcinoma. These results are similar to those found in European and American studies for

disease control rates and one-year survival rates, with our study showing a slightly higher average survival. Observations abroad corroborate our findings that patients with adenocarcinoma have a significantly higher survival rate than those with squamous cell carcinoma. The ISEL study, using a placebo control group, found a significant increase in survival rates for patients treated with gefitinib only in Asian populations. Nevertheless, it is possible that with a complex molecular diagnostic approach, gefitinib therapy may prove effective even in first-line treatment in a specifically defined group of patients.

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ZOLTÁN SÜTTŐ (2008)

Novel aspects of ciliary beat regulation in human bronchial epithelial cells

Supervisor: Gábor Horváth

Background: Ciliary function has a pivotal role in airway mucus clearance, but the regulation of ciliary beat frequency (CBF) is still not completely understood. In these studies we focused on two issues. (1) In human airway epithelial cells (HAEC), transmembrane adenylyl cyclases (tmAC) are the only known source of cAMP. Soluble adenylyl cyclase (sAC), a recently cloned enzyme, regulates flagellar motility in sperm. In contrast to tmACs, sAC is insensitive to G-protein activation, but is stimulated by HCO_3^-, independently of H^+. Here we examined the expression and cellular distribution of sAC in HAECs. Because functional experiments of sAC are complicated by the fact that intracellular HCO_3^- concentration and intracellular pH (pH_i) correlate, and neither has ever been examined for ciliary effects, we also studied how pH_i itself influences CBF in HAECs. (2) β-adrenergic agonists are the most important ciliostimulant drugs of airway epithelium. Organic cation transporters (OCT) mediate non-neuronal norepinephrine (NE) uptake and thus inactivate NE at adrenergic receptors. NE uptake by certain OCTs has been reported to be inhibited by glucocorticoids (GSs), thereby increasing NE concentration at receptor sites. We hypothesized that inhaled β-adrenergic agonists could also be potentiated by GSs inhibiting their uptake into HAECs, and thus enhancing ciliostimulation. Therefore we tested the expression of OCTs in HAECs and examined the characteristics, including GS-inhibition, of NE transport.

Results: (1) We have shown that sAC (both mRNA and protein) is expressed in HAECs. Confocal microscopy localized sAC in the ciliary shaft, co-localizing with axonemal tubulin. Preliminary functional studies have suggested that cytosolic HCO_3^- stimulates CBF in HAECs. We also demonstrated that relatively small changes in pH_i result in significant changes in CBF in HAECs (increasing pH_i increases CBF and *vice versa*). Changing pH_i may directly act on the ciliary motile machinery, as the involvement of the kinase/phosphatase system, Ca^{2+} and sAC has been excluded. (2) mRNA for certain OCTs (OCT-1 and

EMT) as well as neuronal epinephrine transporter (NET) are expressed in HAECs. NE uptake of airway vascular smooth muscle cells expressing both OCT-1 and EMT was acutely inhibited by GSs in a non-genomic way. Further experiments are needed to define the role of catecholamine transporters expressed by HAECs.

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ZSUZSANNA SZABÓ (2007)

The cyclooxygenase metabolism in asthma

Supervisor: György Losonczy

The role of cyclooxygenase metabolites, prostaglandins (PGs) and thromboxane A_2, in airway inflammation in asthma is extensively investigated. In the first part of the study we compared the *in vitro* generation of PGE_2 and PGD_2 by bronchial fibroblasts isolated from healthy non-asthmatic, aspirin-tolerant asthmatic and aspirin-intolerant asthmatic (AIA) groups. PGs were measured under control condition and after cytomix (TNF-α+IL-1β+LPS) stimulation. We showed that fibroblasts should be implicated in the pathophysiology of asthma in addition to inflammatory cells. We demonstrated different increase in PGs synthesis caused by proinflammatory cytokines in each group. We observed an intrinsic deficiency in the production of PGE_2 in asthmatic fibroblasts and it was most pronounced in AIA group. This result supports the hypothesis of the elimination of "PGE_2-brake" in AIA promoting leukotriene overproduction. After stimulation the PGE_2/PGD_2 ratio was significantly lower in AIA group which reflects the downregulation of PGE_2-synthase comparing to PGD_2-synthase. The observed deficient PGE_2 synthesis was not due to insufficient COX-2 expression induced by cytomix but we showed the downexpression of constitutive COX-1 protein in AIA fibroblasts. Our results pointed out that deficient PG synthesis in AIA and asthma can be caused not only and directly by the expression of inducible COX-2 key enzyme but also by the enzymes in the downstream of the metabolism and perhaps by the different expression of COX-1. In the second part of the study we demonstrated a significant increase in the concentration of a TxA_2 metabolite, TxB_2 in exhaled breath condensate obtained from asthmatic patients comparing with healthy volunteers. We were the first to show the stable presence of its oxidative metabolite, 2,3-dinor-TxB_2 in the airway condensate. We proved that considerable differences can be measured in the level of a mediator in exhaled breath condensate even in healthy subjects, used different analytical methods (EIA, RIA) and different batches of a kit. In order to further standardize and compare more realible the results of this recently more widely using non-invasive method for investigating mediators directly from the airway, we may recommend measuring all samples with the same kit and always giving a reference point within the samples.

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LILLA TAMÁSI (2006)

Asthma in pregnancy. Studies on cellular immunology and pharmacoepidemiology

Supervisor: György Losonczy

The aim of our study was to evaluate the hypothesis that pregnancy (a T helper-2 polarized state) of asthmatics will enhance the number of circulating T2 lymphocytes, but decrease the subset producing interferon-γ (T1 lymphocytes) and thereby cause a culminating T2 dominance with possible clinical consequences. The aim of our study was also a retrospective analysis of the data of asthmatic pregnant patients nursed at the ambulance of our department. In the following we assessed the risks of congenital abnormalities among informative offsprings of mothers affected by BA during pregnancy using the data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities.

Interleukin-4 or interferon-γ producing T-lymphocytes were determined by flow cytometry in healthy (n=8) and asthmatic (n=13) non-pregnant women and healthy (n=18) and asthmatic (n=48) pregnant women of similar chronological and gestational (2nd–3rd trimester) age and asthma severity (GINA II–III). For the treatment of asthmatic pregnant women the inhalative corticosteroid budesonide, the long-acting beta-agonist formoterol or salmeterol, and the short acting terbutaline were used.

In blood of non-pregnant women—healthy or asthmatic—, the numbers of interleukin-4 and interferon-γ positive T-cells were very low (<10/μl blood). In contrast, in asthmatic pregnant women the cell counts were 182±27 and 39±6 for IFN-γ and interleukin-4 positive T-cells/μl blood, respectively (both $p<0.05$ vs respective control values of non-pregnant asthmatics). Within the asthmatic pregnant group significant negative correlations were revealed between the numbers of interferon-γ or interleukin-4 positive T-cells and maternal peak expiratory flow as well as birth weight of newborns (both $p<0.05$).

Ten of the 48 asthmatic patients had cesarean delivery, 3 developed preeclampsia, 1 diabetes mellitus. The mean gestational age was 38.8±0.3 weeks and the weight of newborns 38.8±0.3 g. No congenital malformations or spontaneous abortions were detected.

The results of the case-control study suggest that maternal bronchial asthma during pregnancy did not increase the risk for major congenital abnormalities.

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• *Somoskovi A, Kodmon Cs, Lantos A, Bartfai Z, Tamasi L, Fuzy J, Magyar P (2000) Comparison of recoveries of mycobacterium tuberculosis using the automated BACTEC MGIT 960 system, the Bactec 460 TB System and Löwenstein-Jensen Medium. J Clin Microbiol 38: 2395–2397.*

GÉZA VASS (2006)

Adenosine in exhaled breath condensate: methodological and clinical aspects

Supervisor: Ildikó Horváth

Exhaled breath condensate is a new, *non-invasive* tool to investigate lower airways. Several questions related to this method have still not been answered. Thus it is not clear for example how inhalation (which can occur via mouth or nose) during sample collection influences parameters of exhaled air. At the same time studies from different laboratories showed that mediators in exhaled breath condensate can help us to screen or follow-up *non-invasively* lung diseases. A number of *in vitro*, murine and human studies confirmed the role of adenosine in asthma. Our goal was to examine how inhalation via nose alters the volume, levels of adenosine and other mediators of exhaled breath condensate in healthy subjects and patients with rhinitis as compared to the "traditional" oral inhalation; furthermore, to determine exhaled adenosine levels of asthmatic and non-asthmatic rhinitic patients as compared to healthy controls. Our data showed that significantly more sample can be obtained if subjects inhale via nose than via mouth. The composition of condensate does not depend on the mode of inhalation in healthy subjects, while in patients with rhinitis exhaled adenosine is significantly higher when they inhale via nose. This elevated adenosine in rhinitis patients at nasal inhalation suggests that adenosine from the inflamed nasal cavities may enter lower airways by inhalation. Exhaled breath condensate adenosine is significantly elevated in patients with asthma and non-asthmatic rhinitis, and shows correlation with exhaled nitric oxide proposing that exhaled breath condensate adenosine may be a potential marker of lower airway inflammation.

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- *Huszár É, Vass G, Csoma Zs, Vizi É, Barát E, Herjavecz I, Horváth I (2002) Adenozin koncentráció egészségesek és atópiás asztmás betegek kilégzett levegő kondenzátumában Medicina Thoracalis 55: 186–192.*
- *Vass G, Huszár É, Barát E, Valyon M, Horváth I (2003) Comparison of nasal and oral inhalation during exhaled breath condensate collection. Am J Respir Crit Care Med 167: 850–855.*

PROGRAM 2/10.

OPHTHALMOLOGY

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Program overview

Vision is essential for human life, but not endangered by several frequent eye diseases, the treatment of which has not been completely established. The Ph. D. Program in ophthalmology addresses the pathophysiology and pathology of the most important eye diseases. The research projects cover the diseases of the light refractive media of the eye, the biochemical and histological alterations important for vision, and the experimental models of the ocular alterations. Ocular blood flow and aqueous humour dynamics are especially important topics of the Ph. D. school. Glaucoma, one of the blinding eye diseases with the highest prevalence is investigated with special emphasis.

Titles of research projects	***Supervisors***
Physiology and pathophysiology of the neuroretina in certain diseases, especially in hereditary retinal diseases	Ágnes Farkas
Clinical—biological—imaging examinations of retinal ganglion cell apoptosis in glaucoma	Gábor Holló
Wound healing of the cornea especially in refractive surgical procedures	Zoltán Zsolt Nagy
Physiology and pathophysiology of vision. The topics include the examination of the refractive layers of the bulb in pathologic conditions and the follow-up of lesions occurring in diseases of the eye	Ildikó Süveges
In vitro culturing of corneal limbal stem cells and examination of their potential in clinical application	Nóra Szentmáry

Ph. D. students		***Supervisors***
Zsuzsanna Antus	pt	Ildikó Süveges
Gábor György Deák	ft	Ildikó Süveges
Eszter Fodor	pt	János Németh
Anita Garas	ft	Gábor Holló
Ákos Kusnyerik	ft	Ildikó Süveges
Enikő Siklódi-Nemes	pt (a)	Ildikó Süveges
András Zsolt Szigeti	ft	Zoltán Zsolt Nagy
Ágnes Ildikó Takács	ft	Zoltán Zsolt Nagy
Erika Tátrai	ft	György Salacz

Ph. D. candidates		***Supervisors***
Orsolya Fiedler	pt	Ágnes Farkas
Krisztina Hagyó	ft	János Németh
Balázs Lesch	ft	Ágnes Farkas
Mónika Popper	ft	Ildikó Süveges
Rita Vámos	pt	Ágnes Farkas

Ph. D. graduates		***Supervisors***
Árpád Bereczki	i	Ildikó Süveges
Béla Erdélyi	pt	János Németh
Mária Éva Ferencz	pt	György Salacz
Katalin Gombos	i	György Salacz
János Hargitai	i	Ágnes Farkas
Andreas Katsanos	it	Gábor Holló
András Papp	i	Ildikó Süveges
Miklós Resch	pt	Ildikó Süveges
Gábor Márk Somfai	ft	György Salacz
Antal Szabó	i	Ildikó Süveges
Viktória Szabó	ft	Zoltán Zsolt Nagy
Gabriella Szatmáry	i	Ildikó Süveges
Márta Tóth	ft	Gábor Holló
László Balázs Varsányi	ft	Ágnes Farkas

a, absolutorium; pt, part-time; ft, full-time; i, individual; it, international

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

ÁRPÁD BERECZKI (2008)

Surgical treatment of macula disease

Supervisor: Ildikó Süveges

Removal of subfoveal neovascular membranes in age-related diabetic macula degeneration: Macular translocation may be an effective method in the treatment of age-related macula degeneration by data published in the literature. Although our experience is moderate due to the low number of cases, we found that visual acuity shows close relation with surgical indication and preoperative ophthalmic state of the patient. In summary we suppose that this kind of surgery may be appropriate in the treatment of certain types of macular degeneration, at the same time the number of complications decreases due to the development of surgery. Unfortunately we should count on higher number of recurrences *(Bereczki et al. 2000, Bereczki and Bíró 2007)*. Surgery of diabetic macular oedema: Prevalence of diabetic macular oedema depends on a number of factors, such as the type of diabetes and diabetic retinopathy, duration of diabetes as well as general status of patient. The protocol on indication and contraindication of surgery shall be established

by later prospective studies. Actually the indication for surgery is made by thorough individual analysis of expected improvement of visual acuity and chances of complications *(Bereczki 2001, Milibák and Bereczki 1999)*. Pars plana vitrectomy performed in topical anaesthesia: Retrobulbar and peribulbar anaesthesia became preferred methods in posterior segment surgeries versus general anaesthesia in short time due to advanced techniques and instrumentation as well as shorter surgery times. Topical anaesthesia may represent appropriate alternative to retrobulbar and peribulbar anaesthesia with thoughtful patient selection in case of three gate pars plana vitrectomy but we think that its wide-spreading use is not expected *(Bereczki and Domsa 2002)*.

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BÉLA ERDÉLYI (2008)

The effects of the tear-film dynamics in healthy subjects and dry eye patients

Supervisor: János Németh

The preocular tear film has a significant optical function, because the air-tear-film interface represents two-thirds of the total refractive power of the eye. This surface shows dynamic changes between two blinks, which is important to recognize as the refractive surgical procedures are performed partly according to the momentary image of the ocular surface. SRI, SAI and PVA proved to be suitable parameters for assessment of the alterations of the tear film. The changes are individual, suggesting that tear film stability might be extremely variable among healthy subjects. The color-coded topographic map of the ocular surface was thought to be a constant quality of the eye. Our investigations demonstrated, that the topographic pattern changes in more than half of the cases when the healthy subjects retained from blinking for one minute. The reproducibility of keratometric measurements significantly worsened 60 seconds after blinking. The standard deviation of the measures exceeded 0.5 diopter. The application of an artificial tear improved the regularity of the ocular surface significantly. Based on this experience corneal topography was found suitable for testing the topographic effect of various artificial tears. With the high-speed videotopograph developed by our workgroup the tear-film related changes can be followed exactly from the opening of the eye. Our examinations showed that the most regular ocular surface is attained in about 3–10 seconds postblink, which we named tear film build-up time first in the world. Subsequently ocular surface regularity deteriorates. Our proposal for capturing a videotopographic image is 4–9 seconds postblink. Tear-film dynamics is altered in dry eye patients. After application of an artificial tear drop healthy dynamics appeared. Following incomplete blinks—common in everyday life—tear-film dynamics does not differ from that observed after complete blinks. Using confocal microscopy we experienced the effects of hypoxia related metabolic disorder in the cornea of dry eye patients, these symptoms were the most pronounced

at the lower corneal periphery of patients with lagophthalmos. The progression of the condition is well indicated by the status of the lid-parallel conjunctival folds.

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MÁRIA ÉVA FERENCZ (2008)

Surgical treatment of vitreomacular diseases, assessment of anatomical and functional results

Supervisor: György Salacz

The improvement of microsurgical techniques in the last decade resulted in better anatomical and functional results in the treatment of some vitreoretinal pathologies. One of the milestones of this development was the introduction of indocyanine green (ICG) dye in the staining of epitretinal membranes of the macula, facilitating their safe and intact peeling. After the first enthusiastic successes, however, concerns were raised because of the presumed toxicity of the dye.

As epiretinal membranes usually occur in elderly patients there is also some degree of cataract formation present which requires combined surgical intervention involving the anterior segment as well for the good visualization of membrane removal. In order to achieve good postoperative visual acuity, accurate intraocular lens planning is required. Unfortunately, in cases accompanied by macular edema formation regular biometry methods may not be appropriate.

In our work we aimed (1) to assess the possible toxic effect of the ICG dye during macular hole surgery and (2) to find a solution for the avoidance of postoperative refractive correction error in eyes undergoing combined cataract and vitreoretinal operations for macular oedema.

We compared postoperative visual acuity and the response densities of the two central rings on multifocal electroretinography (mfERG) in patients operated with macular hole during a 20-month follow-up. In group A ICG dye were used for the removal of internal limiting membrane, while in Group B no dye was used for peeling. In the case of combined operations we compared the postoperative refraction of the patients with the preoperatively planned correction.

Significantly better visual acuities and higher response densities were found in Group B after the 20-month follow-up. Postoperative refraction showed a myopic shift in the case of combined operations.

According to our results it is not advised to use of ICG dye during vitreoretinal surgical interventions. Other techniques should be employed in the future which do not have any retinal toxic effect. The reason for the difference in the planned postoperative refraction in eyes with combined operations for cataract and macular edema is the use of shorter

axial length measurements due to the presence of macular oedema in the SRK/T formula. With the use of our described corrected axial length the postoperative refractive error may be avoided.

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KATALIN GOMBOS (2007)

New opportunities for research in local ophthalmic anaesthesia

Supervisor: György Salacz

Since the 1970s, the methods of surgical procedures in ophthalmology have changed due to technical developments. New methods have been introduced and, as a result, the requirements for anaesthesiology have also changed. The question is: which method can provide better surgical conditions and is better for patients?

1. Our aim was to compare Topical Anaesthesia (TA) with anaesthesia via injection (RBA) during the course of cataract removal by phacoemulsification. We wanted to examine the activation of the adrenocortical system and any changes in patients' psychological state. We also wanted to identify using statistical analysis the parameters that increase the chance of the patient experiencing pain during the surgery.

2. Our second aim was to develop appropriate anaesthetic methods for extended, painful ophthalmic surgery, which could enable the administration of the anaesthetic solution into the appropriate space, at any time and for any length of time required, without interrupting or changing the procedure. We developed the method through experiments on cadavers, the practical application of a method for administering the anaesthetic via cannule, and the examination of the serum level of lidocain using continual administration.

1. We established that the cataract surgery with phacoemulsification procedure is more painful and the systolic blood pressure is significantly higher using TA than using RBA. The cortisol level of serum changes significantly according to pain, while the adrenalin level of serum changes significantly according to the type of anaesthesia. Using a logistic regression model, we were able to estimate the probability of pain with 93% certainty. Based on this, we can change the planned anaesthetic method and choose the most appropriate method for a given patient.

2. We developed a relatively simple and easy-to-learn technique to introduce a thin, flexible plastic cannule into the orbit.

When used in practice, this method achieved anaesthesia over whatever period was required. By using this technique in lengthy procedures we were able to avoid general anaesthesia. The position of the cannule can be checked by ultrasound before the administration of the anaesthetic solution, so the side-effects of intraocular administration can be avoided. It is also possible to use the cannule for post-operative pain relief.

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JÁNOS HARGITAI (2008)

Novel diagnostic methods and therapeutical approaches in Stargardt macular dystrophy

Supervisor: Ágnes Farkas

Stargardt disease is the most common juvenile-onset macular dystrophy. The disease is characterized by a severe reduction of central vision, often occuring before the age of 20 years. Several clinical data are available to assess the status of a patient, however the prognosis is still hard to predict. The molecular background of this complex pathology is still unclear, and no definitive therapy is available yet.

Optical coherence tomography and scanning laser ophthalmascope are two non-invasive methods capable to bring new and valuble information about the patient. Linear correlation was observed between visual acuity and foveolar thickness and between visual acuity and macular volume.

SLO examination of STGD patients showed strong correlation between the amount of pigment in the fovea and visual acuity.

Genetic analysis of 35 Hungarian STGD patients showed homogeneous distribution of disease associated mutations in contrast with other ethnical groups, thus it favours genotype-phenotype correlation studies in the Hungarian population. This would help us to determine the prognosis of our—juvenile—patients at an earlier age.

Rapid advancing of genetics has allowed us to treat several inherited diseases already. In our basic research we successfully transduced the gene of reporter protein into the retinal pigment epithelium using lentivirus vector both *in vitro* and *in vivo* nature. The immunorejection caused by the foreign protein was succesfully overcome by using immunosuppression. According to our findings, gene therapy seems to be a promising candidate for the treatment of Stargardt macular dystrophy in the future.

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ANDREAS KATSANOS (2006)

Polarimetric measurement of the retinal nerve fibre layer

Supervisor: Gábor Holló

Glaucoma is an irreversible progressive optic neuropathy which is one of the leading causes of blindness worldwide. Assessment of the retinal nerve fibre layer thickness (RNFLT) with scanning laser polarimetry (SLP) is a novel, non-invasive and clinically important, evolving method in the glaucoma diagnosis and long-term care. SLP has been developed and introduced into clinical practice in the last decade but it is still under continuous development. In my research I focused on the clinical application of the newly developed customized corneal polarization compensation in SLP (SLP-C), which technically offers advantages compared to the classic fixed angle and magnitude compensation (SLP-F).
I investigated the influence of corneal LASIK surgery on the results of SLP-F and SLP-C; the relationship between retinal sensitivity and the corresponding RNFLT as measured with SLP-F and SLP-C; the relationship between RNFLT as measured with SLP-C and the corresponding retinal sensitivity as measured with a non-selective (SAP) and an M-cell selective test (FDP); and the influence of subfoveal choroidal neovascularisation (CNV) on the polarimetric macular image. Appropriate number of glaucomatous and healthy eyes was imaged in each study, and appropriate statistics were used.
Our results show that LASIK induced alteration of corneal polarization become stable after the third post-LASIK month when SLP-F was used. However, when SLP-C was used the LASIK-induced effects were neutralized even in the early post-LASIK period. These results suggest that SLP-C is superior to SLP-F for the neutralisation of corneal changes. Measurements with SLP-C correlated better with retinal sensitivity than those with SLP-F. We found a similar structure-function relationship between RNFLT determined by SLP-C and retinal sensitivity as measured with SAP and FDT. This is especially important information for long-term clinical research and structure-function investigations. Our results showed that SLP-C measurements may be disturbed in eyes with CNV due to the CNV-induced artifacts of the macular polarimetric image. To reduce these artifacts, the use of SLP-F is still justified.

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MIKLÓS RESCH (2006)

The role of extracellular matrix in corneal wound healing

Supervisor: Ildikó Süveges

The wound healing of the avascular and hypocellular corneal stroma, composing 90% of the cornea leads to the decrease in corneal transparency and visual acuity. Corneal wound healing is associated with the irregular arrangement of the extracellular matrix. The effect of stromal extracellular lipid deposition on corneal wound healing was examined by corneal topography in a prospective study and a case report of eyes with arcus lipoides corneae undergone cataract extraction. Clear corneal incision phacoemulsification was found safe in eyes with arcus lipoides in corneal topographical aspects, however delayed normalisation of the corneal surface can be expected. In higher grade arcus lipoiedes incision greater than 4.0 mm can result in high corneal astigmatism and surface irregularity. The role of the extracellular matrix in healthy corneas was investigated after different refractive surgical interventions. The fact and postoperative time course of intraoperative corneal edema was shown by ultrasound pachymetry in Laser *in situ* keratomileusis (LASIK) performed in healthy human eyes. The flap and stromal edema appeared intraoperatively and remained significant until postoperative day 5 by subtraction pachymetry. Immunohistochemical analysis of glycosaminoglycan and keratan sulfate changes was performed in healthy rabbit corneal buttons undergone myopic photorefractive keratectomy. Our experiments provided evidence, that the superficial excimer laser treatment results in a prolonged and deeper involvement of the stromal extracellular matrix compared to the cellular changes in form of apoptosis, proliferation and inflammatory response.

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GÁBOR MÁRK SOMFAI (2008)

Clinical and laboratory assessment of diabetic microvascular complications

Supervisor: György Salacz

The increasing incidence of diabetes means a growing burden on the societies of developed countries because of the increasing need for disease treatment along with the treatment of complications. In developed countries, among them also in Hungary, the leading cause of legal blindness and visual impairment is diabetes. Optical coherence tomography (OCT) is an imaging method capable of near-histological resolution scanning of retinal tissue. With the use of a custom-built software it is possible to measure not only the total thickness of the retina in the macular area, but also the thickness of the retinal layers. The structural changes of the retina are caused by biochemical-physiological alterations,

among which an increasing body of evidence is pointing towards the role of an enzyme, called semicarbazide-sensitive amine oxidase (SSAO). The aim of our work was to investigate how macular volume and central foveal thickness can show early macular changes secondary to diabetes, and also to investigate their relationship with visual acuity in macular edema. We also investigated the risk factors for the occurrence of serous macular detachment (SMD). We investigated the sensitivity of the OCTRIMA (Optical Coherence Tomography Retinal IMage Analysis) software towards operator related image acquisition pitfalls (depolarization, defocusing, the combination of depolarization and defocusing and decentration of the scan). We assessed the activity of soluble SSAO, which is known to be correlated with the degree of retinopathy in humans, and examined its correlation to the tissue-bound aortic SSAO enzyme activity, chronic subclinical inflammation and oxidative stress in streptozotocin-induced male Whistar rats, treated under semi-intensive and intensive insulin regimes. Our results have shown that (1) macular volume seems to be a more sensitive indicator of pathological changes in diabetes than central foveal thickness and (2) macular volume and central foveal thickness are strongly correlated to visual acuity in diabetic macular edema. Eyes with serous macular detachment have better visual acuity compared to eyes with similar central foveal thickness and macular volume but without SMD. The risk of developing SMD may be higher in the presence of cystoid macular edema and long diabetes duration (longer than 15 years). OCT image segmentation by OCTRIMA was significantly influenced by the combined cases of decentration and depolarization. The activity of aortic tissue-bound and soluble SSAO were inversely related, poor glucose homeostasis increased the activity of soluble SSAO and also increased the level of C-reactive Protein (CRP, indicator of chronic subclinical inflammation) and oxidative stress (marked by a decreased Total Antioxidant Status, TAS). The levels of CRP, TAS and soluble SSAO activity were strongly correlated and their pathological changes were normalized only by intensive insulin treatment. Our results show the usefulness and importance of macular OCT examinations in early diabetes and also in diabetic maculopathy. Careful fine-tuning of imaging settings is important to obtain a best-possible scan in order to make reliable measurements with the use of the OCTRIMA software. Our results give indirect evidence for the source of soluble SSAO activity and emphasize the importance of tight glucose control in patients with diabetes.

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ANTAL SZABÓ (2008)

The effect of laser on the retina in experimental and pathological conditions

Supervisor: Ildikó Süveges

When performing retinal laser photocoagulation the laser beam causes different effects at the level of the retinal pigment epithelial (RPE) layer, where it is mostly absorbed.
Besides the direct effects of the laser beam there is a secondary effect, a temperature elevation, that we could measure at the level of the RPE layer after laser treatment. The temperature increase measured in the center and at the border of the laser spot had the same tendency.
In our experiment the biochemical reactions of the RPE monolayer changed after laser photocoagulations. Using 1600 mW energy the glutamate uptake of our cells decreased as compared to 800 mW. An opposite effect was observed when examining the GABA uptake: the higher the energy for the treatment the higher was the GABA uptake.
We have perfomed a photodynamic treatment (PDT) for a retinal capillary haemangioma (RCH). We looked upon and monitorized the diameter of the feeder and draining vessel of the RCH under clinical conditions.
We have shown that a decrease of the diameter of both feeder and draining vessel was observed. This decrease was present five days after the treatment and did not change six months after the treatment.

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VIKTÓRIA SZABÓ (2008)

Study of mutations and polymorphisms in ocular diseases

Supervisor: Zoltán Zsolt Nagy

The aim of my Ph.D. thesis was to examine genetic mutations and polymorphisms thought to be potentially important in the mechanism of the autosomal dominant congenital stationary night blindness (adCSNB), and in two multifactorial disorders of patients treated with photorefractive keratectomy. In the course of our study on mutational analysis of *GNAT1* gene we identified a novel heterozygous C to G alteration (c.598C>G) in exon 6 of transducin α-subunit gene that should result in the p.Gln200Glu substitution in the highly conserved Switch 2 region of α-transducin. The three dimensional computer modeling of transducin suggests that the mutant protein exhibits impaired GTPase activity, thereby leading to constitutive activation of phototransduction and causing adCSNB. In patients treated with photorefractive keratectomy, first a genetic study of severe subepithelial

corneal haze was performed. Since this clinical condition resembles the lumican-null mouse phenotype, mutation analysis of lumican and keratocan genes was carried out. There was no evidence that germ-line mutation of lumican and keratocan genes participate in the aetiology of subepithelial corneal haze. Our findings suggest that the mechanisms of the development of severe corneal opacity are different in humans after PRK compared to the lumican-deficient knockout mouse model. In patients treated with photorefractive keratectomy, a further association study of steroid-induced ocular hypertension and functional polymorphisms of glucocorticoid receptor was carried out. Since variation in responsiveness to glucocorticoids observed in healthy population is influenced by genetic polymorphisms of the glucocorticoid receptor gene, we investigated whether variants N363S, ER22/23EK, and Bcl I may contribute to steroid-induced ocular hypertension. In cases where prednisolone acetate was administered, we found a significant correlation between N363S heterozygosity and steroid-induced ocular hypertension. Genotyping of N363S polymorphism and identifying high risk steroid responders may allow an individual therapy to avoid steroid-induced ocular hypertension.

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GABRIELLA SZATMÁRY (2007)

The role of perimetry in the diagnosis of neuro-ophthalmic disorders and its implications to neural plasticity of visual perception

Supervisor: Ildikó Süveges

In summary, we conducted two complementary clinical studies in which we investigated the neuro-ophthalmological applicability of recently developed perimetry techniques.
In our prospective study (Study A), we tested a new algorithm: Swedish Interactive Thresholding Algorithm (SITA) Fast against the gold standard perimetry: Goldmann visual field (GVF) in 64 consecutive patients with either severely decreased vision (20/200 or worse) (n=50 eyes) or neurological impairment (n=50 eyes). The recent development of the SITA family of perimetry has allowed for shorter testing time in normal subjects and in glaucoma patients. However, its usefulness for detecting visual field defects in patients with poor vision or neurological disease has not been evaluated. We categorized the results into 1 of 9 groups based on similarities and reliabilities. Overall, GVF and SITA Fast were equally reliable in 77% of eyes and showed similar visual field results in 75% of all eyes (70% of eyes of patients with severe neurologic deficits and 80% of eyes with poor vision). The mean±SD duration per eye was 7.97±3.2 minutes for GVF and 5.43±1.41 minutes for SITA Fast ($p<0.001$). Thus, our results suggest that for the general ophthalmologist and neurologist, visual field testing with SITA Fast perimetry might even be preferable to

GVF, especially if performed by a marginally trained technician, even in patients with severely decreased vision or who are neurologically disabled.
In our pilot study (Study B), we tested a novel technique: functional MRIperimetry (fMRI-perimetry) on a neuro-ophthalmological patient against healthy controls.
We performed three functional MRI experiments on this pilot patient in addition to SITA Standard perimetry, electrophysiological testings, optical coherence tomography (OCT) and contrast sensitivity. We describe the findings of these visual functional and structural tests. We found that performance on fMRI perimetry closely correlates with pattern deviation (PD) performance as assessed by static automated perimetry.
In conclusion, both Study A and Study B provide important results that have relevance in everyday clinical practice of ophthalmic, neurologic and neuro-ophthalmic patients with disease processes affecting any parts of the afferent visual system.

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MÁRTA TÓTH (2008)

Detection of glaucomatous retinal ganglion cell loss with scanning laser polarimetry

Supervisor: Gábor Holló

Glaucoma, one of the leading causes of blindness is induced by progressive apoptotic loss of the retinal ganglion cells. To detect glaucoma early in the disease process and to assess the possible progression, quantitative measurement of the ganglion cell number is needed. Retinal nerve fibre layer thickness measurement with scanning laser polarimetry (SLP) was developed for these purposes. SLP was introduced to the clinical practice fifteen years ago, but it is still under continuous development. In my research I focused on the clinical application of the commercially available software (GDx-VCC) as well as a new software version (GDx-ECC) presently under development. Using the ECC software the image quality improves in cases when the image is "atypical" and therefore difficult to classify with the VCC software.
I investigated the influence of ECC on image atypicality and anterior segment retardation noise; the effect of myelinated retinal nerve fibres on the VCC and ECC imaging; the correlation between GDx-VCC and two types of visual field testing methods, namely standard automated perimetry (SAP), and matrix frequency-doubling perimetry (M-FDT) which is considered specifically sensitive for the detection of early damage; the screening ability of SLP, M-FDT and another structural diagnostic technique, scanning laser tomography (SLT); the suitability of VCC and ECC to the detection of glaucoma progression.
Our results showed that ECC can effectively neutralize both image atypicality and anterior segment's noise; the influence of myelinated retinal nerve fibres on the SLP images varies between the software versions and according to the retinal location of the disorder; SLP

has a better structure-function relationship with M FDT than with SAP; the combined use of SLP and M-FDT can be considered a useful tool for screening, but use of SLT with the present software cannot; finally, the earliest SLP indicator of glaucoma progression is the increase of the long term variability of a GDx-ECC parameter, called NFI.

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LÁSZLÓ BALÁZS VARSÁNYI (2007)

Clinical and molecular fenetic examinations in patients with congenital achromatopsia

Supervisor: Ágnes Farkas

Achromatopsia is a rare, autosomal recessively inherited retinal disorder, characterized by low visual acuity, inability to distinguish colours, nystagmus and photophobia. The background of the disorder is the lack of functioning cone photoreceptors. In the present work, results of twelve Hungarian patients with achromatopsia, obtained from several examinations, including various electrophysiological, psychophysical, morphological and molecular genetic analyses, were analysed.

Causative pathogenic mutations were found in one of the known achromatopsia genes (CNGA3, CNGB3) in each case. Two of these mutations were novel mutations; the pathogenicity was proved using molecular biological methods. In the search for new candidate genes for achromatopsia, linkage analysis was performed on chromosome 14.

Clinical examinations were used to distinguish the complete and incomplete forms of achromatopsia. Subjective psychophysical methods (dark adaptation, relative brightness matching and spectral luminosity testing) showed some residual colour vision function in some patients. We also tried to use full-field ERG with colour stimuli as an objective method, but no detectable residual cone function could have been detected in any of the patients. OCT was used to assess the morphological changes in the patients in vivo. The macular structure was altered, retinal thickness and total macular volume was lower in patients with achromatopsia.

Achromatopsia is a stationery disorder, but to assess the changes during lifetime, we compared the functional and morphological data of the "younger" and "older" patients with achromatopsia. This could help designing a "gene-therapeutic window" for the forthcoming gene therapy. ERGs showed no remarkable difference, but using OCT a significant thinning of the retina in the "older" patients was found, compared to the "younger" ones. This could be due to degeneration of the cone-related structures in the retina. This finding emphasises a need for an "as early as possible" gene therapy. Early proper diagnosis of achromatopsia is absolutely necessary, investigating the early, not specific signs of achromatopsia accurately, also with molecular genetic methods.

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Castellan C, Dollfus H, Legius E, Anastasi M, Bitoun P, Lev D, Sieving PA, Munier FL, Zrenner E, Sharpe LT, Cremers FP, Wissinger B (2005) CNGB3 mutations account for almost 50% of all cases with autosomal recessive achromatopsia Eur J Hum Genet 13: 3–12.

PROGRAM 2/11.

RADIOTHERAPY

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Program overview

Accreditation of a Ph.D. Program in 2001 in radiotherapy was very important, as half of the radiotherapeutists, radiation physicists and radiobiologists with scientific degrees currently active in Hungarian radiotherapy will retire within five years. The Ph.D. Program includes the announcement of Ph.D. courses and the provision of scientific work for the Ph.D. students. Accreditation and initiation of Ph.D. Program in radiotherapy would promote the training of adequately-prepared personnel and the establishment of a critical, comprehensive view for further modernization of the training and research. The organized Ph.D. training will ensure the possibility for the forward advancement of the most talented young specialists. During the past three years, eight Ph.D. students took part at the Program, and two of them have finished successfully their education.

Ph.D. graduate		***Supervisor***
Zsuzsanna Póti	pt	Olga Ésik

pt, part-time

Abstract of Ph.D. thesis successfully defended in 2007

ZSUZSANNA PÓTI (2007)

Radiobiological and clinical investigation on breast brachytherapy (The clinical consequences of radiation induced toxicity)

Supervisor: Olga Ésik

In patients who have undergone breast-conserving surgery, the role of sole brachytherapy of the tumor bed is currently not unambiguous. My aim was to investigate the radiation-induced toxicity and cosmesis in a total of 70 women diagnosed with stage I or II breast

carcinoma who had previously participated in postoperative ^{60}Co MDR-APBI. Throughout the BT, the 50 Gy dose delivered at 5 mm from the surface of the source was administered during 10–22 h-long treatment. Assumption of a 10-mm safety margin surrounding the tumor bed resulted in a 72 cm^3 median CTV irradiated with a reference dose of 28 Gy. In the assessment of the skin and subcutaneous toxicity, the RTOG and my own late radiation morbidity scoring system were applied. The radiosensitivity of the cultured fibroblasts was determined by clonogenic assay to check whether individual radiosensitivity played a role in the development and course of the radiation-induced side-effects. The results revealed a population of 34 subjects at the end of the follow-up study: 27 patients with tumor-free breasts and 7 with breasts erroneously (tumor-free) ablated/excised for misinterpreted radiation-induced sequelae. A total of 97% of the cohort had grade≥2, and 59% had grade≥3 radiation-induced toxicity. Grade 3 telangiectasis was observed 41%, 88% had localized fibrosis of some grade, and 35% had grade≥3 fibrosis. 41% of the cohort displayed fat necrosis, which was often accompanied by grade≥3 fibrosis and/or telangiectasis. The cosmetic results were poor in 50%. The radiosensitivity of the fibroblasts was increased in 8% of the patients, in agreement with data published for the general population. Comparisons our fibrosis prevalence data with those of others allowed an estimate of 0.47 h^{-1} for the rate of recovery from DNA damage in the fibroblasts. The results of my investigation allow the conclusion that ^{60}Co MDR-APBI with a limited CTV (median 72 cm^3) and a low total dose (28 Gy) is associated with a high rate (59%) of grade≥3 radiation-induced toxicity and a high rate (50%) of poor cosmetic outcome at the end of the 12-year follow-up. Although the relatively high BT dose rate (1.3–2.8 Gyh^{-1}) applied during the short overall treatment time (10–22 h) and a possible geographic miss (close to skin implantation) may have contributed to the development of these sequelae, the radiobiologically unfavorable effects of BT on normal tissues are also likely to have contributed to the observed toxicities.

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- *Ésik O, Póti Zs, Nemeskéri C, Mayer A, Szalai G, Sáfrány G, Trón L, Antal G, Glavák C, Repa I (2004) Suboptimal planning of earlier clinical studies does not exclude the acquisition of useful information for present or future practice: in response to Drs. Vicini, Edmundson and Arthur. Int J Radiat Oncol Biol Phys 60: 345–346 .*
- *Poti Zs, Nemeskeri C, Fekeshazy A, Safrany G, Bajzik G, Nagy ZP, Bidlek M, Sinkovics I, Udvarhelyi N, Liszkay G, Repa I, Galuska L, Tron L, Mayer A, Esik O (2004) Partial breast irradiation with interstitial ^{60}Co brachytherapy results in frequent grade 3 or 4 toxicity. Evidence based on a 12-year follow-up of 70 patients. Int J Radiat Oncol Biol Phys 58: 1022–1033.*

PROGRAM 2/12.

CLINICAL AND EXERIMENTAL RESEARCH IN ANGIOLOGY

Coordinator:
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Program overview

The education of clinicians and researchers having profound scientific background is the basic aim of the postgraduate Program, offering a possibility of research work on organ failures due to vascular disorders and of application of the results in the therapeutic processes. The study of the pathogenesis of the diseases of ischemic origin using the latest techniques is also part of the Program.

Titles of research projects	***Supervisors***
Relations of arteriosclerosis and chronic uraemia	György Acsády
Overview and planning of clinical studies of therapeutic angiogenesis	György Acsády
Physiological and pathological adaptation of venous system in cases hemodinamical stress	György Acsády
Assessment, monitoring of cardiopulmonal perioperative risks and treatment options in cardiovascular surgical diseases	János Gál
The role and problems of endovascular graft implantation in treatment of aneurysms	Kálmán Hüttl
The role of genetic factors in restenosis	István Karádi
Complex examination of left ventricle aneurysm	Béla Merkely
Clinical use of homologous vessel transplantation and experimental research on vessel preservation	Attila Nemes
Examination of vessel structures infected by *Chlamydia pneumoniae*	Zsuzsa Schaff
Molecular biological aspects of dilatative cardiomyopathy and myocarditis of viral origin	Péter Sótonyi
The role of regulatory function of Nociceptin in cardiovascular disease and its control	Péter Sótonyi
Radiological investigation and geometric analysis of aortic aneurysm	Péter Sótonyi
The cardiovascular effects of apelin	Miklós Tóth

Ph.D. students		***Supervisors***
Zsuzsanna Cserép	ft	János Gál
István Hartyánszky	pt	Béla Merkely
Miklós Krepuska	ft	György Acsády
Gábor Ferenc Molnár	ft	Attila Nemes
Zsófia Panna Patkó (Joóné)	ft	György Acsády
Tamás Mirkó Paukovits	ft	Kálmán Hüttl

Csanád Várallyay	ft	Kálmán Hüttl
Zoltán Szeberin	pt (a)	György Acsády
Ph. D. candidates		***Supervisors***
Al-Sieady Ali Mohsen	it	György Acsády
Gábor Bíró	i	Attila Nemes
Ph. D. graduates		***Supervisors***
Ágnes Petrohai	pt	György Acsády
Gábor Vallus	i	István Karádi

a, absolutorium; pt, part-time; ft, full-time; i, individual; it, international

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

ÁGNES PETROHAI (2007)

Cardiac allograft vasculopathy after heart transplantation

Supervisor: György Acsády

Heart transplantation has been available for more than ten years in Hungary for serious, terminal stage heart failure patients, as a last resort treatment. They are young people, who are finally capable to get back to their family after operation and they are able to work and get incorporated fully to the society. I wish to contribute with my present dissertation to the devoted work of all my colleagues engaged with the heart transplantation by summing up and collating my time honoured clinical work and experience.

The most important factors, which *significantly* affect the outcome of the heart transplantation:

1. Gender of the donor
2. Infection after heart transplantation
3. Gender of the recipient.

The most important factors, which not significantly, but *greatly* affect the outcome of the heart transplantation:

1. Rejection
2. Accelerated graft arteriosclerosis
3. Lymphoproliferativ disease after heart transplantation
4. High blood pressure after heart transplantation
5. Hyperlipidemia after heart transplantation.

New methods, which would improve our results:

1. The citrate synthase test, as important heart function marker, is to be run from serum
2. The citrate synthase test is to be also run in the histopathology
3. Monitoring of autoantibody serum level by measurement
4. Donor and recipient HLA cross-match test after operation
5. Monitoring of immunsuppressed transplant patients by flow cytometric examination.

- *Petrohai A, Nagy G, Bosze Sz, Hudecz F, Zsiros E, Paragh Gy, Nyarady Z, Nemeth P, Berki T (2005) Detection of citrate synthase-reacting autoantibodies after heart transplantation: an epitope mapping study. Transpl Int 17: 834–840.*
- *Nyárády Z, Czömpöly T, Bősze Sz, Nagy G, Petrohai Á, Pál J, Hudecz F, Berki T, Németh P (2006) Validation of in siloco prediction by in vitro immunserological results of fine epitope mapping on citrate synthase specific autoantibodies. Mol Immunol 43: 830–838.*
- *Harmati L, Karlócai K, Petrohai Á (2001) A szívátültetés gyakorlata Magyarországon. Családorvosi Fórum 2: 23–26.*

GÁBOR VALLUS (2008)

Certain genetic risk factors of restenosis in peripheral atherosclerotic disease

Supervisor: István Karádi

The apolipoprotein E genotypes and CETP play a central role in lipoprotein metabolism. The apolipoprotein E genotypes and the genotypes of CETP Taq1B and I405V gene polymorphisms were investigated in patients with accelerated atherosclerosis and restenosis requiring reoperation after femoropopliteal reconstructive surgery. Prothrombotic factors contribute to thrombotic complications not only at the venous site of the circulatory system but may promote arterial restenoses and accelerated atherosclerosis, too. In a part of the above mentioned patient group the frequency of factor V leiden mutation was detected compared to healthy controls.

Our results: There was no difference in the distribution of CETP Taq1B genotypes in vascular patients compared to controls. The same results were found in ischemic heart disease, therefore this gene polymorphism has no effect on atherosclerosis and related restenosis. The number of carrying V allele of CETP I405V genotypes in vascular patients was significantly increased compared to age and sex matched control subjects. This is the first observation in peripheral vascular disease. The same data were published for V allele frequency in patients suffering from ischemic heart disease. The presence of V allele increase the risk of restenosis in patients operated on for femoropopliteal atherosclerosis. The carrier state of ε4 allele of apolipoprotein E gene polymorphism in patients with accelerated atherosclerosis increases the risk for later restenosis.

The presence of factor V Leiden mutation can be observed in the majority of venous thromboembolic diseases (40–70%). We detected a significantly higher frequency of Leiden mutation in patients with accelerated atherosclerosis and reoperated for restenosis compared to healthy controls. This observation calls the attention to consider in these patients a long-distance low molecular weight heparin administration, which may decrease the chance for later restenosis.

- *Horvath A, Fust G, Horvath I, Vallus G, Duba J, Harcos P, Prohaszka Z, Rajnavolgyi E, Janoskuti L, Kovacs M, Csaszar A, Romics L, Karadi I (2001) Anti-cholesterol antibodies (ACHA) in patients with different atherosclerotic vascular diseases and healthy individuals. Characterization of human ACHA. Atherosclerosis 156: 185–192.*
- *Karadi I, Vallus G, Nagy B, Dlustus B, Skopal J, Nagy Z, Papp Z, Romics L (2001) Factor V Leiden mutation in severe femoropopliteal atherosclerosis with restenosis. Eur J Int Med 12: A194.*

• *Vallus G, Dlustus B, Acsády Gy, Papp Z, Skopál J, Nagy Z, Prohászka Z, Romics L, Karádi I, Nagy B (2007) Factor V Leiden and apolipoprotein E genotypes in servere femoropopliteal atherosclerosis with restenosis. Clin Chim Acta 377: 256–260.*

PROGRAM 2/13.

HORMONAL REGULATORY SYSTEMS

Coordinator:
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Program overview

There are numerous important interactions among the hormonal system and neural, immune and other regulatory mechanisms, by which hormones may influence the physiology or pathophysiology of organs or organ systems. The program includes research projects dealing with interactions between hormones and other regulatory mechanisms, such as neuroendocrine regulation of thyroid and gonadal function, and regulation of pituitary and adrenal hormone secretion. Other research projects include studies on the molecular mechanisms of hormone sensitivity, pathomechanism of metabolic bone disorders associated with endocrine disorders, hormonal disturbances associated with inborn metabolic errors, and the development of microsensors for the detection of gene variants involved in hormonal regulation.

Titles of research projects	*Supervisors*
Effect of endocrine alterations on the epidemiology of certain diseases	Nándor Ács
Neurohormonal regulation of thyroid function	Csaba Balázs
Neurohormonal interactions	Ida Gerendai
Functional genomic studies in the pathogenesis of adrenal tumors especially focusing on the expression of cytokines and their receptors	Péter Igaz
Micro-RNA studies in endocrine tumors	Péter Igaz
Disorders of genes encoding mitochondrial electron trasport enzymes in tumors of the endocrine system	Attila Patócs
Molecular mechanisms determining glucocortocoid sensitivity	Károly Rácz
Interactions of regulatory mechanisms in diseases of the pituitary and andrenal glands	Károly Rácz
Clinical and pathophysiological studies in gynecological and reproductive endocrine disorders and in experimental models	János Rigó

Mechanisms of endocrine disease associated bone metabolism disorders	Miklós Tóth
Steroids as antioxidants	Gábor Békési

Ph. D. students		***Supervisors***
Henriett Butz	ft	Károly Rácz, Attila Patócs
Karolina Feldman	ft	Károly Rácz
Nikoletta Lendvai	ft	Attila Patócs
Balázs Stenczer	ft	János Rigó
Péter Szabó	ft	Péter Igaz
Márk Szeverényi	pt	Nándor Ács
Zsófia Tömböl	ft	Péter Igaz

Ph. D. candidates		***Supervisors***
Rita Bertalan	ft	Károly Rácz
Belema Boyle	ft	Csaba Balázs
László Fűtő	pt	Miklós Tóth
Péter Gergics	ft	Károly Rácz
Gábor Csongor Mezei	i	János Rigó
Ágnes Mondok	ft	Károly Rácz
Péter Reismann	i	Károly Rácz
Márta Sereg	ft	Miklós Tóth
Ágnes Szappanos	ft	Miklós Tóth
Judit Tőke	ft	Miklós Tóth

Ph. D. graduates		***Supervisors***
Zita Halász	i	Károly Rácz
István Likó	i	Károly Rácz
Andrea Luczay	i	Károly Rácz
János László Tanyi	i	János Rigó

p, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

ZITA HALÁSZ (2008)

Genetic causes of hereditary multiple hypophysis hormone deficiency. Investigation of *PROP1* gene mutations in Hungarian patients

Supervisor: Károly Rácz

In this work I analysed the outcome of growth hormone (GH) replacement treatment in patients with inherited form of multiple pituitary hormone deficiency (MPHD) and examined disease-causing mutations of pituitary transcription factor genes which may underlie MPHD. The results showed that after treatment for a longer than 7-yr period with a GH-preparation available under well-controlled distribution, the mean height of children with GH-deficiency reached the normal national reference range adjusted for age and sex. After establishment of clinical criteria for screening *PROP1* gene mutations, I performed mutational analysis of all coding exons of this gene in 35 patients with MPHD. With these studies, diseases-causing *PROP1* gene mutations were detected in 15 of the 35 patients (43%). It was also found that more than 80% of mutant alleles were accounted for by those containing the 150delA and 301–302delGA mutations of the *PROP1* gene. Importantly, these findings indicated a high relevance of mutational "hot spots" of the *PROP1* gene in Hungarian patients with MPHD and they also offered an opportunity for the development of rational and cost-effective screening strategy. When clinical and hormonal findings of MPHD patients with and without *PROP1* gene mutations were compared, the results showed that GH-deficiency was diagnosed at earlier age of life in patients with *PROP1* gene mutations, but the severity of growth retardation at the time of diagnosis of H deficiency or the age of patients at the time of manifestation of other pituitary hormone deficiencies (TSH, LH, FSH and ACTH) were similar in the two groups of patients. In 15 MPHD patients without *PROP1* gene mutations, the exon 6 of the *POU1F1* gene containing a mutational "hot spot" was also examined but no mutations were found. Thus, these results do not support a significant role of the mutational "hot spot" of the *POU1F1* gene in Hungarian MPHD patients. Finally, I introduced a method for the detection of mutations of the *PITX2* gene, a pituitary transcription factor that plays a role not only in pituitary development and differentiation but also in the lateralization of organs. With the use of this method, I performed mutational analysis of all coding exons of this gene in an exceptionally unique patient who had both situs inversus totalis and MPHD, but no mutation was found. Thus, the findings in this patient failed to indicate that mutation of the *PITX2* gene is involved in the pathomechanism of situs inversus totalis associated with MPHD.

- *Halász Z, Tőke J, Patócs A, Bertalan R, Tömböl Z, Sallai A, Hosszú É, Muzsnai Á, Kovács L, Sólyom J, Fekete Gy, Rácz K (2006) High prevalence of PROP1 gene mutations in Hungarian patients with childhood-onset combined anterior pituitary hormone deficiency. Endocrine 30: 255–260.*
- *Halász Z, Bertalan R, Tőke J, Tömböl Zs, Losonczi L, Patócs A, Sólyom J, Fekete Gy, Rácz K (2007) Multiple pituitary hormone deficiency. Its genetic background and the role of pituitary transcription factors. Magy Belorv Arch 60: 419–424 (in Hungarian).*

• *Halász Z, Bertalan R, Tőke J, Patócs A, Tóth M, Fekete Gy, Gláz E, Rácz K (2008) Laterality disturbance and hypopituitarism. A case report of co-existing situs inversus totalis and combined pituitary hormone deficiency. J Endocrinol Invest 31: 74–78.*

ISTVÁN LIKÓ (2007)

Structure-function analysis of protein variants causing disturbances in glucocorticoid regulation

Supervisor: Károly Rácz

An extensive review of reported clinical cases of the glucocorticoid resistance syndrome indicated, that the majority of the reported mutations are located in the ligand-binding domain of the glucocorticoid receptor gene. Since the three-dimensional structure of the ligand-binding domain of the glucocorticoid receptor gene has been recently elucidated, we applied a comparative protein modelling technique to predict the functional consequences of nucleotide sequence variants of the ligand-binding domain of the glucocorticoid receptor gene causing glucocorticoid resistance syndrome (Ile559Asp, Asp641Val, Gly679Ser, Val729Ile, Ile747Met, Leu753Phe) and to study the potential significance of novel nucleotide sequence variants of the same domain discovered by our *in silico* approach (Met646Thr, Leu647Pro, Ser651Tyr, Ile701Leu, Phe715Cys). We showed that three-dimensional comparative protein modelling of mutations located in the the ligand-binding domain of the glucocorticoid receptor offers an excellent technique to analyse the effect of amino acid replacements on glucocorticoid receptor function and to correlate structural abnormalities to clinical findings. Also, this protein modelling may be of great value to examine the potential significance of previously undescribed sequence variants discovered by our *in silico* approach, although these should be validated by population-based studies. With the use of plasma hormone measurements we showed that previously undiagnosed congenital 17α-hydroxylase/17,20-lyase deficiency may occur in adult patients with hypertension and hypokalemia. We found that congenital 17α-hydroxylase/17,20-lyase deficiency in two unrelated adult patients was due to a novel mutation of the CYP17 gene, which resulted in a change of arginine to cytein at amino acid position 440 of the P450c17 enzyme. The effect of this novel mutation on 17α-hydroxylase/17,20-lyase activity was assessed by *in vitro* studies on the mutant and wild-type P450c17 enzyme generated by site-directed mutagenesis and expressed in nonsteroidogenic eukaryotic cell line. These studies showed that the mutant enzyme had negligible 17α-hydroxylase and 17,20-lyase activities. In addition, we showed that three-dimensional comparative protein modeling of the heme-binding site of the P450c17 enzyme provides a useful tool for the evaluation of the role of arginine at position 440, and that replacement of arginine by cystein predicts a loss of the catalytic activity of the enzyme.

• *Liko I, Igaz P, Patocs A, Toth S, Pazmany T, Toth M, Racz K (2004) Sequence variants of the ligand-binding domain of the glucocorticoid receptor gene and their functional consequences on the three-dimensional protein structure. Curr Med Chem 24: 3229–3237.*
• *Patocs A, Liko I, Racz K (2005) Gene symbol: CYP17A1. Disease: Steroid 17-alpha-hydroxylase deficiency. Hum Genet. 116: 539.*

• *Patocs A, Liko I, Varga I, Gergics P, Boros A, Futo L, Kun I, Bertalan R, Toth S, Pazmany T, Toth M, Szucs N, Horanyi J, Glaz E, Racz K (2005) Novel mutation of the CYP17 gene in two unrelated patients with combined 17alpha-hydroxylase/17,20-lyase deficiency: Demonstration of absent enzyme activity by expressing the mutant CYP17 gene and by three-dimensional modeling. J Steroid Biochem Mol Biol 97: 257–265.*

ANDREA LUCZAY (2008)

Studies on glucocorticoid receptor gene polymorphism resulting in increased glucocorticoid sensitivity and on glucocorticoid overproduction in children

Supervisor: Károly Rácz

Glucocorticoids worldwide are one of the most frequently prescribed drugs. Because their therapeutic doses and the modes of their application are highly variably, it is of particular interest to obtain detailed information on the consequences of their long-term effects resulting in either a moderate or a substantial increase of glucocorticoid activity.

The role of the sensitizing N363S polymorphism of the glucocorticoid receptor was examined in patients with congenital adrenal hyperplasia (CAH). Non-classical CAH (NC-CAH) patients were not found among the carriers of the polymorphism. We found that girls who were carriers for this polymorphism had milder genital virilization at birth than mutation-matched non-carriers. In the carrier group the cortisol-treatment related obesity started at later age and was progressive during the puberty.

The N363S carrier frequency in obese children was similar to that found in the general Hungarian population. We found that the obesity related consequences of carbohydrate metabolism, the prevalence of hypertriglyceridemia and hypertension were different between the carriers and non-carriers, although these differences were not statistically significant.

The consequences of the endogenous cortisol excess were examined in two rare paediatric forms of Cushing's syndrome (ectopic CRH/ACTH producing pancreas acinar cell tumor and primary pigmented adrenocortical disease [PPNAD]). We found that the clinical findings were less dependent on the histological background but the duration of the glucocorticoid excess was an important factor.

• *Luczay A, Török D, Ferenczi A, Majnik J, Sólyom J, Fekete Gy (2006) Potential advantage of N363S glucocorticoid receptor polymorphism in 21-hydroxylase deficiency. Eur J Endocrinol 154: 859–864.*
• *Illyés G, Luczay A, Benyó G, Kálmán A, Borka K, Köves K, Rácz K, Tulassay T, Schaff Zs (2007) Cushing's syndrome in a child with pancreatic acinar cell carcinoma. Endocr Pathol 18: 95–102.*

JÁNOS LÁSZLÓ TANYI (2008)

Lysophosphaditic acid as a target for molecular diagnosis and therapy of ovarian cancer

Supervisor: János Rigó

Ovarian carcinoma has the highest mortality rate of all gynecologic malignancies owing to late diagnosis and a lack of effective tumor-specific therapeutics. Ovarian carcinogenesis and metastasis is accompanied by a complicated cascade of genetic, molecular, and biochemical events. Indeed over the last several years an ever expanding collection of aberrations has been identified in this tumor. Abnormal lysophosphatidic acid (LPA) production, receptor expression, and signaling are frequently found in ovarian cancers suggesting that LPA plays a role in the pathophysiology of the disease. Moreover, the LPA pathway may provide novel molecular targets, illustrating how the development of new therapeutic and diagnostic strategies can contribute to disease management. The recent identification of the metabolizing enzymes that mediate the degradation and production of LPA and the development of receptor selective-analogues open a potential new approach to the treatment of this deadly disease.

Understanding the physiologic aberrations that originate from genetic alterations of ovarian cancer will lead to the development of new therapeutic approaches for treating ovarian cancer. In this study it is clearly proven that aberrations of LPP production contribute to the progression of ovarian cancer, just as overexpression of these metabolizing enzymes return the physiologic situation and inhibit the growth of the cancer cells. Further manipulating the metabolism, increasing the expression of the enzyme involved in LPA production, can override the effect of the metabolizing enzymes. LPPs exert their effect through metabolizing extracellular LPA. This also clearly explains the excellent "bystander effect" seen in this study. LPA through its production, metabolism, and receptors may provide an excellent target for the development of molecular therapeutics, and the early detection of molecular forms of LPA, other lysolipids, and the activities of LPA pathway receptors and enzymes may facilitate both diagnosis and monitoring the response of a given patient to therapy. The impressive development of knowledge about the pathway regulating LPA production and the identification of selective LPA-receptor agonists suggest that targeting the LPA cascade could be a real addition to the management and treatment of this still-deadly disease. Additional studies of the LPA cascade and other phospholipids in ovarian cancer are essential to further elucidate their critical roles.

- *Tanyi JL, Morris AJ, Wolf JK, Fang X, Hasegawa Y, LaPushin R, Auersperg N, Sigal YJ, Newman RA, Felix EA, Atkinson EN, Mills GB (2003) The human lipid phosphate phosphatase-3 decreases the growth, survival, and tumorigenesis of ovarian cancer cells: Validation of the lysophosphatidic acid signaling cascade as a target for therapy in ovarian cancer. Cancer Res 63: 1073–1082.*
- *Tanyi JL, Hasegawa Y, Lapushin R, Morris AJ, Wolf JK, Berchuk A, Lu K, Smith DI, Kalli K, Hartmann LC, McCune K, Fishman D, Broaddus R, Cheng KW, Atkinson EN, Yamal JM, Bast RC Jr, Felix EA, Newman RA, Mills GB (2003) Role of decreased levels of LPP-1 in accumulation of lysophosphatidic acid (LPA) in ovarian cancer. Clin Cancer Res 9: 3534–3545.*

• *Umezo-Goto M, Tanyi JL, Lahad J, Liu S, Yu S, Lapushin R, Hasegawa Y, Lu Y, Trost R, Beyers T, Jonasch E, Aldape K, Liu J, James RD, Ferguson CG, Xu Y, Prestwich GD, Mills GB (2004) Lysophosphatidic acid production and action: Validated targets in cancer? J Cell Biochem 92: 1115–1140.*

PROGRAM 2/14.

CLINICAL AND EXPERIMENTAL RESEARCH ON UROLOGICAL DISEASES

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Program overview

The program offers research on several fields of clinical and experimental urology and andrology.

Titles of research projects	***Supervisors***
Hereditary and acquired disorders of the external genital and urological tracts	Zsolt Kelemen
Diagnostic and therapeutic challenges in urine retention and evacuation diseases	Péter Nyirády
Up to date clinical experimentel research of andrological diseases	Péter Nyirády
Reconstructive urology	Imre Romics
Novel diagnostic and therapeutic options in urological tumors	Imre Romics
Etiology and etiopathogenesis of urinary tract stones, challenges in drug therapy, surgical treatment and prevention	Imre Romics
Inflammatory disorders of the urinary organs	Imre Romics
Congenital anomalies of the kidney, urinary tract and external genital tract in newborn infants	Éva Görbe

Ph. D. students		***Supervisors***
Katalin Bedi	ft (a)	Imre Romics

Ph. D. candidates		***Supervisors***
Fares Mohammed Osman	it	Péter Nyirády
Péter Riesz	i	Imre Romics
Attila Szendrői	i	Imre Romics

Ph. D. graduates		*Supervisors*
Sándor Lovász	i	Péter Nyirády
Attila Majoros	i	Imre Romics

a, absolutorium; ft, full-time; i, individual; it, international

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

SÁNDOR LOVÁSZ (2008)

Urodynamic examination of pressure-flow relations in the upper urinary tract

Supervisor: Péter Nyirády

We set the aim of developing a urodynamic equipment especially for urodynamic studies of the upper urinary tract and a software for accurate measurement of pressure-flow relation, finding out the most appropriate method, defining the rate of postrenal obstruction quantitatively, and developing simplified methods to aid wide spread of urodynamic studies of the upper urinary tract.

Andromeda Ltd., Germany produced for us the prototype of the urodynamic equipment according to our design. We measured the flow rate belonging to stepwise rising hydrostatic filling pressure by using electronic drop sensor, and we calculated corresponding intrapelvic pressure from signals of electronic pressure sensor by subtracting actual flow resistance from measured pressure values. Accurate measurements were allowed by using filling solution of body temperature and software for calculation of mean values.

We established the method of multistep pressure-flow studies allowing us to accurately define pressure-flow relation, and we proved its parabolic character. We developed a novel PC software for calculation of coefficient of the parabolic curve that characterizes the rate of postrenal obstruction by a single number; the obstruction coefficient. This proved significant correlation with Whitaker-test and ureteric opening pressure. We introduced a new, easy-to-define and more reliable reference pressure, the perirenal pressure. In order to let spread our method we developed diuretic-test, which let us define obstruction coefficient even without the need of any urodynamic equipment.

- *Lovász S (2004) Új fejlesztésű urodinámiás készülék a felső húgyúti nyomás-áramlás vizsgálatok céljára. Magy Urol 16: 27–33.*
- *Lovász S, Nyirády P, Romics I (2004) A new concept for active ureteric occlusion during percutaneous nephrolithotripsy: the 'counter-flow' principle. BJU Int 93: 1355–1356.*
- *Lovász S, Riesz P (2005) Urodinámiás alapvizsgálatok a felső húgyúti obstrukciók megítélésében. III. A retroperitonális tér nyomása meghatározásának módszerei és jelentősége a felső húgyúti urodinámiás vizsgálatokban. Magy Urol 17: 150–156.*

ATTILA MAJOROS (2007)

The etiology and examinations of urinary incontinence

Supervisor: Imre Romics

Urinary incontinence after radical retropubic prostatectomy may cause a severe decrease in the life quality of the patient. The etiology of this symptom has been discussed by several studies so far, however no consensus has been accepted yet as for the causes of incontinence after prostatectomy. In this essay our aim was to determine the possible causes of incontinence after RRP, the independent risk factors for incontinence, and any possible anticipating methods to predict postoperative urinary incontinence. The functional, anatomic and preoperative factors leading to postoperative incontinence have been examined through the help of prospective and retrospective examinations. Our results proved that RRP has significant effect on the function of urethral sphincter, however, it has no significant impact on the bladder and pelvic muscles. Incontinence after surgery appears in stress incontinence mainly, which could be explained by the weakness of urethral sphincter. We can anticipate a more serious incontinence in those patients where the complex weakness of pelvic muscles or isolated weakness of urethral sphincter was observed before the surgery. The anal sphincterometry is capable of assessing the global status of pelvic mucles thus the possibility of severe postoperative incontinence; in addition this examination is less invasive and except for our study it has not been performed to assess incontinence so far. In the sphincteric function the maximal voluntary urethral pressure besides the maximal rest urethral pressure is considered to be very important, since in case the former one is normal, postoperative continence could be reached with a proper rehabilitation, regardless of a weaker rest urethral pressure before the surgery. The examinations proved that the total length of the posterior uretha after the surgery could be regarded as a predisposing independent risk factor for incontinence or reaching continence later. The total posterior urethra length is calculated on the sum of the sphincter and the length of urethral stump situated proximally from the sphincter. Older age will not inhibit reaching continence, however will delay it. Those patients under 65 who had no symptoms, complaints, urodynamical abnormalities, have good sphincteric function do have a good chance of reaching immediate postoperative continence regardless of tumor stage.

- *Majoros A, Bach D, Keszthelyi A, Hamvas A, Romics I (2006) Urinary incontinence and voiding dysfunction after radical retropubic prostatectomy (prospective urodynamic study). Neurourol Urodyn 25: 2–7.*
- *Majoros A, Bach D, Keszthelyi A, Hamvas A, Mayer P, Riesz P, Seidl E, Romics I (2007) Analysis of risk factors for urinary incontinence after radical prostatectomy. Urol Int 78: 202–207.*
- *Majoros A, Bach D, Keszthelyi A, Hamvas A, Mayer P, Seidl E, Riesz P, Romics I (2005) Befolyásolja-e a radikális prostatectomia utáni kontinenciát a betegség pathológiai stádiuma ? Uroonkológia 2: 71–74.*

PROGRAM 2/15.

MOLECULAR GENETICS, PATHOMECHANISMS, AND CLINICAL ASPECTS OF METABOLIC DISORDERS

Coordinator:
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Program overview

The program consists of 13 research sub-programs completed with appropriate theoretical courses for postgraduate students. Molecular as well as pathological and clinical aspects of different metabolic diseases are studied including metabolic bone diseases and disturbances of calcium metabolism and lipid metabolism, disorders in onco-hematology processes, endocrine glands, diabetes mellitus and vascular diseases. Ph.D. students are working under the supervision of a qualified scientist but also participate in the work of the laboratoty. Publication in peer-reviewed international journals is a requirement for a successful Ph.D. thesis.

Titles of research projects	*Supervisors*
Molecular genetics, pathomechanism, early diagnosis, prevention and therapy of chronic liver diseases	Margit Abonyi
Regulation of endocrine functions of fat tissue and its relation to insulin resistance	Károly Cseh
Metabolic aspects of malignant hematological disorders	Judit Demeter
Investigation of disorders associated with macro- and microvascular complications and risk factors of atherothrombotic vascular diseases	Csaba Farsang
Pituitary gland dysfunction: Clinical and experimental studies. Ghrelin and cell proliferation	Miklós Góth
Identification of genes participating in the stimulatory effect of ghrelin on cell proliferation	Miklós Góth
Effect of calcium and bone metabolism disorders and the drugs influencing mineral content, quality and mechanical properties of bone tissue	Csaba Horváth
Investigation of disorders associated with macro- and microvascular complications and risk factors of atherothrombotic vascular diseases	Zoltán Járai
Gestational diabetes as preexisting condition for type 2 diabetes and metabolic syndrome	Zsuzsa Kerényi
Thyroid disorders and their effects on bone metabolism	Péter Lakatos
Molecular genetics, pathomechanism, early diagnosis, prevention and therapy of chronic liver diseases	Péter László Lakatos
Molecular mechanisms in bone metabolism	Attila Mócsai
Extraskeletal effects of key genes and proteins of bone metabolism	Gábor Speer

Epidemiology, pathogenesis and diagnosis of thyroid cancers	István Takács
Pathophysiological aspects of normogylcemic control of diabetes mellitus	Gyula Tamás
Prevalence and incidence of diabetes and pathophysiological points of normoglycemic treatment	Gyula Tamás

Ph. D. students		***Supervisors***
Barbara Bokor	ft	Csaba Horváth
El Hadj Othmane Taha	i (a)	Zoltán Járai
Henrik Csaba Horváth	pt (a)	Gábor Speer
László Krivanek	ft	Csaba Farsang
Rita Magenheim	ft	Gyula Tamás

Ph. D. candidates		***Supervisors***
Bernadett Balla	ft	István Takács
János Kósa	i	Gábor Speer
Magdolna Krasznai	i	Károly Cseh
Áron Lazáry	ft	István Takács
Eszter Madarász	ft	Zsuzsa Kerényi
Judit Nádas	i	Károly Cseh
Éva Palik	i	Károly Cseh

Ph. D. graduates		***Supervisors***
Péter Fuszek	pt	Gábor Speer
Gyula Ádám Tabák	i	Gyula Tamás

a, absolutorium; pt, part-time; ft, full-time; i, individual; it, international

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

PÉTER FUSZEK (2007)

The epidemiology and pathogenesis of colorectal cancer (CRC). The redistribution changes in the localization of the CRC. The role of calcium metabolism and calcium sensing receptor and vitamin D receptor (VDR) gene of pathogenesis of CRC

Supervisor: Gábor Speer

Hungary is among the first in Europe regarding colorectal cancer (CRC) mortality. Epidemiology studies of the last 20 years found a change in CRC location (increasing of the proximal proportion of the CRC). Genetical as well as environmental factors, such as diet have important role in the development of CRC. There are several known genetic variations that may play a role in the development of this disease. Among these are the genetical variation such as a calcium sensing receptor (CaSR) gene A986S and of vitamin

D receptor (VDR) BsmI polymorphisms. Our goal was to study the change in the localization in our patient group (n=1738) between 1993 and 2004. In our CRC patients (n=70) we studied their calcium homeostasis, the genetical polymorphism of their CaSR and how these affect each other. We measured the connection between levels of the AFP, CEA, CA19-9 prognostic factors and the genotypes, and patients' calcium-metabolism. Our aim was to record the distribution of the CaSR/VDR polymorphism in patients with rectal tumor. We also studied the connection between the above genotypes and erb-2, EFGR, ras, p53 expression—as prognostic and diagnostic factors of CRC—furthermore with UICC staging for 5 years. Analysis of our data showed an increasing frequency of CRC in both sexes. There was no change in localization, but there were more rectal tumors among distal tumors. In our work we showed that patients with newly discovered CRC had serum calcium and ionized calcium levels below normal. There was no connection between CaSR A986S polymorphism and calcium metabolism or calcium levels. Frequency of CaSR and VDR polymorphism was identical between in the CRC and the control groups. The serum ionic calcium level was inversely proportional with the CA19-9 prognostic marker levels. We showed that in the presence of the VDR gene allel B (i.e. Bb and Bb genotypes) expression of the erb-2 gene is significantly higher, unlike in bb genotype. Among the patients with rectal cancer who died during five year follow-up, AA genotype of the CaSR gene was significantly more frequent compared to survivors at 5 years.

We conclude that it is of paramount importance to consider CRC-as as a priority in Hungary. Various attempts should be made to decrease incidence. Screening should be started. In Hungary it would be indicated to start this at age 50 in males and at age 55 in females. Our work points out the role of Vitamin D/VDR and calcium/CaSR system in the pathogenesis of colorectal cancers. Chemoprevention would be useful; using calcium and Vitamin D. Low level of these may play a role in the pathogenesis of CRC. Normalizing serum calcium level may influence slow malignant transformation in the colon, or would increase local vitamin D synthesis, having a similar effect. We can presume that by reaching the recommended daily calcium intake of 1000–1500 mg for a lifetime, we could decrease the alarming prognosticated frequency of colorectal cancers in our country, and at a very limited cost.

- *Fuszek P, Lakatos P, Tabak A, Papp J, Nagy Z, Lakatos PL, Speer G (2004) Significance of serum calcium levels in colorectal cancer. Orv Hetil 145: 1561–1565.*
- *Fuszek P, Lakatos P, Tabak A, Papp J, Nagy Z, Takacs I, Horvath HC, Lakatos PL, Speer G (2004) Relationship between serum calcium and CA 19-9 levels in colorectal cancer. World J Gastroenterol 10: 1890–1892.*
- *Lakatos PL, Halasz J, Keresztes K, Fuszek P, Jackel M, Poros A, Morvay K, Lakatos L (2004) Familial adenomatous polyposis coli. Case report. Orv Hetil 145: 813–817.*

GYULA ÁDÁM TABÁK (2008)

Quality control in diabetes mellitus

Supervisor: Gyula Tamás

In 1989 the acceptance of the St. Vincent Declaration underlined the public health importance of diabetes, urged the development of treatment standards and health care recommendations and aimed to reduce diabetes related morbidity and mortality. According to the Declaration a Basic Information Sheet (BIS) and its computer version were developed and became widely accepted all over Europe. To collect quality control data willing centers participated in the DiabCare Hungary Project. Between 1994 and 2000 altogether over 11 thousand records were entered into the DiabCare Hungary database. All presented analyses were carried out along two main aspects of care: process and outcome. Participating diabetes care centers were provided with feed-back on quality indicators immediately upon receiving their data. Yearly quality improvement and educational meetings were organized for the participants and anonymous bench-marking data were published. The completeness of basic data was acceptable. The collected data seemed to be representative of the care provided in diabetes care centers, thus giving insight into the care of type 1 diabetes and some information on the care of insulin treated type 2 diabetes. We tested indirect standardization to compare centers with different patient populations. The adherence to current hypertension and lipid guidelines were evaluated longitudinally. The proportion of patients with unacceptable blood pressure and LDL values decreased significantly between 1994 and 2000. We compared the completeness of the ADA standards and outcome indicators among representative samples of diabetic patients from the DiabCare Hungary database and from representative surveys from the US. These data suggest the superiority of centralized diabetes care over decentralized care. Patients treated by general practitioners rarely receive the recommended standard of care in either of the countries. Risk factors for erectile dysfunction were evaluated in a cross-sectional analysis using the quality control data collected during the DiabCare program. We confirmed the association between erectile dysfunction and age, diabetes complications, antihypertensive treatment. The tools and interventions tested during the years of the DiabCare project are readily available for quality assurance and improvement. Our results showed the effectiveness of 2 these interventions along with the wide gap between clinical reality and recommended care. The collected data in addition to quality control purposes might be used for epidemiological hypothesis generation and public health planning.

- *Tabák ÁGy, Kerényi Zs, Pénzes J, Tamás Gy (1999) Poor level of care among diabetic patients: Is that a unique picture? (letter). Diabetes Care 22: 533–535.*
- *Tabák ÁGy, Tamás G, Zgibor J, Wilson R, Becker D, Kerényi Zs, Orchard TJ (2000) Targets and reality: A comparison of health care indicators in the U.S. (Pittsburgh Epidemiology of Diabetes Complications Study) and Hungary (DiabCare Hungary). Diabetes Care 23: 1284–1289.*
- *Tamás Gy, Tabák ÁGy, Stella P, Hidvégi T, Pénzes J, Földesi I, Kerényi Zs, DiabCare Hungary Group (2002) Assessing and comparing quality of diabetes care: a standardized European monitoring system (DiabCare) and a Basic Information Sheet for Diabetes and Pregnancy. Diabetol Hung 10 (Suppl 2): 60–66.*

PROGRAM 2/16.

DERMATOLOGY AND VENEROLOGY

Coordinator:
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Program overview

The Ph.D. program of the Department Dermatology-Venerology and Skin Oncology at Semmelweis University Ph.D. School aims to fill a gap in development of skin and venereal diseases that will provide support for scientific research, education and postdoctoral training of the specialty. The foundation of this new doctoral program stems from a subprogram that belonged to the Molecular Medicine Ph.D. School since 1998. Graduates from this sub-program have already started an independent research work. As a result of the scientific activity of the group, it gained accreditation as a joined research group of the Hungarian Academy of Sciences. The previous Ph.D. program "Basics of Human Molecular Genetics and genetic Diagnostics" under which our sub-program was listed could no longer harbor all that diverse clinical and research activities that include venereology, STD-related microbiology, genetics, dermatologic immunology and dermatologic oncology. Our department has accumulated a very extensive dermato-venereologic clinical data that requires further research that eventually will benefit to our patients in understanding their diseases and will result in providing better healthcare. Our program is being transferred from the Molecular Medicine Ph.D. School and will continue the research of monogenic inherited skin diseases. Our department is also the home of the National Epidermolysis Bullosa Center that carries out studies in the genetics of this severe inheritable group of diseases and has established successful prenatal diagnostics in the lethal forms. Diagnostics is now provided for more than 20 genes at the moment that are responsible for epidermolysis bullosa, Darier's disease and Hailey-Hailey disease and ichthyosis. The analysis of the genotype-phenotype relation as well as macro- and microalterations will give further insight into the pathophysiologic events in keratinocytes. Our facilities and expertise enable us to carry out clinical as well as basic science. A close scientific partnership is reflected by the introduction of two co-program leaders on the field of stem cell research. The skin is the largest organ in our body and also serves as the largest organ of our immune system. The skin is easily accessible and has great regenerating potential. The therapy of inherited, immunologic and all erosive skin diseases could benefit from a better understanding of the nature of *epidermal stem cells*. We wish to join the hot research area of stem cells with the tracking of stem cells of bone marrow transplanted recipient patients and with the use of an animal model. Our future aim, along with investigating skin differentiation and the dynamics of keratinocytes, is to explore the potential in gene therapy. In the current situation with the closing of the National Institute for Dermato-Venerology the university clinic got the obligation to further care of STD patients in form of a state center for *STD diseases* with national coverage

that is based on the previous expertise from the above mentioned institution. To this area of dermatology is given special attention in our Ph.D. program. Beside the classical STD's, the altered immune reactions of HIV positive patients and opportunistic infections that frequently occur among HIV positive individuals are being investigated. This program is strongly supported by a complete microbiological laboratory, including the National Mycology Reference Laboratory, which also belongs to the department. This activity provides a diagnostic background for rare infectious diseases and also is in the process of introducing novel molecular biology diagnostic tools that yield new research data and scientific achievements. A long-lasting successful research activity on *autoimmune blistering skin diseases and gluten sensitive diseases*, like dermatitis herpetiformis and celiac disease, is well indicated by the fact, that in this field one Ph.D. work had been completed, and two further Ph.D. works are in progress. The large number of patients, the regular and careful study of circulating and tissue bound autoantibodies render good possibilities for the project. *Pharmacogenomics*, a new resarch area of the institute, is also based on the large number of patients with drug induced side effects on the skin. Within the planned biobanking, genetic and bioinformatic studies we started to elaborate material and data from patients with drug side effects. Our further goal is to focus to the predictivity of drug induced damages. Colleting data we plan to get important information about the pathomechanism of toxicodermas as well. With our *Pharmacogenomics* Ph.D. course we join the molecular toxicology, bioinformatics and pharmacology as well. Two years ago the Dermato-Venerology Clinic changed its name incorporating the *Skin Oncology* words as well, to underline the extended activity of the institute on the field. Scientific goal: the rapidly growing number of melanoma malignum forces us to organize extended preventive programs with organizing auto-investigation of the skin, and dermatological screening of the Hungarian population. The UV induced carcinogenesis, the develoment of skin tumors and its molecular biological background is also one of our ongoing studies. The Center of the Lymphoma Group of the Hungarian Dermatological Society is also in our clinic. Clinical, immunohistochemical, therapeutical and pathological features of cutaneous lymphomas will be worked up. Epidemiology would be part of different themas: incidence of STD diseases, skin tumors, melanomas, cutaneous lymphomas is planned to be evaluated.

Titles of research projects	***Supervisors***
Clinical and immunological studies in autoimmune bullous dermatological diseases	Sarolta Kárpáti
Pharmacogenomics: pharmacogenomic investigation of molecular mechanisms in toxicoderma	Sarolta Kárpáti
Stem cell research in dermatology	Sarolta Kárpáti
Occurrence, prognostic and etiological factors and investigation of therapeutic modalities in cutan lymphoma	Márta Marschalkó
Molecular genetic investigations in genodermatosis	Márta Medvecz
Stem cell research in dermatology	Éva Mezey
Microbial organisms as pathogens, cofactors and opportunistic infections in retroviral infections	József Ongrádi
Health related quality of life and disease burden assessment in chronic conditions, with special focus on dermatologic diseases	Mária Péntek
Do MRSA strains form a distinct subspecies in the *Staphylococcus* genus?	Ferenc Rozgonyi

Prevalence, resistance to antibiotics, *in vitro* and *in vivo* pathogenic characteristics of coagulase negative staphylococci in nosocomial infections	Ferenc Rozgonyi
Application of molecular microbiological methods in the rapid microbiological diagnostics	Ferenc Rozgonyi
Molecular genetic diagnosis of *Neiserria gonorrheae* infections and resistance to antibiotics	Ferenc Rozgonyi
Molecular pathogenic and taxonomic examination of Methicillin-resistant *Staphylococcus aureus* (MRSA)	Ferenc Rozgonyi
Elaboration of a prevention program to improve the early recognition of melanoma	Beáta Somlai
Allergic skin diseases	Erzsébet Temesvári

Ph. D. students		***Supervisors***
István Almási	pt	Ferenc Rozgonyi, Ian M. Gould
Andrea Horváth	ft	Ferenc Rozgonyi
Gábor Malmos	ft	Ferenc Rozgonyi
Veronika Tóth	pt	Beáta Somlai

Ph. D. candidates		***Supervisors***
Nóra Erős	i	Márta Marschalkó
György Pónyai	i	Sarolta Kárpáti
Zsuzsa Szabó	ft	Ferenc Rozgonyi
Tibor Vág	i	József Ongrádi

Ph. D. graduates		***Supervisors***
Antal Zsolt Blazsek	i	Sarolta Kárpáti
Péter Holló	i	Márta Marschalkó
Klaudia Preisz	i	Sarolta Kárpáti
András Ujházy	i	Sarolta Kárpáti

pt, part-time; ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006 and 2008

ANTAL ZSOLT BLAZSEK (2008)

A possible role of Torque teno virus infection an a triggering agent in autoimmune bullous dermatoses

Supervisor: Sarolta Kárpáti

The TT virus (TTV), a member of the single-stranded DNA virus genus *Anellovirus* as been shown to commonly infect humans, yet with no detectable pathogenicity. Recent studies imply that TTV may contribute to provoking autoimmune progresses in the pathogenesis of systemic lupus erythematosus and idiopathic inflammatory myopathies —diseases able to cause frequent, in some cases severe skin manifestations. Since TTV was demonstrated to be present in the skin, we wanted to investigate, whether this virus is able to trigger skin-specific autoimmunity. Viruses had been also hypothesized to contribute to disease pathogenesis in autoimmune bullous skin disorders including bullous pemphigoid (BP), pemphigus vulgaris (PV), and dermatitis herpetiformis (DH)—but this thesis has never been solidly proven. We analyzed known antigen determinants of BP (BP180 NC16 and C-terminal domains; BP230), PV (desmoglein3, Dsg3), PF (desmoglein1, Dsg1) and DH (tissue and epidermal transglutaminase [TGM2 or TGc] and [TGM3 or TGe]) for similarity to TTV originated proteins. Online BLAST comparisons were done at different levels, on longer but less matching (proteinprotein BLAST) and on short exact matches (SEM BLAST). TTV similarities on large scale were only detected for BP while TTV SEM results were found for all antigens. When BLAST results were compared to predicted antigenic sites within the targeted autoantigens only BP180 was found to show overlaping antigenic and TTV similarity sites. We assessed the prevalence of TTV DNA in these disorders and compared them to results from healthy blood donors. Detection of TTV was carried out by nested PCR and real-time PCR in the sera of 93 patients with bullous skin disorders, 95 age and sex matched healthy blood donors, and 50 healthy blood donors age and sex matched for the bullous pemphigoid patient group. Identity of the PCR products was confirmed by sequencing. We found, that the detection rate of TTV DNA in the whole bullous group of patients was comparable with that in healthy controls (74/93 (77.89%) vs. 62/95 (65.26 %); $p<0.076$), as was in the group of PV where patients had no increased prevalence of TTV (19/33). Patients with DH showed a slight, but not significant tendency to carry TTV DNA (17/20). The TTV DNA prevalence in the BP group was significantly elevated (36/40; $p<0.032$). Age, gender, activity, presence of antigen-specific auto-antibodies or disease duration had no influence on TTV positivity in either group. We further analyzed whether lymphocytes of BP patients recognize a conserved region of the TTV proteins. For this we used a synthetized peptide sequence in a lymphocyte transformation test. Lymphocyte cultures of 4 BP patients and 3 otherwise healthy controlls were assayed with different peptide concentrations. While all BP patient lymphocytes reacted strongly with the peptide, only one control sample showed a minor reaction at only one peptideconcentration. We concluded, that only the BP lymphocytes recognized the peptide. Our data suggests that the persistence of TTV in the human body may contribute to the pathogenesis of BP.

- *Gergely P Jr, Blazsek A, Dankó K, Ponyi A, Poór Gy (2005) Detection of TT virus in patients with idiopathic inflammatory myopathies. Ann NY Acad Sci 1050: 304–313.*
- *Gergely P Jr, Pullmann R, Stancato C, Otvos L Jr, Koncz A, Blazsek A, Poor G, Brown KE, Phillips PE, Perl A (2005) Increased prevalence of transfusion-transmitted virus and cross-reactivity with immunodominant epitopes of the Hres-1/P28 endogenous retroviral autoantigen in patients with systemic lupus erythematosus. Clin Immunol 116: 124–134.*

PÉTER HOLLÓ (2006)

Prognostic and pathogenetic factors in psoriasis. Analysis of the efficacy of synchronous balneophototherapy using immunological and neuroendocrine parameters

Supervisor: Márta Marschalkó

Immunological and neuroendocrine local regulatory mechanisms of the skin play an important role in the pathogenesis of psoriasis. No clinical or laboratory parameters are used widespread to monitorize and predict the clinical outcome of the disease.

The aim of my clinical study was to find prognostic data for the outcome of the synchronous balneophototherapy treatment and find correlation between clinical subtypes of psoriasis and their answer to treatment. Except confluating type there was no significant difference between answer to synchronous balneophototherapy of the clinical subtypes, patients with confluating skin eruptions have worse chance to clear.

Laboratory investigations targeting peripheral mononuclear cell cutaneous lymphocyte associated antigen (CLA) expression and peripheral blood beta-endorphin level examined the correlation of these parameters with the clinical outcome of the disease.

On the basis of the results of mononuclear cell CLA expression analyses, patients could be divided into two groups. Responder patients remained symptom-free at the follow-up control, their mononuclear cell CLA expression showed consequent decrease. The second group of patients had a clinical relapse at the follow-up control, their CLA expression increased during treatment and decreased while having clinical relapse. Responder patients showed a significant higher mononuclear cell CLA expression before treatment. Peripheral blood beta-endorphin level showed no significant change before and after synchronous balneophototherapy treatment.

The results show that clinical type of the skin symptoms and initial CLA expression and its change are predictors of the disease outcome during synchronous balneophototherapy.

- *Holló P, Gonzalez R, Kása M, Horváth A (2005) Synchronous balneophototherapy is effective for the different clinical types of psoriasis. JEADV 19: 578–581.*
- *Holló P, Marschalkó M, Temesvári E, Gonzalez R, Hársing J, Horváth A (2005) Follow-up analysis of circulating mononuclear cell CLA expression in patients with psoriasis. J Derm Sci 39: 131–133.*
- *Holló P, Bender T, Marschalkó M, Gonzalez R, Barna I, Horváth A (2004) No significant change of plasma β-endorphin levels of psoriasis patients after synchronous balneophototherapy. Photodermatol Photoimmunol Photomed 20: 205–209.*

KLAUDIA PREISZ (2006)

New observations in autoimmune bullous skin diseases

Supervisor: Sarolta Kárpáti

Clinical and immunofluorescence studies in autoimmune bullous diseases are presented. Between 1998 and 2003 I evaluated 2180 direct and 4435 indirect immunofluorescence (IF) studies in the immunofluorescent laboratory of the Department of Dermatology, Venereology and Dermatooncology of Semmelweis University. I had the possibility to investigate some interesting, rare cases in details.

I identified new autoantigens: bullous pemphigoid (BP) 180, desmocollin (Dsc) 2 and Dsc 3 by examining some paraneoplastic pemphigus (PNP) patients' sera.

I also detected circulating IgA and IgG autoantibodies against pulmonary tissue in the sera of one PNP patient. These antibodies were possibly involved in the pathomechanism of the severe lung disease.

Beside routine IF examinations of the 116 dermatitis herpetiformis (DH) patients treated by our clinic, I joined to the "DH research group" of our laboratory.

I examined small bowel biopsy samples from DH and coeliac patients with double fluorescence labeling. The binding of monoclonal anti-tissue transglutaminase (TGc) antibody and the deposition of IgA gave similar staining pattern in the IF studies. With confocal microscopy I found a clear colocalization of extracellular tissue-bound IgA and TGc in all patients with DH and coeliac disease.

In 64% of the 116 DH skins studied, a significant vascular staining accompanied the DH-specific granular IgA fluorescence. With dual IF labeling, skin IgA colocalized with epidermal transglutaminase (TGe) in the vessel walls, and within the DH-bodies. The frequent occurrence of IgA and C3 vascular fluorescence mostly in the superficial, small vessels of DH patient's skin, and the colocalization of IgA and TGe, support the possible role of immune complex precipitation in the pathomechanism of skin symptoms.

- *Holló P, Preisz K, Nemes L, Bíró J, Kárpáti S, Horváth A (2003) Linear IgA dermatosis associated with chronic clonal myeloproliferative disease. Int J Dermatol 42: 143–146.*
- *Preisz K, Horváth A, Sárdy M, Somlai B, Hársing J, Amagai M, Hashimoto T, Nagata Y, Fekete S, Kárpáti S (2004) Exacerbation of paraneoplastic pemphigus by cyclophosphamide treatment: detection of novel autoantigens and bronchial autoantibodies. Br J Dermatol 150: 1018–1024.*
- *Preisz K, Sárdy M, Horváth A, Kárpáti S (2005) Immunoglobulin, complement and epidermal transglutaminase deposition in the cutaneous vessels in dermatitis herpetiformis. J Eur Acad Dermatol Venereol 19: 74–79.*

ANDRÁS UJHÁZY (2008)

The prevalence and risk factors for the most frequent lower genital tract infections among adolescent and young females

Supervisor: Sarolta Kárpáti

Genital *Chlamydia trachomatis* infection is of public health importance due to its well documented late consequences on the upper genital tract. Early diagnosis is a prerequisite for the prevention of salpingitis, tubal damage, ectopic pregnancy and chronic pelvic pain in females.

My aim was to investigate the prevalence of chlamydia among a young female population in Hungary and to evaluate the association between genital chlamydial infection and behavioral, historical, demographic correlates and to determine the role of mucopurulent cervicitis in predicting *Chlamydia trachomatis* infection. In addition, a possible association between endocervical chlamydia infection and vaginal infection was also tested

Each participant completed a self-administered and anonymous questionnaire. A total of 408 consecutive, unselected, self-referred sexually active nonpregnant women aged 16–24 years were enrolled, but only 387 patients filled out the questionnaires properly. A standardized gynecologic examination was done, including a colposcopy of the lower genital tract, oncocytology and bimanual pelvic examination. Identification of *C. trachomatis* was by polymerase chain reaction *(Amplicor PCR CT kit, Roche Diagnostic Systems Inc.)*. Vaginal microbial disorder was evaluated by light microscopy.

The prevalence of chlamydial infection in the study population was 7% (27/387). The prevalence of endocervical *C. trachomatis* infection was higher among those with as compared to those without signs and symptoms of lower genital tract infection ($p=0.036$; OR: 2.33; 95% CI: 0.98–5.63). The prevalence of chlamydial infection below 20 years of age was 7.9% (17/214). In this age group the prevalence of endocervical chlamydia was also higher among those with as compared to those without symptoms of vaginitis ($p=0.002$; OR: 5.17; 95% CI: 1.51–22.42). Mean age at first sexual intercourse (coitarche) was 16.5 years in patients infected by *C. trachomatis*, and 17.2 years among those not infected ($p=0.032$). Women who had at least three life-time partners were more likely to have *C. trachomatis* in the cervix compared to those who had two or less partners ($p<0.001$; OR: 22.25; 95% CI: 3.56–917.47). Urethral discharge in the sexual partner was associated with endocervical chlamydia being significantly more common among those infected than not infected ($p=0.019$; OR: 7.38; 95% CI: 1.11–36.77). Below 20 years of age a significant difference was observed between the chlamydia positive and negative patients with regard to the number of sexual partners they had had in the preceding 3 months. The frequency of chlamydial cervicitis was significantly higher among adolescent females with 2 or more sexual partners during the preceding 3 months ($p<0.05$; OR: 5.75; 95% CI: 1.14–23.41). In patients with a positive endocervical chlamydia finding, the prevalence of bacterial vaginosis was significantly higher (33.3%) compared to those not harboring chlamydia in the cervix (11.6%) ($p<0.005$; OR: 3.79; 95% CI: 1.46–9.63). Overall, 55.6% of patients with *C. trachomatis* infection had co-existing vaginal disorder compared to 30% of the chlamydia negative patients ($p<0.01$; OR: 2.92; 95% CI: 1.24–6.9). Among patients with bacterial vaginosis the rate of endocervical chlamydial infection was significantly higher (17.6%) than among those without vaginal disorder (4.5%) ($p<0.005$; OR: 4.5; 95% CI: 1.63–12.4). Beside the 7% prevalence of chlamydia, the sensitivity, specificity and posi-

tive predictive value for microscopic diagnosis of mucopurulent cervicitis to predict the presence of *C. trachomatis* were: 51.8%, 71.7%, and 12.1% respectively.
The above mentioned risk factors might help to select those women in whom testing for endocervical *C. trachomatis* infection with PCR is suggested.
The prevalence of bacterial vaginosis was higher among females younger than 25 years (30.6%) compared to those above (18.2%) (p=0.01; OR: 1.99; 95% CI: 1.11–3.57). In the study the prevalence of the postoperative pelvic inflammatory disease following artificial abortion was only 1.3% (4/298). According to our data the universal perioperative antibiotic prophylaxis is not necessary.
Topical boric acid should be the first choice as a maintenance therapy in the prevention of relapses of recurrent vulvovaginal candidosis among non-pregnant women.

- *Ujházy A, Csaba Á, Máté Sz, Papp Z, Sziller I (2007) Chlamydia prevalence and correlates among female adolescents in Hungary. J Adolescent Health 41: 513–515.*
- *Ujházy A, Kristóf K, Sembery P, Jeney Cs, Széll A (2005) A méhnyak Chlamydia trachomatis fertőzését kísérő hüvelyfertőzések fiatal nőkben. Magy Nőorv L 68: 385–390.*
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SCHOOL OF PH. D. STUDIES

3. PHARMACEUTICAL SCIENCES

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General overview

The Doctoral School of Pharmaceutical and Pharmacological sciences focuses on two scientific disciplines:

- *Pharmacological research* is needed to select new active substances, to develop and use medicinal products. In addition, new scientific results and discovered relationships may help to understand functions of the living human organism.
- *Pharmaceutical research* is related to drug research, development of drug delivery systems as well as it is a prerequisite to produce and apply pharmaceutical preparations. Although pharmaceutical science involves the knowledge of other disciplines (e. g. chemistry and medical science), but the evaluation of medicinal products requires specialised knowledge from the viewpoint of this interdisciplinary science.

The objective of the Doctoral School is to train qualified experts with an internationally recognized scientific degree (Ph.D.) for pharmacological and pharmaceutical research. Scientific results of the above mentioned research topics will be summarized in doctoral thesis and research papers which will be published in international journals of high impact. Research topics provide students with theoretical and practical experience in different fields of pharmaceutical and pharmacological sciences. Special problems are covered by the research projects of the educational Program:

- Study of bioactive substances of plant origin in connection with phytochemical and biological evaluation as well as biotechnological production; pharmaceutical chemistry and analysis; design, manufacturing and biopharmaceutical evaluation of novel dosage forms; clinical pharmacy and pharmacoeconomics; study of organic compounds with potential bioactivity; investigation of medical and pharmaceutical aspects of biology and environmental protection;
- Pharmacodynamic investigations; pharmacokinetic and drug metabolism; influence on neurochemical transmission; study of neurodegenerative and neuroprotective mechanisms; cardiovascular pharmacological investigations; separation methods and their applications; study of drugs affecting on calcium and bone metabolism; human study of cytostatic drugs; role of ion transport mechanisms controlling neurochemical transmission.

PROGRAM 3/1.

MODERN TRENDS IN PHARMACEUTICAL SCIENCES

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Program overview

The scientific-education scope of the participating 15 institutes/departments provides the eligible professionals (pharmacists, medical doctors, chemists, biologists, physicists, chemical engineers) with a wide selection of topics in the fields of fundamental and specific drug-oriented research, including current problems of inorganic, organic, physical, analytical, bioinorganic, bioorganic, and coordination chemistry, biology, biophysics, biotechnology, botany, microbiology and virology.
The specific topics of drug- and pharmaceutical research are drug design and synthesis, structure-activity relationships, mechanism of action of drugs, drug-receptor binding, isolation of active compounds from natural sources, drug metabolism, biochemical toxicology, relationships between physicochemical properties and biological function, pharmacognosy, elucidation of biosynthesis of natural compounds, pharmacokinetics, drug-drug interactions, transport mechanisms, biopharmaceutics, pharmaceutical technology, physical pharmacy, chemical pharmacy and social pharmacy.

Titles of subprograms and research projects	*Supervisors, coordinators*
Subprogram 1.	
Production of bioactive compounds by biotechnological methods	Éva Szőke
Optimization of active substance formation by biotechnological methods (fermentation, bioregulation, gene transformation) in tissue and cell cultures of medicinal plants	Éva Szőke, László Kursinszki
Study on role of endogenous formaldehyde in C1 metabolism and biosynthesis related to C1 fragments	Lehel Hullán
Research and production of bioactive lignanes of plant origin by *in vitro* cell cultures for therapeutical use	Miklós László, István Gyurján, Zoltán Krisztóf
Study on protective response of plants induced by elicitors in case of *in vivo* and *in vitro* systems	Károly Bóka
Subprogram 2.	
Phytochemical and biological evaluation of bioactive substances of plant origin	Éva Lemberkovics
Active ingredient content assays of medicinal plants and their preparations	Éva Lemberkovics, Éva Szőke
Formation of bioactive compounds in medicinal plants	Éva Szőke, Andrea Balázs

Research of plant-derived active substances for phytotherapeutical purpose	Ágnes Kéry
Symptoms of heavy metal contaminations in medicinal plants	Béla Böddi
Metals and metal ions in medicinal plants and their extracts in consideration of dosage forms	Klára Szentmihályi
Subprogram 3.	
Pharmaceutical chemistry and drug analysis	Béla Noszál
Microspeciation of bio- and drug molecules	Béla Noszál
Study on cyclodextrines regarding the ability to form inclusion complexes	Ágnes Barcza-Buvári
Appplication of chiroptical, CD/UV and NMR spectroscopy in analysis of chiral and natural compounds	András Gergely
Development and application of high resolution separation methods for analysis of bioactive molecules and drug candidates	Miklós Idei
Rational drug desing in signal transduction therapy	György Kéri
Preparation of kinase inhibitor molecules by rational drug design	György Kéri
Study on novel molecules with selective tyrosine kinase effect: modelling relationships between chemical stucture and biological effect	György Mészáros
Study on pathobiochemical processes of cancerous and inflammatory diseases regarding to role of kinases and to development of drug candidates	Tibor Vántus
Investigations of nonlinear chemical phenomena	Krisztina Kurin Csörgei
Role of combinatorical chemistry and informatics in the design and preparation of new drug candidates	László Őrfi
Study on relationships between molecular properties and chemical structure, role of lipophilicity	Krisztina Novák Takács
Chromatographic analysis of amino acids and amines	Ibolya Molnár Perl
Development of microanalytical methods and speciation of elements for studying biological systems	Gyula Záray
Subprogram 4.	
Design and preparation of modern dosage forms	István Antal
Investigation of excipient systems used as drug carriers	Judit Dredán
Investigation of drug carrier systems with controlled and programmed drug release	István Antal
Study and optimization of pharmaceutical technological procedures	István Antal
Investigation of competitive interfacial processes in colloidal drug carriers	Ferenc Csempesz
Subprogram 5.	
Biopharmaceutical considerations of design and evaluation of pharmaceutical preparations	Sylvia Marton
Investigation of drug carrier systems with improved bioavailability	Sylvia Marton
Investigation of drug carrier systems with controlled and programmed drug release	Sylvia Marton
Analytical assay of drug carrier systems	Krisztina Ludányi

Study of intelligent, thermoresponsive drug carrier systems	Gabriella Csóka
Subprogram 6.	
Pharmacoeconomics and clinical pharmacy	Zoltán Vincze
Pharmacoeconomical investigations	Zoltán Vincze
Studies in clinical pharmacy	Romána Zelkó
Application of novel dosage forms in the clinical pharmacy	Romána Zelkó
Health care-economics, technological analysis	Ágnes A. Mészáros
Subprogram 7.	
Study of potentially bioactive organic compounds	Péter Mátyus
Study of potentially bioactive organic, heterocyclic compounds	Péter Mátyus
Design and synthesis of potentionally bioactive compounds	György Keserű
Subprogram 8.	
Pharmaceutical considerations of biology and environmental protection	Éva Ádám
Pharmacological and molecular biological investigations of histamine, its receptors responsible for action, histamine agonists and antagonists regarding the role in cell proliferation	Sára Tóth
Biological and clinical principles of chemotaxis	László Kőhidai
Study on action mechanism of pharmaceutical substances influencing membranes	Gabriella Csík
Study of molecular dynamic interactions on model membranes by spectroscopical methods	Pál Gróf
Study of glycoproteins and biomarkers by mass spectrometry	Károly Vekey
Structural bases and medical aspects of protein aggregation	Judit Fidy, László Smeller
Computer study of protein dynamics: role of dynamics in ligand binding	Erika Balog
Significance of glutamaterg neuron phenotype in parvicellular and magnocellular neurosecretoric systems	Ferenc Tölgyesi
Study on structural bases of functional interactions in proteins	Judit Fidy, László Smeller, Levente Herényi, Szabolcs Osváth
Study on structural bases of functional interactions in proteins	András Kaposi
Molecular imprinting polymers	György Horvai

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Gergely Völgyi	ft	Krisztina Novák Takács
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Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

SZABOLCS BÉNI (2007)

Equilibrium and structural characterization of imatinib protonation, cyclodextrin complex formation and liposome encapsulation

Supervisor: Béla Noszál

Equilibrium and structural aspects of protonation, cyclodextrin complex formation and liposomal interactions were studied for imatinib (Gleevec®), the active ingredient against chronic myeloid leukemia, introduced in 2001.

Acid-base properties of the tetravalent base imatinib were investigated by pH-potentiometric and ^{1}H NMR-pH titrations. The *N*-methyl-piperazinyl nitrogen has been identified as the most basic site, protonating in the physiological pH range. The site-specific equilibrium constants quantitate that the second hydrogen ion is attached 3.8 times preferably to the pyridyl nitrogen than to the "inner" nitrogen atom of the already cationic piperazine ring. Protonation of the phenylamino-pirimidine site occurs in highly acidic medium. The pertaining equilibrium constant could be accurately determined using dichloroacetic acid, as an *in situ* NMR-pH sensor molecule. The results allowed the calculation of con-

centration distribution for the variously protonated imatinib species at any pH value in aqueous solution or biological compartments.
A tenfold increase in the solubility of neutral imatinib was achieved with the aid of β-cyclodextrin. Good agreement has been found between complex stability constants determined by pH-potentiometry, NMR-cyclodextrin titration and phase-solubility UV studies. Both β-CD and its random methylated derivative (RAMEB) were shown to form the most stable complex with the uncharged form of imatinib. Interestingly, the affinity of its mono-, di- and tricationic species to β-CD gradually increases in the respective order, while their RAMEB complexes exhibit the expected descending order of stability. The ROESY NMR spectrum suggests that the *p*-disubstituted phenyl ring of imatinib is included in the cavity of β-CD.
Unexpectedly significant interaction was detected between imatinib and the membrane of DPPC liposomes. The drug increases the membrane rigidity for small unilamellar vesicles (SUV), whereas a slight increase in membrane fluidity was observed for multilamellar vesicles (MLV). Effects of neutral and monocationic imatinib on MLV membranes are identical, due to their similar interactions with the head groups of lipids. The decrease in SUVs' membrane fluidity is caused by fusion and micellarization, corroborated by both light scattering and freeze-fracture electron microscopy.

- *Szakács Z, Béni Sz, Varga Z, Őrfi L, Kéri Gy, Noszál B (2005) Acid-base profiling of imatinib (Gleevec) and its fragments. J Med Chem 48: 249–255.*
- *Béni Sz, Szakács Z, Csernák O, Barcza L, Noszál B (2007) Cyclodextrin/imatinib complexation: Binding mode and charge dependent stabilities. Eur J Pharm Sci 30: 167–174.*
- *Béni Sz, Budai M, Noszál B, Gróf P (2006) Molecular interactions in imatinib-DPPC liposomes. Eur J Pharm Sci 27: 205–211.*

JUDIT BALOGH (2007)

Preparation and examination of TPN systemys for the individual clinical therapy

Supervisor: Romána Zelkó

Lipid emulsions have been used in routine clinical practice for more than 40 years. Intralipid, the first well tolerated lipid emulsion, is still the most commonly used lipid emulsion worldwide containing long-chain triglycerides (LCT) with a fatty acid chain length of 16–20 carbon atoms (long-chain fatty acids, LCFA). Structured triglycerides, in which both medium-chain fatty acids and long-chain fatty acids are esterified to the same glycerol molecule, have positive metabolic effects, which make them competitive or even more efficient as an energy source compared with conventional fat emulsions. The purpose of my thesis was to compare the kinetic stability of two admixtures containing different lipid components. A further aim was to collect more evidence for the stabilizing effect of structured triglycerides, with special concern to the ionic concentration of the mixtures.
Kinetic stability of two total nutrient admixtures prepared with different lipid emulsions (Intralipid and Structolipid, respectively) was tracked under different storage conditions with an array of physicochemical methods. Several methods were applied for the assessment of physical stability of lipid emulsions, including particle size analysis via photon correlation spectroscopy, and microscopy. While these methods can follow physical

changes, zeta-potential and pH measurements are able to indicate chemical processes that take place along with storage. Dynamic surface tension measurements could provide additional information concerning the physicochemical processes that take place on the surface of the lipid droplets. Electrolytes play an especially important role from this point of view, as they are present in all admixtures and have a major effect on the zeta potential of the emulsions. Very likely, the formation of a "mixed" interfacial layer formed from the medium and the long chain fatty acids in case of structured triglycerides is responsible for the more efficient stabilization. Droplet size distribution and surface tension data showed that the emulsions containing structured lipids proved to be more stable, especially at lower storage temperatures. Higher electrolyte concentrations of the mixtures can adversely influence this stabilizing effect. The obtained results indicate that besides the advantageous metabolic effects of structured triglycerides, their application is recommended to improve the physical stability of TPN mixtures.

- *Balogh J, Bubenik J, Dredán J, Csempesz F, Kiss D, Zelkó R (2005) The effect of structured triglycerides on the kinetic stability of total nutrient admixtures. J Pharm Pharmaceut Sci 8(3): 552–557.*
- *Balogh J, Kiss D, Dredán J, Puskás I, Csempesz F, Zelkó R (2006) Tracking of the kinetic stability of two types of total nutrient admixtures containing different lipid emulsions. AAPS Pharm Sci Tech 7 (4): 98.*

MIKLÓS BOROS (2007)

Synthesis of NMDA and its amide derivatives. Determination of the site- and rotamer-specific basicities

Supervisor: Béla Noszál

NMDA (*N*-methyl-*D*-aspartate) is a selective glutamate receptor agonist, a widely used *in vitro* test compound in pharmacologycal studies. NMDA has also been identified as an endogenous molecule in mammalian central nervous systems, acting as a local hormone. NMDA receptors are important in memory formation, synaptic plasticity, whereas their malfunctions (mostly over-excitation) are related to barely curable neurodegenerative disorders such as Huntington's disease, CNS ischemia, and Alzheimer's disease. Developing specific and subtype-selective antagonists is therefore a major goal of drug design.

In this work, protonation equilibria of NMDA and its derivatives are characterized at the macroscopic and microscopic levels. ^{1}H NMR-pH and pH-potentiometric titrations were carried out to determine macroconstants that quantitate the association of hydrogen ions at the molecular level.

Protonation of the three ionizing moieties takes place in a partly overlapping pH ranges. NMDA microconstants were obtained by appropriate combination of acidity and NMR parameters of the parent compound and its three synthetic derivatives. These derivatives were close models of the NMDA minor microspecies, allowing the calculation of all the 12 microconstants, the pH-dependent concentrations of the 8 microspecies, and 3 site-interactivity parameters. Reliability of the microconstants was assessed by 3 independent test methods. pH-dependent distribution diagrams of the 8 NMDA microspecies allow the identification of the most populated ones. According to recent pharmacological studies, active species on the NMDA receptor are part of the major protonation pathway.

Rotamer populations and rotamer-specific protonation microconstants of NMDA were also determined from ^{1}H NMR vicinal coupling constants using Karplus-type equations. pH-dependent distribution of rotamers with different protonation stages are calculated. Pharmacophore models resulted in distance values between atoms of the receptor-active conformer of NMDA. We have calculated all the possible distances between atoms of the rotamers by molecular mechanic calculations, and compared with the reported values. Excellent agreement was obtained in one of the gauche rotamers.
A novel practical method for the synthesis of NMA (*N*-methyl-*DL*-aspartic acid) and new syntheses for NMA amid and ester derivatives are also described. Thin layer chromatography systems were developed to separate the important compounds as well.

- *Boros M, Kökösi J, Vámos J, Noszál B (2006) Complete resolution of the microscopic protonation equilibria of N-methyl-(D)-aspartic acid and related compounds. J Pharmaceut Biomed Anal 43: 1306–1314.*
- *Boros M, Kökösi J, Vámos J, Kövesdi I, Noszál B (2007) Methods for syntheses of N-methyl-DL-aspartic acid derivatives. Amino Acids 33: 709–717.*
- *Boros M, Vámos J, Kökösi J, Szokán Gy, Rácz A, Noszál B (2003) Amidcsoport szelektív kialakítása dikarbonsavakban. Acta Pharm Hung 73: 51–59.*

ORSOLYA CSERNÁK (2008)

The effect of protonation on the stability of cyclodextrin inclusion complexes

Supervisor: Ágnes Barcza-Buvári

In the present work, the effect of protonation on the stability of cyclodextrin inclusion complexes was investigated. For this purpose, the complex formation of various organic acid and base protonation forms and cyclodextrins was studied to assess their stability. The behaviour of protonation forms of the following acids has been investigated in the presence of β-cyclodextrin: homologous series of aliphatic α,ω-dicarboxylic acids from oxalic acid up to adipic acid, diethylmalonic acid, maleic acid and fumaric acid. The formation constants were determined by pH-potentiometry combined with competitive UV-Vis spectrophotometric measurements. Based on the measured constants some very interesting conclusions could be drawn on the role of intra- and intermolecular H-bonds in stabilization of inclusion complexes. Although β-cyclodextrin generally forms more stable complexes with undissociated acids than with their strongly hydrated, deprotonated derivatives, a relatively high and unexpected increase of inclusion complex stability could be detected in some half-dissociated species with intramolecular H-bonds. This is probably due to the better space filling of the compact structure caused by the intramolecular H-bonds.
When investigating organic bases stability of cyclodextrin complexes of some alkaloid salts used as medicaments was determined in different protonation forms. The poor water solubility of the free base and the high dissociation constant (K_A) often hinders the assay of alkaloid salts. Different cyclodextrin derivatives form complexes of appropriate stability to keep the base in solution and at the same time to shift favourably the protonation equilibrium. Based on these findings we have elaborated a new method of alkaloid titration that can be carried out in aqueous media by choosing the most appropriate CDs depending on the solubility and the basicity of the free base and the size of the molecules.

Therefore the use of cyclodextrins can provide an alternative, environment friendly assay for many salts of weak bases in aqueous media beside the pharmacopoeial methods.

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ÁDÁM ZOLTÁN DÁVID (2006)

Application of microwave and near-infrared electromagnetic radiation in recent pharmaceutical technological procedures and investigations

Supervisor: István Antal

The aim of my work was to broaden the applicability of microwave and NIR techniques in the pharmaceutical technology. Spectroscopic methods relying on the NIR wavelength range can be applied in many analytical investigations connected to pharmaceutical technology, while microwaves are beneficial when controlled heating or moisture detection of total volumes are in concern. During my investigations I have studied the possibility of completive application of these two techniques in pharmaceutical technology.

I have investigated the process of scale-up in a microwave vacuum granulating instrument, with special interest in the radiation dose–effect relations. As a result, it was possible to numerically characterize the nonadditive permittivity properties of multicomponent systems.

With the combined application of microwave moisture determinations and NIR spectroscopy I have investigated the changes during the moistening of a model active ingredient. Relying on the findings it can be concluded that during moistening chemical reactions take place as well due to secondary chemical (H-bridge) bonds.

I have shown the disparate behavior of pharmaceutical excipients during microwave drying. Relying on the rate constants derived from the drying kinetic experiments, I have sorted the materials into different groups. This grouping was in good correlation with the behaivor that the materias showed durin moistening and drying.

With nondestructive NIR spectroscopy I have determined the active ingredient and excipient content of powders and tablets compressed from them. Applicability of the method was verified with HPLC measurements.

A new off-line coupling of overpressured layer chromatography (OPLC) to NIR spectroscopy has been developed for qualitative and quantitative pharmaceutical analyitcal NIR spectroscopic determination of model substances following rapid separation. With the new coupled technique OPLC can be amplified with NIR spectral informations.

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MARIANN EGYEKI (2007)

Photosensibilization of the DNA and nucleoprotein complex. The mechanism of the photodynamic action and its application

Supervisor: Gabriella Csík

Lately photodynamic therapy is widely used as a treatment for cancer and antimicrobial (bacteria, fungi, viruses) inactivation. Photodynamic virus inactivation represents a very promising method in disinfection of blood and blood products. We wanted to verify that photoactivation of hematoporphyrin (Hp) is able to induced damages in DNAs of various accessibilities such as superhelical Bluescript plasmid DNA, isolated B-conformation DNA of T7 phage, T7 nucleoprotein complex, nuclear DNA of HeLa cells and viral DNA of simian virus (SV40). When isolated nucleic acid was dissolved in Hp containing buffer solution, porphyrin mediated photoreaction caused damages both in superhelical and B-conformational DNA. The reactive species generated by the photoactivated Hp were not able to reach DNA molecules surrounded by protein capsid or localized in the cell nucleus. We investigated the efficiency and the mechanism of action of two cationic and a tetraphenyl porphyrins in their photoreaction with T7 bacteriophage as surrogate of non-enveloped DNA virus. Alteration of photophysical parameters of the cationic derivatives indicated that they bind to the T7 nucleoprotein complex. The DNA-bound porphyrin reduced the viability of T7 phage in the dark. After irradiation, all the three investigated porphyrins were able to sensitize the model virus and irradiation leaded to significant decrease in the viability. The efficiency of the potoreaction was influenced by the ratio of bound and free porphyrin and by the aggregation and photodegradation of the derivatives. Our results suggest that both Type I and Type II reaction play a role in the phage inactivation. In the presence of the cationic porphyrins, the damage in the protein capsid and in the DNA can be responsible for the photodynamic inactivation of T7 phage. The photoreaction of tetraphenyl porphyrin altered the stability of the protein capsid.

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OLIVÉR ÉLIÁS (2006)

Synthesis of polycyclic diazines

Supervisor: Péter Mátyus

[*d*]-Annelated pyridazines have primarily been of interest as structurally related analogues of 4,5-disubstituted pyridazines possessing remarkable biological activities. These achievements have prompted us to elaborate new methodologies toward the syntheses of novel polyfused diazines. In the study, the following reaction and substance classes, respectively, have been particularly investigated.

The easily accessible 4,5-dichloro-2-methyl-6-nitropyridazin-3(2*H*)-one has been proven to be a convenient starting material for the preparation of novel series of [*c*]- and [*d*]-annelated pyridazino-oxazines and -oxazepines. The bicyclic fused pyridazines were converted into imidazo[4,5-*c*]- and pyrrolo[2,3-*d*]pyridazine systems. Moreover, by application of the *tert*-amino effect, some new tetrahydropyrido[2,3-*d*]pyridazines could be obtained. The mechanism of the latter isomerization reaction was carefully investigated, and it was concluded that some geometrical parameters of the starting vinyl compounds, determined by X-ray and/or NMR, correlated well with their cyclisation tendency. 5-iodo-2-methyl-pyridazin-3(2*H*)-one and 6-chloro-1,3-dimethyl-5-nitropyrimidin-2,4(1*H*,3*H*)-dione were the key intermediates for the syntheses of pyridazino[4,5-*b*]indole and the pyrimido[5,4-*b*]indole ring systems by the sequential combinations of two Pd-catalyzed reactions or one Pd-catalyzed reaction with a nitrene insertion reaction.

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HAJNALKA HANK (2008)

Data for characterizing genetically transformed cultures of *Atropa belladonna* L.

Supervisor: Éva Szőke

Atropa belladonna L. (deadly nightshade) contains several agents with pharmacological importance; it is an official drug in the VIIIth Hungarian Pharmacopoeia. The plant contains tropane- and pyrrolidine-base alkaloids; its main alkaloid is thyoscyamine which has parasympatholytic effect. Nowadays gene technological methods are widely used to increase the production of active ingredient beside the conventional tissue culturing procedures. During our experiments we investigated the growth of the genetically modified and nontransformed cultures of *in vitro* and *in vivo A. belladonna*, and its tropane alkaloid composition, specifically its hyoscyamine, scopolamine and 6OH-hyoscyamine content (LCMS- MS, HPLC). Genetical transformations mediated by *Agrobacterium rhizogenes* (R1601) in cultures was demonstrated by using polymerase chain reaction (PCR) and opine detection.

However several methods can be found in the bibliography for determination of alkaloid content using HPLC assessment, we elaborated a new, simple validated method that is suitable for the analysis of numerous and complex samples and that make possible a quick, accurate, qualitative and quantitative determination of alkaloid content. Alkaloid contents (0.52%, 0.40%, 0.35%) of the investigated hairy root clones (#K4, #K5 and #K8) reached or exceeded that of the root of the *in vivo* plants. According to our results in the universal and special metabolism and alkaloid production of the genetically transformed cultures, considerable differences can be observed among clones with different genetical

structure that formations resulted by random bacterial tDNA infiltration. Several additional factors can be beneficial for the formation and alkaloid production of the hairy root cultures; therefore we studied in details the effect of light and the carbohydrate content, nitrogen source, and macroelement content of B5 culture medium on the formation, tropane alkaloid content and production of the genetically transformed tissues.
All three hairy root clones spontaneously organized using liquid B5 culture medium. The *in vitro* transgenic plants were cultivated in vivo after micropropragation. Phenotypes of #K4 and #K8 clones differed from that of the mother plant and its morphological characteristics were typical for the transgenic plants. It has been established that the plants produced more alkaloids *in vivo*. Among *in vitro* cultured clones the #K4 (3.59 mg/g) clone, while among *in vivo* cultured clones the #K5 (5.76 mg/g) clone had the highest hyoscyamine content. In order to know more about the metabolism of tropane-base alkaloids, and to understand more accurately the changes during abiotic stress effects, the measurable endogeous HCHO content of the samples has been determined (OPLC, HPLC). Our biological experiments that have been performed in BioArena-system demonstrated that the investigated components of *A. belladonna* most likely affects through HCHO.

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ATTILA SÁNDOR HALÁSZ (2006)

The role of nitric oxide synthase and pyridine dinucleotides in toxicity of monoaminergic neurotoxins

Supervisor: Kálmán Magyar

The analysis of action of neurotoxic compounds plays a major role in the investigation of the development of neurodegenerative disorders. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine (METH) have detrimental effect on dopaminergic neurons, while N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) impairs the norepinephrine containing neurons. The possible contribution of reactive oxygen and nitrogen species (RONS) to neurodegenerative processes came up after investigations with these neurotoxins.
The first aim of our study was to examine, whether altered nitric oxide synthase (NOS) enzyme activity can be involved in the damaging effect of these neurotoxins. We also investigated the change in the level of pyridine dinucleotides after the administration of neurotoxins to assess the shift in the redox state of neurons. This shift can sensitize the neurons against other harmful actions.
According to our results, the activity of NOS transiently increased in mouse striatum after administration of MPTP. Elevated glutamate transmission induced by MPTP administration could be responsible for this phenomenon.

We observed a transitional decrease of NOS activity in mouse hippocampus as well as in striatum after administration of METH, possibly due to uncoupled enzyme function of NOS. In the uncoupled reaction NOS produces superoxide anions which is accompanied by decreased NO production.
After administration of DSP-4 we could not observe any significant changes in NOS activity.
We have developed a sensitive method for the measurement of oxidized and reduced pyridine dinucleotides.
We have found elevated NADH/NAD^+ ratio in mouse striatum after administration of a single dose of MPTP. Mitochondrial complex I blockade could be responsible for this shift in the redox status of the neurons towards a more oxidative state.
After administration of a single dose of METH we have observed a transitional shift in the pyridine nucleotide redox status of the cells towards a more reducing state. Multiple dose administration of METH reduced the level of NADPH in mouse striatum and hippocampus possibly due to the oxidative challenge induced by METH.

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BEÁTA DAJKA-HALÁSZ (2008)

Synthesis of polycyclic pyridazines by application of C-C bond formation reactions. A mechanistic study of the *tert*-amino effect

Supervisor: Péter Mátyus

[d]-Annelated pyridazino ring systems are of great importance due to their valuable biological activities. One of the most efficient synthetic pathways to obtain such systems involves the type 2 tert-amino effect. (Thermal cyclization of ortho-vinyl-tert-anilines and their analogues.) In this way several, otherwise hardly accessible pyridazine derivatives could be prepared. The mechanism of the isomerization reaction was also investigated: effects of substituents at positions -4, -5 and -6; acyclic or cyclic electron-withdrawing groups at the vinyl moieties; amino substituents were studied. We also compared the influence of benzene and pyridazine ring on the reaction rate. Incorporation of the terminal vinylic carbon into a trioxopyrimidine ring accelerates the cyclization. Effects of substituents at the amino groups are also dependent on the arene ring. An aryl group at position -6 of the pyridazine ring increases the rate of the ring closure reaction. The presence of nitrogen atoms decreases the electronic density on the heterocyclic ring, thereby the pyridazinone derivatives cyclise slower than the benzene derivatives. Studies on deuterated derivatives confirmed an intramolecular pathway for rearrangement. Significant rate and yield enhancements were observed under solvent-free microwave and solvent-free conventional heating conditions.

The pyridazino[4,5-b]indole ring system was prepared from pyridazinone precursors utilizing the combinations of a Buchwald-Hartwig amination with an intramolecular Heck-type reaction. The benzofurane analogue was synthesized via nucleophilic substitution reaction of 5-chloro-2-methyl-6-phenylpyridazin-3(2H)-one with phenol derivatives followed by Pd-catalyzed cyclodehydrohalogenation.

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ZOLTÁN KALÓ (2006)

Economic evaluation of renal replacement therapies and immunosuppressants of renal transplantation

Supervisor: Zoltán Vincze

The thesis presents a comprehensive overview on the economic evaluation of renal replacement therapies and immunosuppressants for renal transplantation, mainly based upon the results of a Markov model developed by the author.

Renal transplantation is more cost-effective than dialysis in the treatment of patients with end-stage renal disease (ESRD). Consequently renal transplantation should be applied for all ESRD patients if there is an available organ for transplantation and the surgery is not contraindicated for medical-professional reasons.

The increasing social and economic burden of dialysis can be reduced by increasing the number of organs available for transplantation, and by extending the survival of transplanted allografts.

Several organ donation programmes have been developed to address the first objective. The widespread introduction of these programmes would result in significant health gain for ESRD patients and cost-savings for the society and health care payers.

An objective method is depicted in the thesis to answer the dilemma how long waiting for an ideal donor results in equal health gain with an immediate transplantation of a marginal allograft from an extended criteria donor.

Finally the thesis summarizes the economic issues of improving the graft survival by better posttransplant care and modern immunosuppressants. Pharmaceutical manufacturares with an interest in the field of transplantation need to invest more and more into the early phase pharmacoeconomic modelling of new drug candidates, as they must demonstrate the cost-effectiveness of their new immunosuppressants at launch. Failing to do so, no reimbursement is granted to the new drug, and so its market access remains restricted.

The presented results are highly important from the health political and industrial political point of view. Similar approaches and methods should also be applied to other fields of health care and other therapeutic areas.

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ANDRÁS ZOLTÁN KAKASY (2007)

New phytochemical data on *Dracocephalum* species

Supervisor: Éva Lemberkovics

Most recent publications relating to *Dracocephalum* species have discussed the phytochemistry and pharmacology of non-volatile and volatile compounds with promising pharmacological effects, such as rosmarinic and caffeic acids, flavonoids with cytotoxic activity, or diterpenes with trypanocidal activity. The aim of our work was to contribute new results concerning the phytochemical characterization of some dragonhead species. We have studied samples of *D. moldavica* L., *D. ruyschiana* L., *D. argunense* Fisch. ex Link, *D. bipinnatum* Rupr., *D. diversifolium* Rupr., *D. grandiflorum* L., *D. peregrinum* L., *D. renati* Emb. and *D. rupestre* Hance., all cultivated by ourselves. In the essential oil of *D. renati* Emb. by means of GC and GC-MS we detected monoterpenes (limononene, carvone, neral, geranial and linalyl acetate), methyl chavicol and β-caryophyllene, caryophyllene oxide and bicyclovetivenol). The sesquiterpenes (essential oil of *D. ruyschiana* L. contains mainly pinocamphone and β-caryophyllene, isopinocamphone and smaller amounts of sesquiterpenes (β-pinene, myrcene, β-cubebene, germacrene-D and elemol), caryophyllene oxide, limonene, p-cymene and methyl chavicol. The essential oil of *D. grandiflorum* L., β-caryophyllene, was found to contain mainly sesquiterpenes (aromadendrene, β-bourbonene) and carvacrol, together with β-cubebene and caryophyllene oxide, β-asarone and methyl chavicol. As compared with species smaller amounts of belonging in the Lamiaceae family, in which ursolic and oleanolic acids were detected in higher amounts (exceeding 3% and 1.5%, respectively), in the dragonhead species we found only average quantities. Ursolic and oleanolic acids, measured by a GC method (after silylation) were found in relatively high quantities in *D. diversifolium* Rupr. (0.81/0.25%) and *D. peregrinum* L. (0.72/0.26%). The main anthocyanidins of the purified and hydrolysed extract of *D. moldavica* L. (investigated by HPLC analysis) proved to be delphinidin and cyaniding; pelargonidin was detected merely in traces. The GC-MS analysis of the constituents of the extracted matrix (before and after hydrolysis) of *D. moldavica* L. and *D. ruyschiana* L., in the form of their trimethylsilyl (oxime) ether/ester derivatives led to the identification and quantification of more than 30 newly identified constituents, including sugars, sugar alcohols, flavonoids, and aliphatic and aromatic carboxylic acids, together with their esters. Phenoloids, such as rosmarinic acid (26.76 mg/g), tannins (11.2%) and flavonoids (0.5%) accumulate in *D. moldavica* L. in the early stages of ontogeny. Higher quantity of caffeic acid (0.5 mg/g) and essential oil (0.40–0.83% v/m) were measured during flowering. An infusion prepared

from Moldavian dragonhead herb contains large quantity of tannins and up to 20–30% of the phenolcarboxylic acids and flavonoids present in the herb. Alcohol, used as a solvent to prepare a tincture, dissolves primarily the essential oils of the herb. The main volatile terpenoid of the infusion is geraniol, while in the tincture geranyl acetate predominates. We have established, that during the process of extraction of Moldavian dragonhead herb by supercritical carbon dioxide, oxygenated monoterpenes are extracted at the beginning of the extraction process; less volatile components and fatty acids were extracted later.

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DOROTTYA KISS (2007)

Formulation of floating tablets containing metronidazole

Supervisor: Romána Zelkó

The aim of the present work was to develop a sustained release, gastroretentive dosage form, which is capable of floating upon the gastric contents, thus providing the site-specific delivery of the active ingredient.

I used metronidazole as a model drug, because its role in the eradication of *Helicobacter pylori* justifies its application in a dosage form characterized by the above mentioned properties.

In the course of the preformulation studies, I selected the excipients that proved to be compatible with the model drug according to differential scanning calorimetry investigations. I also determined their thermal properties, flowability, compressibility and morphological characteristics, which are of great importance from the aspect of the manufacturing process. I prepared 36 kinds of tablets using low density foam polymer in half of the formulations and gas-generating excipients in the rest of the tablets in order to assure the appropriate density of the dosage forms. I evaluated the tablets by investigating the effect of the amount of the foam powder, the ratio of the matrix-forming polymers, the amount of the active ingredient and the amount and ratio of the fillers on the hydrodynamic properties and drug release of the formulations. In the course of the optimization of the composition and production of the tablets, I found that only dosage forms based on gas-generation can be reproduced properly.

Upon investigating the stability of the drug release of the tablets, I found that structural changes of the amorphous region of the matrix-forming agent caused by storage resulted in the increase of the extent of drug release in the case of the low molecular weight matrix-forming polymer.

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ANDRÁS KLEBOVICH (2008)

Utilization of olanzapine and risperidone in Hungary with special concern to the treatment of schizophrenia in the psychiatric rehabilitation

Supervisor: Romána Zelkó

Because olanzapine and risperidone have similar efficacy and tolerability in the treatment of schizophrenia and these two agents are the most widely used atypical antipsychotics in Hungary, the utilisation of the drugs is a relevant consideration. The purpose of this study was to follow the utilisation trend of olanzapine and risperidone in Hungary and in the Gálfi Béla Hospital, which is a specialised institute on psychiatric rehabilitation. Data regarding dosages and psychiatrists' clinical preferences were collected through studying hospital charts in the Gálfi Béla Hospital during the period 1999–2007. Utilisation of the agents indicated that there was an increase of use for the whole examined period in the Gálfi Béla Hospital, although unambiguous growth began only in 2001. Hospital chart analysis shows that both drugs were used to a large extent and risperidone use is increasing more rapidly, which is justified by the gerontopsychiatric profile of the hospital.
The further aim of the study was to compare the risk of diabetes in a Hungarian schizophrenic population treated with atypical antipsychotics with that of the non-schizophrenic population. We wished to reveal the effects of gender and age. A schizophrenic population was examined by studying hospital charts in the Gálfi Béla Hospital. Data were given by the National Health Insurance Fund registry in the period of 2000–2006, while the Hungarian Central Statistical Office presented data on the prevalence of diabetes in the Hungarian population. Binomial distribution was used for the hypothesis testing. The examination shows higher prevalence of diabetes among schizophrenic patients (12.72%) in comparison with the Hungarian population (6.85%) in the age group of 18–64 among both sexes, while there was no higher risk of diabetes found in the age group of above 65 among both sexes. As a conclusion, we emphasize that continuous weight, glucose and lipid level monitoring should be considered during treatment with antipsychotics in all age groups, even if our study does not show higher risk of diabetes among elderly schizophrenic patients. The results of my investigations did not show higher risk of diabetes among elderly schizophrenic patients treated with atypical antipsychotics, while continuous weight, glucose and lipid level monitoring should be indicated during treatment with antipsychotics in other age groups.

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KRISTÓF KÓCZIÁN (2007)

Microspeciation of pharmaceutical ampholytes of unusual basicity

Supervisor: Béla Noszál

The physico-chemical properties of bio- and drug molecules greatly influence their interactions in the body and strongly effect the mechanism of drug action. Among these properties, macroscopic and site-specific protonation constants are of crucial importance. Latter one is the tool to calculate the relative concentration of the various microspecies in the compartments of the body at different pH values, and also, it is the versatile parameter to improve the pharmacokinetic properties of a new molecule in a particular family of drugs. In the present thesis work, the microspeciation of three molecules of great pharmaceutical importance and unusual acid-base properties, were carried out. The microconstants of tenoxicam, the non-steroidal anti-inflammatory drug, were described, introducing a novel deductive method using Hammett constants. For this purpose, a total of 8 tenoxicam and piroxicam derivatives were synthesised. To the best of our knowledge, the microconstant of tenoxicam obtained thus is the lowest enolate basicity value, which, however, can be well explained by the effects of the intramolecular environment. The developed evaluation procedure is suitable for microconstant determination of compounds in other molecule families. Besides, prodrug-type compounds and analogues similar to the structures of selective COX-2 isoenzyme inhibitors were synthesised. ONklog The other two molecules studied, the 6-aminopenicillanic acid and 7-cephalosporanic acid, the core molecules of the two most important beta-lactam antibiotic-types were derivatised and investigated by 1D and 2D NMR techniques. The NMR-pH titration on the parent compounds and their ester derivatives, combined with *in situ* pH-measurements allowed the microspeciation of these easily decomposing molecules. One of the protonation constant of 7-ACA (=4.12), to the best of our knowledge, is the least non-aromatic basic amino-site among the natural compounds.

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GÁBOR KRAJSOVSZKY (2008)

Synthesis of hetero-ring fused diazines

Supervisor: Péter Mátyus

[*d*]-Annelated pyridazines have primarily been of interest as structurally related analogues of 4,5-disubstituted pyridazines possessing remarkable biological activities. These achievements have prompted us to elaborate further new methodologies for the syntheses of novel polyfused diazines, utilizing Suzuki cross coupling and subsequent ring closure reactions.

Substrate-, reagent- and temperature-dependent halogen-displacement reactions of halopyridazinones with iodide were studied. The monohalogen compounds thus obtained were used as starting materials for cycloaddition and electrocyclization reactions.

1,3-Dipolar cycloaddition reactions of 5-ethylsulfonyl- as well as 5-chloro-2-methyl-3(2*H*)-pyridazinones led to simple and convenient preparations of pyrazolo[3,4-*d*]pyridazinones. The results of these experiments were also analyzed by FMO theory.

Suzuki reactions of halopyridazinones and the subsequent ring closure reactions of their products via nitrene insertion or nucleophilic substitution, were elaborated as efficient synthetic routes for pyridazino[4,5-*b*] and -[3,4-*b*]indoles. This methodology was extended to reactions of chloropyrimidine and chloropyrazine, and pyrimido[5,4-*c*]cinnoline, pyrazino[1,2-*b*]indazole, and pyrazino[2,3-*b*]indole ring systems were synthesized. By this procedure various substituents could be easily introduced into the products using appropriately substituted precursors.

Tandem Suzuki-aza-Wittig, and tandem Suzuki condensation reactions were developed as further methods for the syntheses of pyridazino[4,5-*c*]isoquinolines.

Pyridazino[4,5-*c*]quinoline system was obtained via carbodiimide intermediate by tandem aza-Wittig electrocyclization reaction, whereas pyrimido[5,4-*c*]quinoline was also available *via* ring closure reaction utilizing a carbodiimide. The different reactivities of pyridazines, pyrimidines, and pyrazines could be well explained taking their different electronic properties into consideration. The constitution of each product was proven by spectroscopic investigations.

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ZSUZSANNA KOVÁCS (2007)

Species-specific analytical and equilibrium characterization of morphine and its derivatives

Supervisor: Béla Noszál

Knowledge of the protonation constants is essential for the prediction of pharmacokinetics parameters and the development of separation methods. I have determined the macroscopic and microscopic protonation constants of major analgetic morphine, and its most important metabolite.

I have observed that for morphine the major protonation pathway includes in noncharged species, so at any physiological pH this protonation isomer is the dominant one contributing to the entrance of morphine to the central nerves system. The protonation microequilibria of morphine-6-glucuronide, the active metabolite, were characterized and major protonation pathway was proved to be identical to that of morphine The acid-base properties of two semi-synthetic morphine derivatives, codeine and pholcodine, were characterized. Codeine has only one basic site so its protonation process can be described by a macroconstant that showed good agreement with literature data.

I determined protonation of macro- and microconstants of pholcodine, also utilizing newly-synthetized N-methyl derivatives. The electrophoretic behavior of these compounds was investigated by using background electrolytes with different pH.

I have observed that in this pH range the change in the effective mobility of these compounds is influenced not only by their charge, but also by their different solvation properties.

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ORSOLYA LÁNG (2007)

Specificity of chemotactic ligand-receptor interaction: chemotaxis and drug targeting

Supervisor: László Kőhidai

Chemotaxis is a cell-physiological response of migratory cells induced by chemical gradients. It has an underlined significance in phylogeny: in the early phases chemotaxis provides a survival factor as it supports detection of food molecules and helps to avoid toxic substances. In higher levels chemotaxis is a basic process of several physiological and pathological processes (e.g. sexual reproduction, inflammation). Chemotaxis is also considered as a significant factor of molecular evolution of signal molecules.

In the first part of our work L-amino acids and peptide libraries of small peptides (W/SXWS and formyl peptides, tuftsin derivatives) were tested in the eukaryotic ciliate *Tetrahymena pyriformis*. Relationships of chemotactic activity and the chemical, physico-chemical characters of the ligands were analysed. Chemotactic character of SXWS peptides was explained by experiments based on molecular conformational studies. Then, the validity of structure-function relations described before were tested in human cells lines (mostly in monocytes).

Theory of a novel drug targeting technique—chemotactic drug targeting (CDT)—was suggested based on chemotactic behaviour of target cells.

There was a good correlation between the physico-chemical characters (solvent exposed area, hydropathy index, etc.) and the chemotactic ability, chemotactic selection potential of the ligands. Among others, solvent exposed area (SEA) index proved to be predictive of chemotactic ability of oligopeptide ligands. Wide amplitude of responsiveness to attractants and a narrow one to the repellent molecules were detected in this group of ligands and it was described as "Chemotactic range fitting".

Model experiments of chemotactic drug targeting (CDT) were done by screening structures for the best efficiency in drug-targeting. In this system chemoattractant ligands provide fast, close and selective targeting, while chemorepellents could be also used as protectors from degradation by non-target cells.

Carrier components of the tested conjugates were oligotuftsins. Formyl peptides were substituted as chemoattactants and methotrexate as the drug component to the carrier. In the experiments it was proved that due to their chemotactic characters and other cell-physiological parameters, the novel molecular complexes are good candidates for chemotactic drug targeting.

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PÉTER ONODY (2006)

ERBB family in breast cancer: detection methods, clinical and prognostic significance, under-recognized mechanisms in their function

Supervisor: Ottó Dobozy

Historically the definition of an ERBB2 positive status was based on arbitrary assumptions. Even with the current level of clinical validation, it is reasonable to assume that it will be further refined into a more precise definition in the future. There is a need to develop alternative approaches to ERBB receptor family, in the first place to ERBB2 testing to overcome the limitation of the current assays and meet the demand of clinical practice. Ideally new ERBB assay should be accurate, robust, reliable, simple to use, cost-effective,

easy to standardise and capable of high sample throughout. Our research project has carried out different methods and tests, trying to contribute to this persistent requirement. We approached this problem by comparison of molecular and immunolo-histochemical techniques to evaluate ERBB2 status at DNA, at mRNA and at protein level. We found high degree of concordance between ICH and FISH and mRNA techniques. Further we studied the relationship and eventual correlation between ERBB2 status and different clinical, pathological and biological factors. We studied and demonstrated the ERBB2 status on various populations as node negative, post-menopausal or metastatic breast cancer. Our team developed and validated a new method to measure ERBB2 gene expression at mRNA level, a method, which seems to fulfil and able to response the above mentioned criteria. At last, we widened this technique to investigate the mRNA expression for each member of the ERBB family and to examine their prognostic values in breast cancer. Our data illustrated different levels of expression of the ERBB genes in mammary carcinoma. These facts suggest to widen the use of classical detection test (ERBB2) to all members of the ERBB family, thus optimising the therapeutical approach of breast cancer patients.

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ÁGNES SÁRKÖZI (2007)

Novel data contributing to our knowledge of the greater celandine (*Chelidonium majus* L.)

Supervisor: Ágnes Kéry

Greater celandine is a medicinal plant well-known for the spasmolytic, anti-inflammatory, antimicrobial and antitumor effects of its isoquoinoline alkaloids. Although this medicinal herb is a subject of recent investigations its successful therapeutic applications has not been corroborated so far by scientific results. Re-evaluation of the aerial parts of the plant has become especially timely with its adoption as an official drug in the VIIIth Hungarian Pharmacopoeia. We wished to offer new data to the plant database, in order to ensure the pharmaceutical and therapeutic quality of *Chelidonium* drugs and preparations.

The application of *Chelidonium* drugs obtained by collection always involves higher risks than those of cultivated drugs, therefore herbal samples from our own collection have been qualified according to pharmacopoeia.

Determination of the total alkaloid content by spectrophotometry has been modified so as to allow the measurement of tertiary and quaternary alkaloids as well. The main steps and results of determination have been confirmed by HPLC assessment.

For the investigation of main alkaloids—usually carried out by HPLC method, a highly accurate but rather laboursome procedure—taking the advantage of the fluorescence

property of quaternary alkaloids we developed a simple, low-cost TLC-densitometry method. This is applicable for routine determination of a large amount of samples (sensitivity: RSD= 0.67–1.24% and accuracy: RSD =3.3–4,8%).
For a closer knowledge of alkaloid ratios, important from the aspect of total alkaloid content and therapeutic effect we supplied data obtained by chromatographic methods. Measurement of the total alkaloid content of the plant parts gave the following results: generative organs (1.54%), root (1.43%), leaf (0.71%), stem (0.68%). The main alkaloid of the aerial part was determined as coptisine. In the root part, chelidonine can be found in highest amount, but the concentration of sanguinarine, coptisine, chelerythrine and berberine is also considerable. Highest total alkaloid content was measured in samples collected during the rest period, therefore this time is most appropriate for the collection of both the herb and the root.
In order to contribute to the phytotechnology of *Chelidonium* preparations, we measured the results of alkaloid dissolution from traditional extracts of the herb.
The mineral element composition of *Chelidonium* drugs and their traditionally applied extracts were analysed by ICP-OES for Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn content.
Our biological studies performed by the BioArena method confirmed the antibacterial activity of *Chelidonium* alkaloids and also support the assumption that the reaction mechanism is realized by the formation of formaldehyde due to demethylation.

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DÁVID SZEGVÁRI (2007)

The applications of chiroptical spectroscopy for the determination and the detection of steroids and for the examination of their cyclodextrin-mediated enantioselective solubility

Supervisor: András Gergely

Chiroptical spectroscopy is one of the most developing areas of chiral analysis which role has been enhanced in recent years. In the thesis new applications of circular dichroism (CD) and optical rotatory dispersion (ORD) methods are presented via some examples.
A quick and accurate CD spectroscopic method was developed for the simultaneous determination of ethisterone and its Δ^5-isomer (Δ^5-ethisterone). The method is based on the selective negative Cotton effect of the Δ^4-3-oxo group of ethisterone. The concentrations of the two isomers can be easily determined by ellipticity measurements at two different wavelengths without the application of any separation technique. CD detection for the separation of ethisterone isomers by high performance liquid chromatography (HPLC) is suitable even for the determination of the Δ^5-ethisterone, that contains isolated oxo groups.

The separation of dehydroepiandrosterone (DHEA), dehydroepi-androsterone sulfate (DHEA-S) and related steroids was also elaborated by a CD detected HPLC technique. CD detection is a good tool for the determination of saturated ketones.
Enantioselectivity was observed when the solubility of raceme norgestrel was examined in aqueous solutions of γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin. A CD spectroscopic method, based on the measurement of g-factor was applied for the determination of the enantiomer ratio obtained. Levonorgestrel, the effective enantiomer was dissolved in greater extent using either cyclodextrins. The obtained solubility of norgestrel was greater using γ-cyclodextrin, although the enantioselectivity was more significant when hydroxypropyl-γ-cyclodextrin was applied. Phase-solubility examinations were appropriate for the verification of 1:1 stoichiometry and that the enantiomer ratios were independent of the cyclodextrin concentrations. A new calculation method was also elaborated, suitable for the direct determination of the concentrations of norgestrel enantiomers in aqueous cyclodextrin solutions.

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ZSOLT SZÉKELYHIDI (2006)

Design and synthesis of pyrazole and thiophene derivatives with kinase inhibitory activity

Supervisor: György Kéri

During my Ph. D. research I participated in a development project of a novel pharmaceutical agent. In the process, in order to validate the target I synthesized pyrazole leads with highly active p38 MAP kinase inhibitory activity, as well as α-terthiophene lead molecules with protein kinase C (PKC) inhibitory activity, that are acknowledged in the literature. Our research group examined these lead compounds on different targets and on humane A431 EGFR overexpressing tumor cell line. The biological measurements showed that two 1-substituted-5-pyrazolyl urea derivatives have high activity of RIP kinase inhibition, while two α-terthiophene derivatives have a high A431 tumour cell growth inhibitory activity. I synthesized a focused molecule library with patentable structures around the lead molecules of new biological activity—taken into consideration the ADME parameters expected from the pharmaceutical agent and the structure-activity relationships known from the literature—and our research group examined the biological profile of these derivatives. Those groups of compounds of 5–10 molecules are considered as focused molecule library, the members of which are structurally similar to the lead molecule. By changing the substituents or in some cases, introducing new molecule fragments, or by changing

given molecule fragments, patentable structures of more efficient biological activity can be created, that are not known in the literature so far. If biological test is carried out on these derivatives, new potential candidates for pharmaceutical agents might be obtained. During the biological test of the focused molecule library built around the pyrazolyl urea and the terthiophene structure we found that several analogue exhibited similar or enhanced inhibitory activity compared to the initial lead molecules. On the contrary, in the case of some derivatives—though they exhibited high structural similarity to the lead molecules—we found complete change in their biological profile.
The precise exploration of the structure-activity relationship of these derivatives and the optimalization of their biological activity require further research.

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- *Kéri G, Székelyhidi Z, Bánhegyi P, Varga Z, Hegymegi-Barakonyi B, Szántai-Kis C, Hafenbradl D, Klebl B, Muller G, Ullrich A, Erős D, Horváth Z, Greff Z, Marosfalvi J, Pató J, Szabadkai I, Szilágyi I, Szegedi Z, Varga I, Wáczek F, Őrfi L (2005) Drug discovery in the kinase inhibitory field using the Nested Chemical Library technology. Assay Drug Devel Techn 3: 543–551.*

TAMÁS TÁBI (2006)

Examination of the metabolic N-oxidation of deprenyl by chiral capillary electrophoresis

Supervisor: Éva Szökő

Selegiline or R-(-)-deprenyl, a selective and irreversible inhibitor of monoamine oxidase (MAO) B enzyme, is widely used in the treatment of Parkinson's disease and other neurodegenerative disorders. In the recent years neuroprotective, neuro-rescue and antiapoptotic activities of the compound were also reported, and supposed to be, at least partly, independent from its MAO-B inhibitory effect, though still showing high stereoselectivity. The majority of these favorable effects requires the metabolic transformation of the compound; however, the known metabolites were found only partially effective. Recently, besides desalkylation another metabolic route has been proposed, that results in the generation of *N*-oxidized metabolite.
The objective of our study was to investigate the extent of generation of this new metabolite during the *in vivo* and *in vitro* metabolism of deprenyl enantiomers. As a new asymmetry center is created during *N*-oxidation of the prochiral *tert*-nitrogen containing deprenyl, the stereochemistry of the conversion was intended to be examined as well.
To accomplish our aims, we have developed chiral capillary electrophoresis methods for the simultaneous separation of deprenyl enantiomers and their metabolites. The methods have been validated for the determination of the metabolites in rat urine and microsome

preparations. In case of both R-(⁻)- and S-(⁺)-deprenyl, the *N*-oxidation has been demonstrated *in vivo* and *in vitro*. In the rat the metabolism has been found to be stereoselective, the generation of the *NS*-isomers was preferred.
In case of the isoforms of flavin-containing monooxygenase (FMO), the enzyme having primary importance in *N*-oxidation, significant differences have been found in the metabolism of deprenyl enantiomers. Both isomers proved to be better substrates for FMO 1 that catalyzed the formation of the *NS*-isomers in 6–13 fold excess, while in case of FMO 3 the generation of the *NR*-isomers was preferred. The observed differences between the isoenzymes can result in significant species variations in deprenyl metabolism.

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GERGELY VÖLGYI (2008)

Development of methods for determination of physico-chemical parameters (pK_a, log$P$) of water-insoluble compounds in early phase of drug discovery

Supervisor: Krisztina Takács Novák

A new, multicomponent cosolvent mixture consisting of equal volumes of methanol, 1,4-dioxane and acetonitrile referred to as MDM (from MeOH, Dioxane and MeCN) is selected that significantly improves the solubility of most pharmaceutical compounds. The utility of this system is that it enables pK_a measurements to be performed on a wide range of compounds where measurements would be impaired in aqueous solution. We measured the physicochemical characteristics of MDM-aqueous mixtures (density and relative permittivity). The validation of pK_a determination in MDM-water mixtures is also presented. The cosolvent dissociation constants (p$_s K_a$) of 50 chemically diverse compounds (acids, bases and ampholytes) were measured in 15–56 wt% MDM-water mixtures by potentiometric or spectrophotometric titration and the aqueous pK_a values obtained by extrapolation. The Yasuda-Shedlovsky extrapolation procedure was proposed to obtain the aqueous pK_a values. The extrapolated results are in good agreement with pK_a values measured in aqueous medium. Further we also present that the single point estimation based on measurement in 20%/v MDM-mixture using a general calibration equation may be suitable for rapid pK_a determination in the early phase of drug research.
A reversed-phase thin-layer chromatographic method consisting of two optimized chromatographic systems (named as TLC/logP_{0-3} and TLC/logP_{3-6}) were developed for the estimation of lipophilicity of chemically diverse neutral compounds or weak acids and bases. RP-diC1 silanized silica gel plates were applied as stationary phase. The acetone-water mixture was found to be optimal mobile phase in both systems with respect of the corre-

lation of R_M values to the octanol/water partition coefficients. The method uses general calibration equations obtained with chemically non-related compounds. The universal applicability of the optimized chromatographic systems was validated using 20 randomly selected structurally diverse compounds. Mainly, there was good agreement between the log*P* values obtained by shake-flask method and by RPTLC technique. The proposed two TLC experiments with the automation of the sample application and imaging detection of the compounds can be considered as a possible alternative for fast and acceptable accurate estimation of lipophilicity of drug candidates in the early phase of drug research.

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PETER ADOLF WEISHEIMER (2006)

Healthcare occupational diseases in Germany

Supervisor: Sándor Nagylucskay

Hepatitis is still among the infectious diseases with the highest worldwide incidence levels, despite blood donor screening and prophylactic vaccination. The WHO estimates that about two thousand million persons from the world's total population have already suffered an HB virus infection. HBV is a recognized occupational disease among healthcare staff. The objective of this thesis on the hepatitis B virus is to help avoid the attendant risks of infection. This high-risk group is subjected to increased levels of exposure and thus requires special protective measures.

In a collaborative study effort with the Semmelweis University of Budapest, 3,943 physicians and nurses working in hospitals and university clinics, as well as persons from the normal population of Budapest, were examined for HBV markers. The risk of contamination increases with age. The causal factor involved here is time. The probability of occurrence of HBsAg-positive persons is greater in cases in which there is a direct risk of contact infection. No essential differences were determined between the different vaccines available. Active immunization thus provides reliable and well-tolerated vaccination protection against hepatitis B.

Basically, one must conclude that comprehensive vaccination of all risk groups, especially among persons whose professional work involves exposure to the disease, is to be recommended. All economic parameters, cost-to-benefit calculations and ethical considerations —especially in view of fatal hepatitis infections—support a thoroughgoing vaccination programme. Hepatitis B infections are not controllable in all cases, so that the spread of the disease must be contained at all costs.

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PROGRAM 3/2.

EXPERIMENTAL AND CLINICAL PHARMACOLOGY

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Program overview

The topic of pharmacokinetics becomes increasingly important for studying bioequivalence and planning modern drug preparations for optimal drug treatment. The study of the mechanism of drug action by pharmacodynamic tools aims at recognising new active substances and various opioid receptor types and subtypes. It is advised to get acquainted with their physiological and pathophysiological role in the development of opioid dependence or in the protection of gastric mucosa. The Program also covers the research into the metabolism of neurotransmitters in the CNS in order to elucidate the relationship between disturbances of neurotransmission and certain psychiatric disorders. Research in the field of presynaptic regulation of the neurochemical transmission in the peripheral and central nervous system is also included in the Program. Investigation of compounds affecting calcium and bone metabolism is also carried out. The mode of action of cardiovascular drugs and endogenous substances are planned to be tested, as well as antihypertensive agents in order to find optimal treatment of the diseases. Anticancer agents are involved in the studies to improve the effectiveness of the treatment. Structure activity relationship studies, including the chirality of drugs is also part of the Program.

Titles of subprograms and research projects	*Supervisors*
Subprogram 1.	
Drug and mechanism-oriented pharmacodynamic studies	Klára Gyires
Central and peripheral mechanisms as potential drug targets	Zsuzsanna Fürst
The role of ORL-1 receptor-mediated neuromodulation in the central autonomic control	András Rónai
The role of local mediators in vascular reactions, functional integrity of mucosa and the adrenomedullar-functions	Klára Gyires
Receptor-mediated protection of the gastrointestinal mucosa	Klára Gyires

Analysis of the centrally-mediated protection of the gastrointestinal mucosa	László Köles
The role of opioid receptors in the cellular immunmodulation	Julianna Kardos
Subprogram 2.	
Pharmacokinetic and drug metabolism studies	Imre Klebovich
Drug pharmacokinetic studies in humans, and animal experiments	Imre Klebovich
Regulation of extrahepatic cytochrome P450 enzymes; the role of inhibition of drug metabolism in drug interactions	Károly Tihanyi
Studies on induction of cytochrome P450 enzymes	László Vereczkey
Studies on drug interactions	László Vereczkey
Neuropsychopharmacology, drug discovery and development	György Lévay
Selective detection methods in studies of xenobiotic metabolism	István Hazai
Pharmacometric analysis of bioequivalence studies	László Tóthfalusi
Subprogram 3.	
Modulation of neurochemical transmission by drugs; neurodegenerative and neuroprotective mechanisms	Kornélia Tekes
Analysis of correlation among nociceptinerg, nocistatinerg and biogenaminerg systems	Kornélia Tekes
Biochemical basis of affective and anxiolytic disturbances	Kornélia Tekes
Animal models for studies of drugs affecting neurochemical transmission	Júlia Tímár
Neurochemistry of mental diseases	Gábor Faludy
Investigation of neuroprotective and neuroregenerative effects	Kálmán Magyar
Studies on the role of nitric oxide synthase and transcription factors in neurodegenerative and neuroprotective processes	Éva Szökő
Biochemical basis of neuropsychiatric symptoms of chronic hepatic diseases	Ferenc Szalay
Subprogram 4/A.	
Preclinical and clinical cardiovascular pharmacological studies	Valéria Kecskeméti
Effect of cardiovascular or other drugs on cardiac parameters (mechanical, electrophysiological) on isolated cardiac preparations under physiological and pathological conditions	Valéria Kecskeméti
Subprogram 4/B.	
Preclinical and clinical cardiovascular pharmacological studies	Csaba Farsang
Possibilities of drug treatment of macro- and microvascular diseases	Zoltán Járai
The role of imidazoline receptors in haemodynamic regulation in hypertension	Judit Kapocsi
Reduction of cardiovascular risks by antihypertensive drugs in chronic renal failure patients	István Kiss
Experimental and clinical cardiovascular pharmacological studies	Csaba Farsang
Subprogram 5.	
Separation methods and their applications in pharmacological studies	Huba Kalász
Investigation of fate of drugs in the body and their effects by chromatographic methods	Huba Kalász

Subprogram 6.	
Studies on compounds affecting calcium and bone metabolism	Péter Lakatos
Studies on molecules and drugs affecting the calcium and bone metabolism	Péter Lakatos
The effect of diseases and drugs of calcium and bone metabolism on the mineral content, quality and mechanical competence of the bone	Csaba Horváth
Pharmacogenetics of the calcium metabolism	István Takács
Subprogram 6.	
Human trials of anticancer drugs (controlled clinical pharmacological studies)	András Telekes
Human studies on anticancer drugs, their mechanism of action and rational use	András Telekes
Clinical pharmacological studies and rational use of analgesic drugs	András Telekes
Clinical development of anticancer drugs	Sándor Kerpel-Fronius
Subprogram 8.	
The role of ion-transport mechanisms in the pre-synaptic regulation of neurochemical transmission	Tamás Török
The role of ion-transport mechanisms in the pre-synaptic regulation of neurochemical transmission	Tamás Török

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a, absolutorium; f, full-time; pt, part-time; i, individual; it, international; crc, Cooperation Research Centre

Abstracts of Ph.D. theses successfully defended in 2006, 2007 and 2008

KRISZTIÁN BÁCSI (2008)

Role of calcium metabolism in the pathogenesis of osteoporosis and colorectal cancer

Supervisor: Péter Lakatos

Epidemiological studies suggested the preventive role of calcium supplementation in both osteoporosis (OP) and colorectal cancer (CRC). Calcium is necessary to reach normal peak bone mass and to maintain bone, thus it is not surprising that calcium intake decreased bone loss. Calcium also precipitates intestinal secondary bile and fatty acids reducing their carcinogen effect on colon cells. We genotyped lactase phlorizin hydrolase (LCT) 13910 C/T, calcium-sensing receptor (CaSR) A986S and CYP3A7*1C T/G polymorphisms that are thought to be associated with altered calcium metabolism. Also, we examined the effect of calcium supplementation on MC3T3-E1 osteoblasts. We showed that in postmenopausal women LCT 13910 CC genotype leads to higher frequency of milk aversion, decreased serum calcium level, body height and bone mineral density (BMD) at both cortical and trabecular bones suggesting the important role of LCT gene in the development of OP. Furthermore, we found that CaSR 986 SS genotype is connected to milk aversion, but it had an effect on BMD only in a smaller study group. We have observed decreased lumbar spine BMD for homozygous CYP3A7*1C GG genotype independently from serum DHEAS level, suggesting that it might influence bone mass via other CYP3A7 hormonal substrates as estrogens and/or androgens. We found that in CRC patients LCT 13910 CC genotype is related to distant recurrence, moreover, in male subjects it is associ-

ated with worse DFS. Also, corroborating our previous findings in postmenopausal women, this genotype was accompanied with reduced serum calcium level in female patients. The CaSR 986 SS genotype was associated with higher CRC incidence probably acted through the altered apoptotic signal transduction, but it was not related to cancer progression. CYP3A7*1C polymorphism was not related to the incidence or progression of colorectal cancer. In MC3T3-E1 osteoblasts calcium supplementation increased alkaline phosphatase activity, stimulated type II procollagen alpha 1 expression through transforming growing factor beta (TGF-β) pathway. In conclusion, both LCT and CaSR gene polymorphisms play a role in OP and colorectal carcinogenesis. CYP gene alters BMD possibly via estrogens and/or androgens but it is not related to colorectal carcinogenesis. Based on these results, calcium supplementation, especially in the lactose intolerant population, might be a cheap and effective measure in the prevention of osteoporosis and colorectal cancer.

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TÍMEA BORBÁS (2007)

Insulin as a regulator of flavin-containing monooxygenase enzyme in streptozotocin-induced diabetic rats

Supervisor: Károly Tihanyi

The flavin-containig monooxygenase enzyme (FMO) family is one of the major microsomal monooxygenase enzyme systems involved in drug metabolism. Its physiological role in mammals, aside from the transformation of trimethylamine into trimethylamine N-oxide, is unknown. FMO1 and FMO3 isoforms are predominant in the liver of experimental animals and humans, respectively. The activity of FMO changes in certain pathophysiological conditions, for example in diabetes. Our main goal was to study whether insulin has a role in FMO regulation. For this purpose we induced experimetal diabetes in rats using streptozotocin, and then the diabetic rats received insulin supplementation. Changes in FMO function were determined at the level of enzymatic activity and gene expression. FMO activity was measured using an FMO specific substrate, benzydamine. The FMO1 and FMO3 gene expressions were observed by q-RT-PCR. Along that, the changes in abundance and activities of hepatic cytochrome enzyme system were characterized in order to support and complete the results in our experimental model system. It was shown that both, FMO activity and FMO1 mRNA level increased approximately 2-fold in diabetic rats. These levels were restored to the control level upon insulin supplementation and no change was observed upon insulin treatment of non-diabetic animals. A repressor func-

tion was proposed for insulin, because FMO activity was induced in insulin-deficiency, restored on insulin supplementation to control level and had no influence *per se*. As a high correlation was found between the FMO activity and the blood glucose level of diabetic rats, blood glucose level was suggested to be a good marker for elevated FMO activity. Furthermore, we have recognized that FMO1 and FMO3 isoforms showed distinct sensitivity to insulin-deficiency.

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KATALIN FARKAS (2006)

Assessment of endothelial function and microvascular reactivity by laser Doppler flowmetry

Supervisor: Csaba Farsang

Scientific results in the last 25 years demonstrated that endothelium is much more than a barrier. It is an organ controlling the coagulation and fibrinolytic system, platelet and leukocyte adhesion and regulating the vascular tone. Dysfunction of endothelial cells plays an important role in the development of different pathological states, like atherosclerosis, diabetic angiopathy and hypertension. Laser Doppler flowmetry (LD) is a non-invasive, objective and reproducible method to estimate skin microcirculation in different vascular disorders. LD gives the possibility for the objective assessment of different treatments affecting the microcirculation.

Using a new LD method, developed and introduced in the clinical praxis by our team, we demonstrated the presence of endothelial dysfunction in the forearm skin microcirculation in patients with essential hypertension, end stage renal disease (with hypertension), hypercholesterolemia and peripheral artery disease. We demonstrated the effect of BRX-235 on endothelial dysfunction in hypertensive patients and the beneficial effect of LDL-apheresis (H.E.L.P. treatment) on the microcirculation in homozygous hypercholesterolemia.

In the literature we firstly described the effect of clopidogrel, pentoxifylline and lumbar sympathectomy on microvascular reactivity in peripheral artery disease. In diabetic patients we demonstrated that microcirculatory disturbances can be recognised in an early, asymptomatic stage by LD investigation.

Based on our results, the non-invasive assessment of endothelial dysfunction and of impaired microvascular reactivity may contribute the selection of more efficient drug therapy in the clinical practice.

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VIKTÓRIA FERENCZ (2008)

The relationship between histamine and bone metabolism

Supervisor: Csaba Horváth

Histamine is a factor which has role in both immun system and bone metabolism, however, the mechanism of action is not fully understood. Our aim was to investigate the relationship between histamine and bone metabolism.
Bone effect of histamine deficiency was investigated in histidine-decarboxylase gene knock-out mice, measuring bone mass and serum calcitriol levels. Bone turnover markers, bone mass and fracture prevalence was determined among patients (children, postmenopausal women and men) suffering from disease characterised by histamine overproduction, such as allergic rhinitis. Bone effect of H1 receptor (H1R) antagonists and inhalated corticosteroids was also investigated.
(1) We have found a greater (not significantly) bone mass in HDC-KO mice, and oestrogen deficiency after ovariectomy did not cause decreasing in bone mass in case of histamine deficincy. (2) Serum calcitriol level was found to increase in histamine deficiency. (3) No correlation was detected between the markers of bone formation and bone resorption among allergic children who were not treated with H1R antagonists, however, the coupling was restored and decreased bone resorption was measured in multiplex allergic children treated with H1R antagonists. (4) Femoral neck bone mineral density was lower in postmenopausal women suffering from pollen allergy in comparison with non allergic controls matched by age and antropometric parameters. (5) Bone fracture prevalence was greater among non-treated pollen allergic postmenopausal women than among controls. Bone fractures were more frequent in inhalated corticosteroid only treated women than in antihistamine only treated or antihistamine and inhalated corticosteroid treated women. (6) Neither hip, nor clinical vertebral fractures were found in the antihistamine treated groups, independently from corticosteroid administration. (7) Bone fracture prevalence positively correlated with body mass index in untreated pollen allergic women. (8) Bone mass and quantitative bone ultrasound parameters were greater (not significantly) and bone fracture prevalance was lower among H1R antagonist treated pollen allergic men than among non allergic controls.
According to our results we assume the role of histamine in the pathogenesis of bone loss caused by oestrogen deficiency. Histamine deficiency influences bone metabolism probably through vitamin D overactivation. Our findings demonstrate the unfavoriable effect of allergy on fracture risk. Greater bone mass and obesity does not protect against bone fracture in allergic patients. We presume the advantageous effect of H1 antihistamine therapy on bones, that is increased elasticity. Antihistamine therapy not only protects against the unfavorable effect of histamin overproduction on bones, but also furher improves bone quality.

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KATALIN FÜLÖP (2008)

Gastric mucosal integrity and gastric motility: Pharmacological analysis of α_2 adrenoceptor subtypes involved in regulation of gastric motility in the rat

Supervisor: Klára Gyires

Potential correlation between influence of gastric motility, gastric acid secretion and gastric mucosal protection induced by α_2 adrenoceptor agonists has been studied.
Gastric emptying: Gastric emptying was inhibited by clonidine (ED_{30}: 0.54 μmol/kg, s.c.) and the selective α_{2A} adrenoceptor agonist oxymetazoline (ED_{30}: 0.81 μmol/kg, s.c.) given peripherally. The delaying effect of clonidine was inhibited by the α_2 antagonist yohimbine (12.82 μmol/kg, s.c.), but not the $\alpha_{2B/2C}$ subtype selective antagonist prazosin (1.19 μmol/kg, s.c.) and ARC-239 (0.68 μmol/kg, s.c.). The opioid antagonist naloxone (2.75 μmol/kg, s.c.) failed to affect delaying effect of clonidine. Gastric emptying was also inhibited by i.c.v. clonidine (ED_{30}: 29.84 nmol/rat, i.c.v.) and oxymetazoline (ED_{30}: 7.93 nmol/rat, i.c.v.). Gastric emptying was also delayed by i.c.v. morphine (ED_{30}: 118 nmol/rat), the α opioid receptor agonist DAGO (ED_{30}: 5.7 nmol/rat) and the α opioid receptor agonist deltorphin II (56.7 nmol/rat).
Gastric motility: The 2-deoxy-D-glucose stimulated gastric motility was inhibited by clonidine in the dose of 0.75 μmol/kg i.v., and the effect was antagonized by yohimbine, but not by prazosin and naloxone. The inhibitory effect of oxymetazoline (0.185–3.4 μmol/kg, i.v.) was only partially reversed by yohimbine.
Gastric acid secretion: The antisecretory effect of i.c.v. clonidine was blocked by yohimbine and naloxone, but not by prazosin.
Gastroprotection: The protective effect of i.c.v. clonidine on ethanol induced damage was blocked by yohimbine, prazosin, and naloxone. The protective doses of clonidine (ED_{50}: 0.14 nmol/rat, i.c.v.), DAGO (ED_{50}: 0.0068 nmol/rat, i.c.v.) proved to be much lower than the gastric emptying delaying and the antisecretory ones.
In conclusion, (1) $\alpha_{2B/2C}$ subtypes may mediate the gastroprotection, while $\alpha_{2A/2D}$ subtypes may account for the antisecretory and gastric motility and emptying inhibitory effect of α_2 adrenoceptor agonists. (2) Opioid component may be involved in their gastroprotective and antisecretory effects, but not in the inhibitory effect of gastric motility and gastric emptying. (3) The protective doses of opioid and α_2 adrenergic receptor stimulants are much lower, than the gastric emptying and gastric motility inhibitory ones. Consequently,

no correlation is likely to be exist between the gastroprotective effect and affection of gastric motor activity and gastric acid secretion induced by α_2 adrenoceptor stimulants.

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ISTVÁN GACSÁLYI (2008)

Anxiolytic activity and the mechanism of action of deramciclane

Supervisor: Imre Klebovich

Deramciclane, EGIS-3886 is an original compound developed by EGIS Pharmaceuticals Plc. Deramciclane showed strong affinity for central 5-HT_{2C} (K_i=8.7 nM) and 5-HT_{2A} (K_i=11.0 nM) receptors, moderate affinity for σ_1 (K_i=52.0 nM), 5-HT_6 (K_i=0.0 nM), 5-HT_7 (K_i=105.0 nM) and D_2 (K_i=113.0 nM) receptors. Deramciclane did not bind to (K_i>1000 nM) α_1, α_2, β, D_1, 5-HT_{1A}, $GABA_A$, benzodiazepine, CCK_A, CCK_B, and H_1 receptors.

On the basis of our studies, the effect of deramciclane on the peripheral and central 5-HT systems is different. The weaker peripheral and stronger central activities were supported by the receptor binding studies and the results of DOI induced "head twich" test (MED= 1.0 mg/kg p.o.), and the results of 5-HT-induced pawoedema test in rats (MED=30.0 mg/kg p.o.). Further experimental evidence for weaker peripheral efficacy of the compound was obtained from studies on isolated organs of rabbits (isolated aorta strip IC_{50}=420 nM). On the basis of the above-mentioned studies deramciclane has an antagonistic character at 5-HT_{2A} and 5-HT_{2C} receptors.

Deramciclane exerted remarkable anxiolytic activity in three animal models of anxiety (Vogel model in rats, MED=1.0 mg/kg i.p., marble burying test in mice ID_{50}=7.1 mg/kg p.o., and the light-dark test in mice, MED=3.0 mg/kg s.c.).

On the basis of studies performed the 5-$HT_{2A/2C}$ receptors play a dominant role in the anxiolytic efficacy of the compound but the roles of 5-HT_6, 5-HT_7 andσ_1 receptors cannot be excluded.

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ANDREA HORVÁTH (2006)

Changes of the nociceptin system in hepatological disorders, mostly in hepacellular carcinoma

Supervisor: Ferenc Szalay

Nociceptin (N/OFQ) is a 17 aminoacid endogenous opioid, structurally resembles mostly dynorphin A, but it does not bind classical opioid receptors. It modulates the pain perception, cognitive function, kinetic activity, but its function is not known in detail. Expression of N/OFQ has been detected not only in the central nervous system, but also in the liver, and inflammatory cells. We reported elevated plasma nociceptin level in Wilson disease. Based on an accidental observation I investigated nociceptin in human and experimental hepatocellular carcinoma (HCC).

We observed elevated nociceptin level in patients with Wilson disease, PBC, and liver cirrhosis of different etiology compared to the healthy controls. We found strikingly high N/OFQ plasma level in patient with HCC compared to both healthy and also to other liver diseased control groups, independently whether the patients had pain or not. These findings suggested that N/OFQ may be considered as a tumor marker. This possibility was supported by the observation of continuous elevation of plasma N/OFQ parallel with AFP level during the development of HCC in a PBC patient. The tumor appeared after 18 years follow up and caused the death of the patient within 2 years. The nociceptin content was higher in the tumorous liver tissue, than in the corresponding non-malignant liver tissue.

In rats with experimentally induced HCC, we measured higher plasma, cerebrospinal fluid, and tumorous liver tissue N/OFQ content compared to both untreated and to cirrhotic animals. We also found higher N/OFQ content in human hepatocellular carcinoma tissue compared to the surrounding cirrhotic liver tissue.

The high plasma N/OFQ level found in patients with extrahepatic malignancies suggests that the increase in N/OFQ level may be a general accompanying sign of malignant diseases. According to our novel data the high plasma nociceptin level could be an indicator of hepatocellular carcinoma. Our results may stimulate further researches to clarify whether the nociceptin is produced by the tumor itself or the tumor accumulates it by increased receptor expression or the tumor sends a signal to the brain for increasing prepronociceptin and N/OFQ synthesis.

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VERONIKA JENEI (2006)

Intracellular regulation and pharmacological induction of cell adhesion

Supervisor: Kálmán Magyar

The role of cell adhesion and migration is of importance in a number of diseases, such as development of metastatic lesions and neurodegenerative disorders. Elucidating the molecular mechanisms that regulate cell adhesion and migration is fundamental for the design of specific molecular and/or pharmacological therapies to prevent or slow the progression of these diseases. The aim of my work was to investigate the role of the adaptor protein e3B1/Abi-1, as a potential therapeutical target, in the epidermal growth factor receptor (EGFR) signaling, which participates in the regulation of cell proliferation and adhesion. Furthermore, I was aiming to elucidate the possible effect of the antiparkinsonian monoamine oxidase (MAO)-B inhibitor, R-(–)-deprenyl on cell-cell adhesion. Our most important theses are as follows: (1) We confirmed the previously identified role of e3B1/Abi-1 in the EGF-induced activation of the small G protein, Rac1 in NIH3T3 fibroblasts overexpressing e3B1/Abi-1 and EGFR. At the same time, we excluded the possibility that e3B1/Abi-1 inhibits cell proliferation by downregulating the activation of the small G protein, Ras. (2) We were the first to identify that overexpression of e3B1/Abi-1 in mouse embryo fibroblasts sensitizes the EGF-induced activation of another small G protein, Rap1. (3) We proved that this process in adherent cells is strongly dependent on Src family kinases, but independent on Abl or PI-3 kinase, and the Rap1 GEF, C3G. (4) Our results suggest that this mechanism is a result of a collaborative response between the integrins and the EGFR, which can be a possible explanation for our next finding, namely that e3B1/Abi-1 affects cell adhesion. (5) We showed the integrin-dependent activation of Rap1 in isolated human polymorphonuclear neutrophils, which was also dependent on Src family kinases and PI-3 kinase. (6) We demonstrated a novel, MAO-B-independent effect of R-(–)-deprenyl; namely that it induces homotypic cell-cell adhesion of both neuroectodermal and non-neuronal-originated cells. This finding can contribute to the understanding of the protective effect of this drug. (7) This cell-cell adhesion inducing effect of R-(–)-deprenyl is irreversible within a 24-hour recovery period, depends on the proper molecular configuration, and most probably does not require the metabolism of the drug.

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GYÖRGY LENGYEL (2007)

Study of bilirubin conjugation and transport of conjugates in primary collagen sandwich and conventional rigid hepatocyte culture

Supervisor: László Vereczkey

The UGT1A1 conjugation enzyme as well as multidrug resistance associated proteins (MRP2, MRP3) play a critical role in the bilirubin metabolism. Changing of the serum bilirubin and bilirubin-glucuronide (BG) levels truly reflect the physiological and pathological state of the liver. The aim of our study was to model the adaptation of mammalian organisms to the changing chemical environment via investigation of bilirubin metabolism. The stimuli of adaptation were caused by chronic treatment with inductors (rifampicin, clofibrate, phenobarbital, methylcholanthrene, dexamethasone) or acute treatment with inhibitors (indomethacine, probenecid, benzbromarone) of MRPs.

The BG secretion dramatically decreased in the first 4 h of culturing. The reason of this phenomenon may be the internalization of transport proteins from the cell membrane. In our model system the well-known citochrome P-450 inductors (dexamethasone, clofibrate, rifampicin), except methylcholantrene, increased UGT1A1 activity via metabolic enzyme induction (1). A method was elaborated to investigate the MRP3 mediated sinusoidal and MRP2 mediated canalicular disposition of BG in collagen sandwich cultured hepatocytes using bilirubin as source of substrate (2). In hepatocyte sandwich culture the effect of inhibitors on canalicular efflux was higher then the sinusoidal efflux. In the case of RIF, the inhibited canalicular efflux was compensated by enhanced sinusoidal efflux due to failing of the expression of the toxic effect of drug accumulation. The importance of our study lies in that the determination of BG efflux in sandwich hepatocyte cultures is suitable for the *in vitro* prediction of drug-induced cholestasis. The effect of investigated cholestatic drugs (IM, RIF) and transport inhibitors (PR, BB) was detectable via decreased BG efflux. Therefore, preclinical research of drug candidates using primary collagen sandwich hepatocytes could predict serious side effects of certain drugs (3).

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ÁGNES SZILÁGYI (2007)

Clinical aspects of polymorphism and haplotypes

Supervisor: Huba Kalász

Investigation of the genetic predisposing factors of complex inherited disorders is the main topic of recent human genetic research. In our studies, we analyzed the possible role of four candidate genes of the serotonin and dopamine systems in two complex traits, pediatric migraine and substance abuse.

Regarding the observation of pediatric migraine, we described a new endophenotype category characterized by intense vomiting and abdominal pain during the attack. Studying the supposed relation between polymorphisms and pediatric migraine, we have found an association between the STin2 VNTR of serotonin transporter gene and migraine with aura and the newly defined endophenotype as well. Concerning the dopamine system, the G1947A SNP of the COMT gene seemed to contribute to the early onset of migraine. Furthermore, our results indicate that this polymorphism may influence the well-known sex related difference in the prevalence of migraine as well.

Applying the case-control approach, no association was found between the nine relevant polymorphisms of the serotonin and dopamine systems and substance abuse. This negative result can be explained by the heterogeneity of this phenotype. Further investigations showed that untreated childhood attention deficit and hyperactivity can be risk factors in the development of substance use disorder. Two variants of DRD4 gene were identified as genetic components in the background of this trait, underpinning the relevancy of the new endophenotype.

A novel approach was recently launched in the study of the genetic background of complex inherited disorders, the analysis of a newly described variation type, the so called copy number polymorphisms. However, its methodology is still problematic. Using the gene number variation of the human complement component C4 as a model system, we developed a new real-time PCR based method and another single-tube reaction that combines the advantages of a multiplex PCR system and capillary electrophoresis. The two newly developed methods can be reliably applied for the quantification of C4A and C4B genes and can be easily adapted for the determination of any other copy number polymorphisms.

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- *Szilagyi A, Blasko B, Ronai Z, Fust G, Sasvari-Szekely M, Guttman A (2006) Rapid quantification of human complement component C4A and C4B genes by capillary gel electrophoresis. Electrophoresis 27: 1437–1443.*

CSABA SZÁNTAI-KIS (2006)

Structure-activity relationships of kinase inhibitors

Supervisor: László Őrfi

Protein kinases play important role in the pathomechanism of many diseases. During my work we made QSAR models, which can be used for designing new inhibitors against therapeutically relevant kinases. We built a database for storing and collecting the input structures and activity data, which is needed for modeling. This can be accessed via a simple internet browser program. I have applied our in-house developed 3DNET4W software for model building. Model building algorithm was optimized for maximizing the predictive capability. We validated our models externally after the internal validation then we checked the selected descriptors for chance correlation. Based on these results we decided whether the given model can be used for virtual screening. Models, which are used for virtual screening, highly likely extrapolate when predicting molecules with unknown activities. We have compared the power of selection methods of the external validation set using a widely accepted dataset of solubility in water. The comparison of the methods suggested that it is preferable to use perimeter oriented external set selection. Recently analyzing our results we found that a randomly selected external validation set may give more balanced result depending on the size of the input dataset. QSAR models of EGFR, Akt1 and CDK4 inhibition had good predictive capability and there weren't chance correlations of descriptors. These models can be used for virtual screening. The EGFR inhibition model predicted well a set of recently synthesized benzo-thieno-pyrimidin derivatives. Based on PDGFRβ and ROCK-II data I couldn't make well predicting models, which weren't chance correlation of descriptors.

Biological data which are used in QSAR studies usually originate from biochemical assays and molecules selected by virtual screening are also screened in this kind of assays. We have developed Akt1 IMAP assay successfully, which can be used for screening of potential inhibitors. We use a storage and identification system for these compounds which is based on 2D barcode technique. I have made an appliance, which consist of a balance and a 2D barcode scanner connected to a PC, for facilitating the compound weigh-out process to the storage tubes. I have developed an Excel based application for controlling the appliance.

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- *Szántai-Kis C, Kövesdi I, Erös D, Bánhegyi P, Ullrich A, Kéri G, Örfi L (2006) Prediction oriented QSAR modelling of EGFR inhibition. Curr Med Chem 13: 277–287.*
- *Kéri G, Örfi L, Erös D, Hegymegi-Barakonyi B, Szántai-Kis C, Horváth Z, Wáczek F, Marosfalvi J, Szabadkai I, Pató J, Greff Z, Hafenbradl D, Daub H, Müller G, Klebl B, Ullrich A (2006) Signal transduction therapy with rationally designed kinase inhibitors. Cur Sig Trans Ther 1: 67–95.*

MÁRIA TÓTH (2008)

In vitro and *in vivo* pharmacokinetic evaluation of mechanism of tofisopam-tacrolimus drug-drug interaction

Supervisor: Imre Klebovich

Interaction between tacrolimus and the 2,3-benzodiazepine anxiolytic tofisopam has been reported after kidney transplantation. Tofisopam caused 2–5 folds augmentation in plasma concentration of tacrolimus leading clinically relevant renal function impairment. Considering the *in vitro* findings have indicated that CYP 450 3A4 is responsible for tacrolimus metabolism, the *in vitro* evaluation was done using human recombinant CYP 3A4. In *in vitro* experiments the inhibitory effect of tofisopam (IC_{50}=0.8 μmol/l) was lower than that of ketokonazole (potent CYP3A4 inhibitor) with an order of magnitude (0.03 μmol/l). After the *in vitro* examination, the human pharmacokinetic study was performed with involvement of healthy volunteers by reason to reach the best knowing the pharmacokinetic properties of tofisopam before planning of the interaction studies. To evaluate the clinical relevance of the CYP 450 3A4 inhibiting effect of tofisopam, a clinical interaction study was performed using "probe drugs". At first the effect of tofisopam was evaluated on hepatic CYP 450 3A4 enzymes using alprazolam as a probe drug. Based on the results tofisopam slightly modified (20%) the single oral dose pharmacokinetics of alprazolam, indicating modest hepatic blockade of CYP 450 3A4. The magnitude of inhibition was not sufficient to explain the pharmacokinetic interaction between tofisopam and tacrolimus. Tacrolimus is a substrate of the intestinal CYP 450 3A4; therefore interaction between the two drugs may occur at the intestinal level. The pharmacokinetic/pharmacodynamic drug-drug interaction study was done to evaluate the effect of tofisopam on the CYP 450 3A4 in the gut wall, using midasolam as a probe drug. The C_{max}, AUC_0 and $_t$ values of midasolam increased, and the CL_{oral} decreased significantly when midasolam was administered with tofisopam compared to the administration of midasolam alone. Summarizing the results can be stated that the tofisopam-tacrolimus drug interaction observed at transplanted patients caused by the CYP-450 3A4 competitive inhibitory effect of tofisopam at hepatic and also the intestinal level. Nevertheless, based on the FDA recommendation tofisopam could be considered as a moderate inhibitor of CYP 3A4, because it increased the AUC of sensitive CYP3A4 substrates less than five fold. Based on the above mentioned finding the following conclusion can be drawn: Summarizing of the results, in the observed clinical cases, the tofisopam-tacrolimus interaction was cased by tofisopam inhibitory effect on tacrolimus metabolism on CYP 450 3A4 enzyme at hepatic and also at intestinal level. The blood concentrations of drugs that are metabolised by CYP3A4 may be increased if they are used in combination with tofisopam.

- *Tóth M, Bajnógel J, Egyed A, Drabant S, Tömlő J, Klebovich I (2005) Tofisopam hatása a humán rekombináns CYP3A4 szuperszóma enzim aktivitásra. Acta Pharmaceut Hung 75: 25–28.*
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SEVIL YASAR (2006)

Reinforcing and discriminative-stimulus effects of *l*-deprenyl (selegiline) and its isomer in rats and in monkeys

Supervisor: Kálmán Magyar

Rationale: *l*-Deprenyl (selegiline) is used to treat Parkinson's disease and has been proposed as a treatment for smoking cessation and psychostimulant abuse. *l*-Deprenyl is metabolized in the body to *l*-methamphetamine and *l*-amphetamine, suggesting that it may have abuse potential. **Methods:** Wistar rats were trained to discriminate injection of methamphetamine, *d*-amphetamine or cocaine from injection of saline using two-lever choice schedules of food delivery or stimulus-shock termination. Squirrel monkeys were also trained to discriminate methamphetamine from saline injection using a two-lever choice schedule of stimulus-shock termination. Other monkeys were trained to self-administer i.v. cocaine or *d*-amphetamine under fixed-ratio (FR) or second-order schedules with FR components. Other drugs and drug combinations were tested by substitution. **Drug-discrimination results:** *l*-Deprenyl had psychostimulant-like discriminative stimulus effects in rats and squirrel monkeys, but only at very high doses that markedly depress behavior and that are 10 to 20 times higher than doses selective for MAO-B inhibition. Ro 16-1649, a reversible inhibitor of MAO-B without psychoactive metabolites, had no psychostimulant-like discriminative effects and blockade of *l*-deprenyl's metabolism with SKF 525A eliminated *l*-deprenyl's psychostimulant-like discriminative effects in rats. *l*-Deprenyl's psychostimulant-like discriminative effects in rats were reduced when β-PEA synthesis was blocked by NSD 1015, indicating facilitatory modulation of the effects of the *l*-deprenyl metabolites by elevated levels of β-PEA. The *l*-deprenyl analog, *p*-fluoro-*l*-deprenyl, had methamphetamine-like discriminative-stimulus properties in squirrel monkeys at relatively high doses. **Drug self-administration results:** Rates of responding maintained by *l*-deprenyl and by *p*-fluoro-*l*-deprenyl were much lower than for *d*-deprenyl. At doses 10- to 20-times higher than doses selective for MAO-B inhibition, *l*-deprenyl failed to maintain drug-taking behavior above vehicle placebo levels. *p*-Fluoro-*l*-deprenyl maintained drug-taking behavior above vehicle levels, but it was a relatively limited reinforcer of drug-taking behavior compared to cocaine and amphetamine. **Conclusions:** The effects of *l*-deprenyl and *d*-deprenyl on MAO-B clearly do not contribute to their behavioral effects. Production of amphetamine and methamphetamine metabolites is primarily responsible for the psychostimulant-like discriminative effects of *l*-deprenyl seen at high doses and these discriminative effects can be modulated by elevated levels of β-PEA under some conditions. These results suggest that neither *l*-deprenyl nor *p*-fluoro-*l*-deprenyl, have significant human abuse potential, thus are not useful for the proposed treatment for smoking cessation and psychostimulant abuse.

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- *Yasar S, Winger G, Nickel B, Schulze G, Goldberg SR (1993) Preclinical evaluation of l-deprenyl: lack of amphetamine-like abuse potential. Pp 215–233 in: Szelényi I (ed) Inhibition of monoamine oxidase B: Pharmacology and clinical use in neurodegenerative disorders. Birkhäuser Verlag, Basel.*
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SCHOOL OF PH. D. STUDIES

4. MENTAL HEALTH SCIENCES

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General overview

The Ph. D. training and research programs of this Doctoral School of Semmelweis University aim to offer research and methodological training for those interested in psychiatry, clinical psychology and mental health sciences.
The school promotes initiatives related to preventative medicine. One of its goals is to facilitate the Ph. D. candidates' skills for promoting their research topic in public and provides feedback on their academic competence during the Ph. D. training period.
Our research topics highlight the interactions between clinical neurosciences, mental health and social sciences in medicine. Our school is proud to have a history of promoting interdisciplinary research.

PROGRAM 4/1.

CLINICAL PSYCHOLOGY AND PSYCHIATRY

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Program overview

This program offers education and research possibilities for, medical doctors, psychologists and other eligible persons in behavioural sciences, clinical psychology and in the neurobiological, psychosocial, diagnostic and treatment aspects of psychiatric disorders. The research profile of the program has considerably widened in the last few years. New research topics include psychiatric, genetics and psychophysiology, as well as cognitive neuropsychology and social cognition in several psychiatric diseases, including psychotic disorders and adult ADHD. Another important area covers methodological research in the field of clinical psychopharmacology in cooperation with genetics, biochemistry and bioinformatics.

Titles of research projects	*Supervisors*
Genetic factors and gene x environment interactions in psychiatric disorders: The NEWMOOD study?	György Bagdy
Psychosocial and inherited factors in depression and addiction	György Bagdy
Psychiatric disorders in children and adolescents	Judit Balázs
Clinical psychopharmacology	István Bitter
Psychophysiological and neuropsychological mechanisms of psychiatric disorders	Pál Czobor
Survey of electroconvulsive therapy's clinical use	Gábor László Gazdag
Epidemiology, clinical and psychosocial characteristics of the addictive diseases	József Gerevich
Mental deficits in the developmental psychopathology	György Gergely
The application of neuro-cognitive tests in psychiatric disorders	Szabolcs Kéri
Family pathology and communication	Tamás Kurimay
Experimental behavioural physiology and pharmacology	Csaba Nyakas
Post-traumatic stress disorder (PTSD), trauma research	Dóra Perczel Forintos
Suicide prevention	Dóra Perczel Forintos
The research of energetic aspects of mental health by the use of actigraphia	Péter Rajna
Clinical and biological aspects of affective disorders	Zoltán Rihmer
Computer aid in the diganosis and psychotherapy of psychiatric disorders; identifying non-verbal communication and virtual reality therapy	Lajos Simon
The recognition and expression of emotions in psychiatric disorders	Lajos Simon
Psychotherapy in the medical practice	László Tringer

Ph. D. students		*Supervisors*
Anita Benkő	ft	György Bagdy
Nikoletta Bódi	ft	Szabolcs Kéri
Gyöngyvér Dallos	pt	Judit Balázs
Árpád Dóró	pt	Tamás Kurimay
Judit Futó	ft	György Gergely
Nóra Garamvölgyi	pt	Zoltán Rihmer
Krisztina Kocsis-Bogár	pt	Dóra Percze Forintos
Sarolta Komlósi	ft	Pál Czobor
Krisztián Kórász	pt	Lajos Simon
Daniella Kováts	pt	Tamás Kurimay
Krisztina Magyar	pt	István Bitter
Ágnes Mészáros	ft	István Bitter
Mónika Barbara Miklósi	ft	Dóra Perczel Forintos
Eszter Molnár	ft	György Bagdy
Dorottya Pap	ft	György Bagdy
István Pénzes	pt	György Gergely
Csaba Petővári	ft	József Gerevich

Patrícia Polgár	pt	István Bitter
József Szabó	pt	József Gerevich
Anabella Veress	ft	János Füredi

Ph. D. candidates		***Supervisors***
Brigitta Baran	i	István Bitter
Anna Bátki	ft	György Gergely
Lilla Katalin Kozek	ft	László Tringer
Judit Lazáry	ft	György Bagdy
László Mayer	i	Judit Balázs
Tamás Telek	ft	István Bitter

Ph. D. graduates		***Supervisors***
Szabolcs Barótfi	pt	István Mucsi
Pál Czobor	i	István Bitter
Márta Farkas	i	István Bitter
Xénia Gonda	ft	György Bagdy
Zoltán Hidasi	pt	Péter Rajna
Zoltán Kovács	pt	László Tringer
Tamás Tölgyes	i	László Tringer
Zsolt Szabolcs Unoka	i	Lajos Simon
Terézia Zsombók	pt	György Bagdy

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

SZABOLCS BARÓTFI (2006)

Quality of life assessment in patients with chronic kidney disease

Supervisor: István Mucsi

In the evaluation of patient care and therapies in chronic conditions, the assessment of psycho-social parameters and quality of life have become increasingly important recently in addition to classical clinical and laboratory outcome parameters. In end stage renal disease, the disease itself and the applied renal replacement therapy has significant effect on the patients' everyday life, hence on their quality of life as well. Numerous large, multinational studies have been performed to assess quality of life (QoL) of patients with renal disease and factors associated with QoL, however there is little data available in Hungary with regard to this matter. One of the reasons behind this is the lack of appropriately translated and validated QoL instruments in Hungarian language. The Kidney Disease Quality of Life (KDQOL-SFTM) questionnaire is a widely used QoL tool in nephrology, which has been translated and validated in several languages. In our first study we performed the complete validation process of the Hungarian version of the KDQOL questionnaire. Basic

psychometric properties were determined of the Hungarian version on large group of dialysis and kidney transplantation patients. Our results confirmed that the Hungarian KDQOL questionnaire is a reliable and valid tool and appropriate to assess QoL of renal patients in Hungary. In further studies we examined the association between certain components of QoL and some selected sociodemographic and clinical parameters in dialysis patients. Our studies showed that comorbid conditions, the presence of sleep disorders and time from renal disease diagnosis are significant predictors of QoL in dialysis patients. We also confirmed the previous findings that older and female patients are reporting worse QoL than younger and male patients, and higher education and being on a transplantation waiting list is associated with better QoL. Our results confirmed those previous findings that serum albumin and creatinine levels correlates strongly with patients' QoL. In addition we found that illness intrusiveness experienced by patients on dialysis is significantly correlated with their comorbid conditions.

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- *Barótfi Sz, Molnár MZs, Almási Cs, Kovács A, Remport A, Szeifert L, Szentkirályi A, Vámos E, Zoller R, Eremenco S, Novak M, Mucsi I (2006) Validation of the Kidney Disease Quality of Life-SF questionnaire in kidney transplant patients. J Psychosom Res 60: 495–504.*
- *Mucsi I, Molnár MZs, Réthelyi J, Vámos E, Csépányi G, Tompa Gy, Barótfi Sz, Marton A, Novak M (2004) Sleep disorders and illness intrusiveness in patients on chronic dialysis. Nephrol Dial Transplant 19: 1815–1822.*

PÁL CZOBOR (2007)

Basic underlying structure of psychopathological symptoms in schizophrenia and its change over time during pharmacological treatment

Supervisor: István Bitter

Background: Identification of basic underlying dimensions of manifest psychopathology in schizophrenia has been the focus of on-going research for over 50 years. Although various models have been proposed, and countless empirical studies were conducted in order to address this issue, the true intrinsic dimensionality of the observed clinical manifestations remain elusive. In this dissertation, we identified and addressed several major methodological problems that may underlie the continuing uncertainties regarding the factor structure of psychophathological symptoms as indexed by widely used comprehensive psychopathological scales, including the BPRS and the PANSS. We placed a particular emphasis on addressing the lack of longitudinal view that characterized most studies in the literature.

Objective: The principal goal was to investigate whether the same factor structure can provide an adequate representation of symptoms at different time points during psychopharmacological treatment in clinical trials, including a placebo washout period. In light of the time-varying associations among clinical symptoms that have been documented in the pertinent literature, we considered of major interest to investigate whether time invariant latent variables (factors) can describe the data at different points in time.

Method: In order to accomplish this, a 2-step approach was adopted. In the 1st step, exploratory factor analyses were performed at different time points during treatment. In the 2nd step, confirmatory factor analysis was applied.
Results: The main findings indicated that factor structures that have been reported in the literature were reproducible with high fidelity at the pre-placebo time point. However, during placebo fundamental changes took place in the factor structure. Results also indicated a reemergence of the initial (pre-placebo) factor structure for most of the factors during treatment treatment.
Conclusion: These findings support the view that the latent structure of psychopathological factors cannot be conceived as consisting of time invariant and "pure" (non-overlapping) constructs. They also suggest that longitudinal applications of the traditional factors of psychometric instruments that measure a broad spectrum of symptoms should take the issue of factorial stability over time into consideration. The fact that the congruence of empirically derived factors with the clinical factors was the lowest at placebo can be viewed as a reflection of decreased construct reliability at this point in time. Because in many clinical trials data from the placebo (wash-out) time point are used as standard for longitudinal comparisons, we think that replication studies, testing BPRS, PANSS as well as other major rating instruments, could be of considerable practical use.

- *Czobor P, Volavka J (1996) Dimensions of the Brief Psychiatric Rating Scale: an examination of stability during haloperidol treatment. Compr Psychiatry 37: 205–215.*
- *Czobor P, Volavka J (1996) Positive and negative symptoms: is their change related? Schizophr Bull 22: 577–590.*
- *Lindenmayer JP, Czobor P, Volavka J, Lieberman JA, Citrome L, Sheitman B, McEvoy JP, Cooper TB, Chakos M (2004) Effects of atypical antipsychotics on the syndromal profile in treatment-resistant schizophrenia. J Clin Psychiatry 65: 551–556.*

MÁRTA FARKAS (2008)

Psychopathologic and cognitive investigations in schizophrenia

Supervisor: István Bitter

The topics of the thesis are the analysing the long-term course of delusions and hallucinations in patients with psychotic disorders, in particular in the highlight of schizophrenia patients—according to "Budapest 2000" project; and studying the feedback-guided associative learning and acquired equivalence in two groups of schizophrenia patients and matched controls. The aims were (1) to study the severity, the changeability, and likelihood of appearance of delusions and hallucinations in each identified clinical group over time; (2) to differentiate the courses of illnesses along the two psychopathological symptoms; (3) to investigate the cognitive dimension in schizophrenia patients regarding to the feedback-guided associative learning and acquired equivalence: distinguishing the deficit/non-deficit schizophrenia subtypes from each other and controls. **Methods:** 221 female patients were evaluated in the first study. Originally, the subjects were classified according to Leonhardian nosological system into affective, cycloid and schizophrenia groups (at the endpoint of the study was re-diagnosed acc. to DSM-IV) and were assessed at three different times (index, and 5– and 21–33-year follow-up). The Rockland-Pollin Rating

Scale and "List of Specific Symptoms" were obtained at each time point in the study. In the second study 49 male and female schizophrenia patients and 20 matched controls were evaluated. They were assessed by Rutgers Acquired Equivalence Test, some frontal neuropsychological tests, and PANSS. **Results:** (1) The main positive symptoms with some fluctuations persist through the illness courses. Each symptom of positive dimension shows reduction, but the severity of hallucinations shows a significant worsening after the 5-year, while the severity of delusions shows mild, but not significant reduction. The observed symptoms and social functioning are distinguishing the affective, cycloid and schizophrenia groups and certain schizophrenia subgroups were distinguishable based on the correlation of the two symptoms. (2) The acquired equivalence learning was similarly impaired in deficit and non-deficit patients, whereas feedback-guided associative learning was impaired only in deficit patients. Associative learning and acquired equivalence were not related to frontal lobe tests. **Conclusions:** (1) The two subgroups (deficit/non-deficit) of schizophrenia may distinguish based on observed phenomenon of the cognitive dimension. (2) The classical schizophrenic subtypes may differentiate (catatonic, hebephrenic, paranoid) based on the changes of positive dimension through the long-term follow-up investigation.

- *Farkas M, Tolna J, Vizkeleti Gy, Pethő B (2007) Szkizofrénia vagy szkizofréniák? Neuropsychopharmacol Hung 9(1) Suppl: 3–6.*
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XÉNIA GONDA (2008)

The serotonin transporter gene and personality: Association of the 5 HTTLPR s allele, anxiety, depression and affective temperaments

Supervisor: György Bagdy

We investigated the association of the 5HTTLPR polymorphism of the serotonin transporter gene and anxiety, depression and affective temperaments in a psychiatrically healthy female population. The association of this polymorphism has earlier been described in case of psychiatric disorders (anxiety and affective disorders). In case of a healthy population, however, mainly its association with the neuroticism trait was examined. Our aim was to investigate the association of subclinical manifestations of depression and anxiety and possible association of affective temperaments with the above polymorphism. In case of the neuroticism trait an association with 5HTTLPR has earlier been demonstrated. Neuroticism, however, is a complex trait incorporating tendency for anxiety, depression, irritability and mood lability, and thus it was not obvious whether all the above psychological phenomena equally contribute to the association of neuroticism and the s allele, or only some of these play a crucial role in this relationship. In our research we administered questionnaires assessing depression (ZSDS, Zung Relf-rating Depression Scale), anxiety (STAI, State-Trait Anxiety Inventory) and affective temperaments (TEMPS-A,

Temperature Evaluation of Memphis, Pisa, Paris and San Diego), and determined 5HTTLPR genotype by means of polymerase chain reaction (PCR). We found that depression, anxiety and affective temperaments carrying a depressive component (depressive, cyclothymic, anxious, irritable) show significant association with the s allele of the 5HTTLPR, indicating that people carrying the s allele are more prone to increased anxiety, depression and are more likely to carry one of the affective temperament-types incorporating a depressive component. Furthermore, if only physical and vegetative symptoms of depression are taken into consideration this association was even stronger, indicating that physical and vegetative symptoms of depression are also strongly associated with the s allele. Viewed from the aspect of gene-environment interactions our results show that the presence of dominant affective temperaments and personality traits related to neuroticism confers increased vulnerability to chronic stress and other life events which play an important causative role in the development of depression. Our findings open the potential to identify within the community people with temperamental inclination to mood instability with the joint use of measures of putative behavioural endophenotypes and molecular genetic markers. The present findings bring psychiatric genetics closer to the aim of delineating risk profiles for clinically manifested affective disorders in more precise quantitative measures and thus point to the possibility of preventive psychological and biological therapies. This further emphasises the need for future research to delineate the genetic background of traits and characteristics observable in the healthy population as endophenotypes related to neuropsychiatric disorders.

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- *Gonda X, Rihmer Z, Zsombok T, Bagdy G, Akiskal KK, Akiskal HS (2006) The 5HTTLPR polymorphism of the serotonin transporter gene is associated with affective temperament as measured by TEMPS-A. J Affect Disord 91: 125–131.*
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ZOLTÁN HIDASI (2008)

Static and dynamic electroencephalographic studies in Alzheimer's disease

Supervisor: Péter Rajna

In my thesis static and dynamic EEG characteristics were studied in Alzheimer disease (AD), using simple sensory stimuli and cognitive task situations. Electrophysiological markers have increasing importance with respect to new data on prevention, diagnosis, prognosis and therapy of AD. Static and dynamic quantitative EEG measures combined with cognitive tasks are widely used for the assessment of cognitive and pathophysiological changes in Alzheimer's disease. Main aims of my work were the evaluation of simple but informative EEG situations, appropriate quantitative EEG measures and task situations suitable for the investigation of AD, and the comparison of static and dynamic EEG methods in AD. In our first study AD group of mild severity and normal controls were compared with the use of spectral EEG measures and non-linear complexity characteristics, using

both static and dynamic methods. The dynamic approach used eye-opening as a different state. Frequency spectra did not differ in the two groups, whereas significant decrease of coherence values were present in the AD group relative to controls. In the main part of my work, a study comparing patient group with mild to moderate AD and normal controls is presented, using static and dynamic methods. Spectral and coherence values were evaluated, combined with a cognitive task situation. Instead of the analysis of EEG data obtained during the performance of the task, in this study data recorded in the immediate after-task period were analyzed. The performance of the task resulted in an increase of the relative alpha2 band in the AD group, whereas it slightly decreased in the control group. The most prominent coherence differences between AD and controls were found in the alpha1 band, especially for long-range coherence values. Coherence in this frequency band increased in the control group following the task, not seen in the AD group. We conclude that EEG parameters calculated from epochs following the completion of a cognitive task clearly differentiates patients with AD from normal controls. The electrophysiological changes found in AD may correspond to the decrease of functional connectivity of cortical areas and to the malfunctioning of the networks engaged in the cognitive task investigated. In conclusion, dynamic EEG approach, especially with a cognitive task provides extra information in AD. Results are remarkable regarding pathophysiological aspects and early diagnostic possibilities.

- *Hidasi Z, Czigler B, Salacz P, Csibri É, Molnár M (2007) Changes of EEG spectra and coherence following performance in a cognitive task in Alzheimer's disease. Int J Psychophysiol 65: 252–260.*
- *Rajna P, Hidasi Z, Waldemar Sz (2005) Eseményfüggő EEG- és kiváltottválasz-vizsgálatok a klinikai gyakorlatban. Clin Neurosci 58: 380–392.*
- *Czigler B, Csikós D, Hidasi Z, Gaá ZsA, Csibri É, Kiss É, Salacz P, Molnár M (2008) Quantitative EEG in early Alzheimer's patients—power spectrum and complexity features. Int J Psychophysiol 68: 75–80.*

ZOLTÁN KOVÁCS (2008)

Psychometric evaluation of functional and organic gastrointestinal disorders

Supervisor: László Tringer

Objectives, methods: In the first study stressful life events, social support, depressive and anxiety symptoms, dysfunctional attitudes and coping strategies were assessed among irritable bowel syndrome (IBS) and inflammatory bowel disorder (IBD) patients and healthy subjects. In the second study we evaluated psychopathological and gastrointestinal symptoms and health-related quality of life among non-erosive reflux disorder (NERD) and erosive reflux disorder (ERD) patients. In the third study we investigated psychopathological symptoms in patients suffering from sphincter of Oddi dysfunction (SOD) and depression and healthy subjects. In the fourth study we investigated the effect of social, demographical characteristics and psychopathological symptoms on the symptom presentation of SOD patients. The fifth study was directed toward the evaluation of the factor structure of the SCL-90-R in different GI patients' groups. **Results:** IBS patients had more severe symptoms compared to IBD patients and healthy subjects, while IBD patients

had more severe symptoms compared to healthy subjects. NERD and ERD patients did not differ on the assessed scales, but in the group of NERD patients more pronounced correlations were detected among the severity of reflux symptoms and psychological distress, while ERD patients' quality of life was more strongly associated with the fluctuation of symptoms. SOD patients demonstrated less severe symptoms compared to depressed patients, and scores related to the irritability factor were more pronounced compared to healthy patients. The concurrent presence of acute stress and psychopathological symptoms was associated with an increased likelihood of SOD outcome. Instead of the original 9 factors our results give support for a 7 factor model of the SCL-90-R in the group of GI patients, and based on the analysis of the new factors, IBS patients exhibited most severe psychological distress, and the GI patients as group had more severe somatization and irritable depression symptoms compared to healthy subjects. **Discussion:** IBS patients exhibit the more severe psychopathological symptoms among the GI patients. The other functional GI groups did not exhibit more severe psychological symptoms compared to the organic groups, but presented more stronger associations between psychological, social and GI symptoms.

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- *Kovács Z, Kerékgyártó O (2007) Psychological factors, quality of life and gastrointestinal symptoms in patients with erosive and non-erosive reflux disorder. Int J Psychiatry Med 37: 139–150.*
- *Kovács Z, Kovács F, Pap Á, Czobor P (2007) Sphincter of Oddi dysfunction: does psychosocial distress play a role? J Clin Psychol Med Settings 14: 138–144.*

TAMÁS TÖLGYES (2007)

The epidemiological survey of eating disorders and dysfunctional eating behaviours—psychometric method and cognitive approach applied in identifying personality structures in patients suffering from eating disorders

Supervisor: László Tringer

Objectives were: (1) to survey of the prevalence rates for anorexia (AN) and bulimia nervosa (BN) and related adverse dieting attitudes and eating behaviours among secondary school, and college students in Budapest and Pécs; (2) to elucidate whether eating disorder (ED) subtypes—as defined by DSM-IV—exhibit specific characteristics in terms of depression, dysfunctional attitudes, assertive behaviour, coping style, visual shape assesment and early maladaptive schemes (EMS).
Methods: (1) In the process of a two-stage study a series of Bulimia Investigatory Test Edinburgh (BITE), Eating Attitudes Test and Beck Depression Inventory (BDI) were applied for screening and a semi-structured diagnostic interwiew was conducted. The test-battery was administered to 800 representatives out of which 580 test-battery (332 females, 248 males) were returned. (2) The specific characteristics of ED subgroups were measured by BDI, Burns Dysfunctional Attitudes Scale, Rathus Assertiveness Scale, Ways of Coping Questionnaire, Human Figure Drawings Test and Young Schema Qestionnaire. The Questionnaires were filled out by 73 Restrictive AN (RAN), 46 Binge/Purging type of

AN (BPAN), 87 BN patients and by a control group of 67 healthy women. The mean differences between specific groups were identified by general linear model (GLM) analysis. The structure of the EMS factors among the ED was examined by factor-analysis, while the differences among the subgroups by GLM-analysis.

Results and discussion: (1) The overall female sample, 3% revealed anorexic disposition, but no actual cases of AN were detected. 4.5% of the females and 0.8% of the males were classified as subclinical bulimics, whereas 3.6% of the females and 0.4% of the males could be listed in the category of simulated BN. The prevalence rate of BN, defined by DSM-IV, was 0.6% among women which could be proved by the diagnostic interview. (2) EDs were characterized by high depression values, and low values of assertivity and problem-solving coping styles, in contrast with the control group. BNs showed a stronger level of external control, and a higher need for appraisal, than those with the RAN, and control. BNs and BPANs showed a higher level of emotional coping, visual overestimation of physical self image and the preference for a thinner desired physical shape, compaired to that of the controls' and RANs'. RANs and BPANs showed higher scores on the EMS of self-sacrifice, unrelenting standards and punitiveness, while BNs and BPANs on the EMS of entitlement and insufficient self-control.

- *Tölgyes T, Nemessúri J (2004) Epidemiological studies on adverse dieting behaviours and eating disorders among young people in Hungary. Soc Psychiatry Psychiatr Epidemiol 39: 647–654.*
- *Unoka Zs, Tölgyes T, Czobor P (2007) Early maladaptive schemas and body mass index in subgroups of eating disorders: a differential association. Compr Psychiatry 48: 199–204.*
- *Tölgyes T, Simon L, György S (1996) A táplálkozási magatartászavarok kognitív és viselkedésterápiája. Psychiat Hung 11: 299–309.*

ZSOLT SZABOLCS UNOKA (2008)

Investigation of personality traits, symptom dimensions, parental maltreatment and identification of latent vulnerability dimensions in different mental disorders

Supervisor: Lajos Simon

Goals: Our first investigation had two main goals: (1) To investigate the mean differences between normal and psychiatric patient sample in terms of personality traits, childhood parenting experiences, symptom dimensions and canonical vulnerability factors, measured by Temperament and Character Inventory, Young Schema Questionnaire, Young Parenting Inventory and SCL-90 respectively. (2) To identify independent, latent vulnerability-diagnosis dimensions in a mixed sample, where the vulnerability variables were the following: temperament traits, early maladaptive schemas, parenting behaviors. (3) Our second investigation's goal was to study the reliability and factor structure of the Current Psychiatric State interview (CPS-50). **Methods:** Our first investigation's sample contains 157 normal subject and subjects who suffer from depression (395), anxiety disorders (138), bulimic symptoms (79), alcohol dependence (50), borderline (69), mixed personality disorder (60). (1) A mean difference among the diagnostic groups (normal, depressive, anxious, bulimic, borderline) in terms of different variables was investigated by General Linear

Model (GLM) analysis. Diagnostic groups was used as independent variable in the GLM model while the subscales of the used questionnaires and the Vulnerability canonical variates served as dependent variables and gender and age served as covariate. (2) Relationship between vulnerability factors (EMS, parenting experiences and temperaments) and diagnostic groups was analyzed using canonical correlation analysis and gender and age served as covariate. In our second investigation CPS-50 was used to assess 237 patients with a range of psychiatric diagnoses. Comparisons of interrater reliability on each item and on 8 subscales were made and principal component analysis was used to investigate the factorstructure of the CPS-50's items. **Results:** (1) The diagnostic groups showed a significant difference in their temperament and character dimensions, parenting experiences, early maladaptive schemas and symptom dimensions. (2) Association between vulnerability factors (EMS, parenting experiences and temperaments) and diagnostic groups reached significance for the first three pair of canonical factors, indicating a specific relationship between diagnostic groups and vulnerability factors. (3) Acceptable interrater reliability was found for 46 of the 50 items. The principal components analysis factors were similar to the clinical scales. **Discussion:** Our findings indicate that the investigated variables differ significantly among the diagnostic groups and that canonical vulnerability factors differ significantly among the diagnostic groups. The CPS-50 is a reliable standardized assessment of current mental status, and it has a theoretically sound factor structure.

- *Unoka Zs, Tölgyes T, Czobor P (2007) Early maladaptive schemas and body mass index in subgroups of eating disorders: a differential association. Compr Psychiatry 48: 199–204.*
- *Unoka Zs, Rózsa S, Kő N, Kállai J, Fábián Á, Simon L (2004) A Derogatis-féle tünetlista hazai alkalmazásával szerzett tapasztalatok. Psychiatr Hung 3: 28–35.*
- *Unoka Zs, Rózsa S, Fábián Á, Mervó B, Simon L (2004) A Young-féle séma kérdőív: a korai maladaptív sémák jelenlétét mérő eszköz pszichometriai jellemzőinek vizsgálata. Psychiatr Hung 3: 235–243.*

TERÉZIA ZSOMBÓK (2008)

The effect of autogenic training combined with organ formula and motion therapy on spontaneous and provoked headaches

Supervisor: György Bagdy

In my research I studied the effect of Schultz's autogenic training combined with organ formula and motion therapy elements on primary headaches, especially focusing on spontaneous and nitroglycerine-provoked headache. My aim was to establish whether frequent AT practice influences spontaneous headaches e.g. migraine attacks and use of medications in the treatment of migraine, provoked migraine, as well as changes in plasma cortisol levels, blood pressure and pulse frequency during migraine provocation. In our studies we established a 4-month observation period, and patients learned and practiced autogenic training in the next four months. We compared the effects of autogenic training on measured parameters and during nitroglycerin provocation to a control group. During the regular, 4-month practice of autogenic training the frequency of headaches and the use of medications decreased as compared to data during the observation period in all headache groups (migraine, tension and mixed headache). Effect of auto-

genic training appeared earliest and was most pronounced in tension and mixed headache groups, while in the migraine group a significant decrease was found only from the third month of autogenic training practice. While the decrease in headache frequency was strongly associated with the decrease in use of painkillers and antimigraine medications, there was no significant association found in case of the use of anxiolytic drugs.

NO donor nitroglycerine-provoked headache is one of the most widely used human migraine models. In our research we aimed at establishing whether frequent practice of autogenic training and its concomitant application during nitroglycerine treatment is able to influence the migraine provoking effect of the NO donor molecule and the increase of plasma cortisol levels. Our results indicate that nitroglycerine generates migraine attacks in migraine patients which in addition is accompanied by an increase in cortisol concentration. This migraine headache develops on average five hours after nitroglycerine ingestion and it can be clearly distinguished from the transitory headache appearing shortly after nitroglycerine ingestion and lasting about 30–60 minutes. Autogenic training significantly decreased the effect of nitroglycerine on blood pressure and pulse frequency. Despite of these, our results indicate that frequent autogenic training practice influenced parameters of neither immediate/transitory, nor migraine headache and had no influence on the increase of plasma cortisol levels either. These latter can be extinguished only by triptans used as medication for migraine attacks.

Our results indicate that autogenic training is an effective therapy in case of all three patient groups investigated, decreasing both headache frequency and related use of medications. Despite of these, it is unable to significantly influence migraine headache provoked by a strong chemical stimulus (e.g. a NO donor). Therefore autogenic training has a favourable effect on the quality of life of migraine patients, however, drug therapy is indispensable for treating evolving migraine attacks.

- *Zsombók T, Juhász G, Budavári A, Vitrai J, Bagdy G (2003) Effect of autogenic training on drug consumption in patients with primary headache: an 8-month follow-up study. Headache 43: 251–257.*
- *Zsombók T, Juhász G, Gonda X, Vitrai J, Bagdy G (2005) Kognitív- és szimbólumterápiás elemekkel módosított autogén tréning hatása az elsődleges fejfájós betegek kezelésére. Psychiatr Hung 20: 25–34.*
- *Juhasz G, Zsombok T, Gonda X, Nagyne N, Modosne E, Bagdy G (2007) Effects of autogenic training on nitroglycerin-induced headaches. Headache 47: 1–13.*

PROGRAM 4/2.

BEHAVIORAL SCIENCES

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Program overview

Behavioral sciences constitute an integrative field which bridge the paradigms of natural and social sciences. They study human behavior in a biological, psychological and social perspective, and provide an opportunity for establishing and analysing the components of healthy behavior, the psychological and social risk factors of diseases, as well as investigating the background of self-destructive conduct and the development of attitudes to protect health. They examine the regularities and the possibilities of developing human behavior from an interdisciplinary and integrative perspective relying on achievements of Medicine, Psychology, Sociology, Anthropology, Bioethics, Neuroanatomy and Neurophysiology. Nowadays, the prevention and successful treatment of diseases which impact on public health cannot rely entirely on a biomedical approach since the behavioral risk factors are highly influenced by psychological and social factors. The professional Program follows the analogous one of Johns Hopkins University (Baltimore, Maryland, USA).

Titles of research projects	*Supervisors*
The chronic one (conjugal-, work and social) stress, the distressful and depressed symptoms, and the cardiovascular illnesses the examination of the causality contexts of circular	Piroska Balog
Possible methods for measuring and assessing healthcare services and deliveries in Hungary	Éva Belicza
The relationships between sleep, cognitive activity and affective processes	Róbert Bódizs
The function and significance of the social capital in the healthcare system	Péter Gaál
Development of early attachment: Stress reactivity and parental care	Judit Gervai
Genetic and evironmental influences on infant temperament and attachment	Judit Gervai
Relationships between health condition and personality traits among adolescents and young adults with special regard to suicidal behavior	Ágnes Hajnal
Mental health aspects of death, dying and bereavement	Katalin Hegedűs
Behavior redress and health psychology	Mária Kopp
Health and bioethics	József Kovács
The relationship between stress, quality of life and physical-mental health (gender and age specific perspectives). Stress management, health protection and the development of communicative competence	Mónika Erika Kovács

Medical anthropology: Cultural and intercultural aspects of diseases and their treatment	Imre Lázár
Sleep disorders in patients with chronic kidney disease	Miklós Zsolt Molnár
The research of factors relating to the quality of life of patients with organ transplant	István Mucsi
The psycho-social aspects of chronic diseases	István Mucsi
Sleep disorders among chronic kidney disease patients	István Mucsi
Questions of efficiency in curative and preventive communication	Erzsébet Németh
The social and health significance of disorders in sleep and awake state	Márta Novák
Gender differences in the disorders in sleep and awake state	Márta Novák
The significance of mood and anxiety disorders in patients suffering from chronic diseases	Márta Novák
Study of youth's problem behavior and mental health based on the risk and protective theory	Bettina Pikó
The disturbed circadian the role of a rhythm comorbid in the development of psychiatric and internal medicine clinical pictures and its procession	György Purebl
Study of youth's problem behavior and mental health based on the risk and protective theory	Adrienn Stauder
Biological aspects of some sleep disorders	Anna Szűcs
Eating disorders and obesity—clinical aspects and prevention	Irena Szumska
Psychological assessment of eating disorders	Ferenc Túry

Ph. D. students		***Supervisors***
Babusa Bernadett	ft	Ferenc Túry
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Mária Eszter Czira	ft	Márta Novák
Zoltán Cserháti	ft	Andrienne Stauder
Nóra Dudás	pt	Andrienne Stauder
Szilvia Dukay-Szabó	ft	Ferenc Túry
Péter Farkas	pt	Imre Lázár
Ákos Gerencsér	pt	József Kovács
Edmond Girasek	pt	Péter Gaál
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Zita Jeszenszky	pt	Mária Kopp
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Anett Virág Lindner	ft	Miklós Zs. Molnár
Csilla Zita Madarász	pt	Márta Novák
Tamás Martos	pt (a)	Mária Kopp
Júlia Mazzag	pt	Erzsébet Németh

Katalin Fornádi (Molnárné)	pt	Márta Novák
János Pilling	pt	Mária Kopp
Anikó Smudla	pt	Katalin Hegedűs
Anna Susánszky	ft	Mária Kopp
Nóra Erzsébet Szabó	pt (a)	Katalin Hegedűs
Rita Széchey	pt	István Mucsi
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Johanna Takács	pt	Adrienne Stauder
Péter Vajer	pt	Adrienne Stauder
Márta Varga	pt	Ferenc Túry
Márta Molnár (Vargáné)	pt	Márta Novák
Rezső Zoller	ft	István Mucsi
László Zubek	pt	József Kovács

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László Csaba Dégi	ft	Ferenc Túry
András Fogarasi	pt	József Kovács
László Harmat	ft	Róbert Bódizs
Sándor Kalmár	i	Mária Kopp
Noémi Keresztes	ft	Bettina Pikó
Ágnes Zsófia	ft	Márta Novák
Mariann Kovács	pt	Katalin Hegedűs
Sándor Lázár Alpár	pt	Róbert Bódizs
Ibolya Nikolett Nétling	pt	Erzsébet Németh
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Éva Susánszky	i	Ágnes Hajnal
Melinda Sverteczki	ft	Róbert Bódizs
Gábor Szabó	ft	Mária Kopp
András Szentkirályi	ft	Márta Novák
Péter Torzsa	i	István Mucsi
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Ph. D. graduates		***Supervisors***
Piroska Balog	ft	Ágnes Hajnal
Réka Baranyai	ft	Mária Kopp
Emma Birkás	ft	Judit Gervai
Gábor Élő	pt	József Kovács
György Purebl	pt	Mária Kopp
Zsuzsanna Szántó	pt	László Molnár
Miklós Károly Szócska	i	Mária Kopp

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph.D. theses successfully defended in 2006 and 2007

PIROSKA BALOG (2007)

Marital stress and cardiovascular vulnerability

Supervisor: Ágnes Hajnal

Consistent with the literature marital stress can contribute to cardiovascular vulnerability through several different pathways: indirectly by increasing the prevalence and the intensity of psychiatric disorders and through unhealthy behavior, and directly through the physiology of the cardiovascular, neuroendocrine and immune systems. According to some researchers the effect of marital stress on health is stronger for women than for men, but to them the effect of work stress is considerable. Around these questions related to gender differences we sought to investigate marital stress and cardiovascular vulnerability. Given the significance of these gender differences prevention and rehabilitation programs could be developed that target men and women differentially and thus increase their effectiveness. The Stockholm Female Coronary Risk Study included 292 female patients 65 years or younger who were admitted for an acute CHD, and 300 age-matched healthy women. We analyzed the relationship between marital and work stress, and depressive symptoms in those women who were cohabiting and currently working. We have validated the Hungarian version of Marital Stress Scale on coronary patients (N=137) who had undergone cardiac surgery in the Gottsegen Gyorgy National Institute of Cardiology, and we also analyzed psychometric properties of the Shortened Marital Stress Scale. In Hungarostudy 2002, the national representative study (N=12680) we had the possibility to study gender differences. From this population were selected men (2221) and women (1838) who were cohabiting (married or living in a common law marriage) and actively working at the time of examination. Among them 244 men and 283 women reported high marital stress. We analyzed how marital stress and other psychosocial factors and unhealthy lifestyle were related, and we investigated the role of marital stress in connection with hypertension and treated depression.

Our results showed that: (1) in the Swedish population based case-control study we found marital stress, but not work stress to be associated with depressive symptoms in both groups. (2) The psychometric properties of the Shortened Marital Stress Scale comprised of 5 items were found to be satisfactory. (3) In both the Swedish and Hungarian populations in both groups (patients with coronary artery disease and healthy controls), and both sexes, those who reported high marital stress, showed significantly higher scores on depression, anxiety, vital exhaustion and more sleep complaints, compared to men and women with low marital stress. (4) High marital stress in both sexes was related to a higher frequency of binge drinking, and for men with higher number of cigarettes smoked per day, and increased risk of alcoholism. (5) In men high marital stress was found to be related to hypertension, independent of traditional risk factors, and in women high marital stress was related to treated depression. We have concluded that high marital stress is related to a higher risk of cardiovascular vulnerability for both sexes through depression and unhealthy behavior, and for men as an independent risk factor for hypertension.

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- *Blom M, Janszky I, Balog P, Orth-Gomer K, Wamala SP (2003) Social relations in women with coronary heart disease. The effects of work and marital stress. J Cardiovasc Risk 10: 201–206.*
- *Balog P, Mészáros E (2005) Marital stress, depressive symptoms and cardiovascular vulnerability in women. LAM 15: 685–692.*

RÉKA BARANYAI (2007)

Biopsychosocial and behavioral correlates of coronary heart disease

Supervisor: Mária Kopp

Background: Coronary heart disease (CHD) is the leading cause of death worldwide. Moreover, according to the WHO, an even higher prevalence is expected in the future.
Aims: To suggest possible targets improving prevention strategies and to provide health care professionals as well as policy makers specific hints on the special characteristics of the targeted populations, pointing out possible drawbacks and pitfalls of the interventions, our studies focused on the prevalence as well as the interrelationship of conventional and non-conventional risk and protective factors of CHD. We also examined a postulated pathomechanism of the cardiovascular effect of psychosocial parameters.
Methods: A cross-sectional, national survey was performed in 590 university and college students. We applied self-reporting questionnaires assessing health behaviors, health beliefs and psychosocial factors relevant to the aetiology and prognosis of CHD. We examined the relationship of health beliefs and risk of awareness on behaviour, as well as determinants of well-being in a historical and international context (Study 1). Furthermore we evaluated the health behavior and psychosocial factors of Hungarian patients with a history of myocardial infarction aged 45 to 90 (n=365) based on data from Hungarostudy 2002, a national representative survey (Study 2). Finally we examined the platelet function of depressed in-patients (n=10) and healthy controls (n=10) under baseline conditions, as well as following mental and phsysical stress. Platelets were assessed for surface expression levels of CD62 (P-selectin) and CD63 (GP53) using flow cytometry (Study 3).
Results: (1) Health behavior and risk awareness of students have been disappointing with partly deteriorating tendencies in the past decade. Female students show healthier lifestyles than men. There is a close association between beliefs and behavior emphasising the importance of cognitive factors, while knowledge of risk factors does not seem to play a major role. Religiosity, purpose in life and socioeconomic status were associated with behavior. (2) Behavioral risk factors are highly prevalent among coronary patients. Furthermore, the prevalence of mental health problems was higher in patients than in controls. Special therapy-relevant socioeconomic characteristics of patient subgroups can be delineated. (3) Depressive patients exhibited a greater increase in the activation-dependent surface markers P-selectin and GP53 after physical activity than healthy volunteers.
Conclusions: There is an enormous contrast between the recommended, ideal state and the real physical and mental state of the Hungarian population in both students and sub-

jects with and without a history of heart attack. Health education must aim besides informing about risk factors also a change of beliefs, attitudes and intentions. Prevention and rehabilitation programmes should aim at improving self-efficacy, purposes in life, coping strategies and encourage religiosity. Our study underscores the importance of heightened platelet reactivity as a postulated link between depression and coronary heart disease.

- *Baranyai R, Lederbogen F, Gilles M, Menart-Houtermans B, Tschoepe D, Deuschle M (2004) Effect of mental and physical stress on platelet activation markers in depressed patients and healthy subjects—a pilot study. Psychiatry Res 127: 55–64.*
- *Baranyai R, Nonell A, Deuschle M, Lederbogen F (2008) How depression may increase cardiac risk: effect of hypercortisolism on platelet activation markers. Neuropsychobiology 57: 146–150.*
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EMMA BIRKÁS (2007)

The effect of the D4 dopamine receptor and catechol-O-methyltransferase gene polymorphisms on the functioning of attention networks in children

Supervisor: Judit Gervai

The 7-repeat allele of the exon III length polymorphism of the D4 dopamine receptor (DRD4) gene occurs more frequently in children diagnosed with attention deficit hyperactivity disorder (ADHD). This result, confirmed by meta-analyses, inspired studies attempting to discover the role of the polymorphism in individual differences observable in the operation of the executive attention network and the temperament. Some research links this polymorphism with childhood attention and behavioral problems measured on non-clinical samples and with attention processes showing up in elementary acoustic stimulus processing. The catecol-O-methyltransferase (COMT) polymorphism 158Val/Met has been found to be associated with executive functions in adults. However, the effect of the polymorphism in childhood has yet to be fully established.

For this thesis, the effects of the two candidate genes on phenotypes associated with attention systems were examined in 6-year-old children as part of the longitudinal Budapest Infant Parent Study (BIPS). In the six-year-old data collection phase, the BIPS involved 89 first-born children from middle class families (52 boys, 37 girls). The DRD4 exon III repeat polymorphism exerted an effect on externalising behavioural problems (aggressive and deviant behaviour): children carrying the 7-repeat allele were reported by their mothers as having fewer problems. In the presence of the T.7 haplotype containing the 7-repeat and the T allele of the -521 C/T promoter polymorphism on the same chromosome, better performance was observed in the behavioral task measuring distractability. An association with the T.7 haplotype also showed up in the late negativity component amplitudes of brain event-related potentials characterising passive attention processes. In the case of the COMT 158Val/Met polymorphism, boys carrying the Met allele characterised with lower enzyme-activity performed less well in some executive function tests. The results demonstrate that the polymorphisms studied exert an effect on some child-

hood characteristics related to attention functions, although not always in the direction expected. We have also shown that research into attention-related phenotypes should take account of information on allele combinations (haplotypes). The results also confirmed the hypothesis that the dopaminerg system has a role in the modulation of some evoked potential components (late negativity).

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- *Birkás E, Lakatos K, Nemoda Zs, Ney K, Tóth I, Novák A, Sasvári-Székely M, Gervai J (2005) A D4 dopamin receptor gén hatása a 6 éves kori viselkedési problémákra. Neuropsychopharmacol Hung 7: 125–131.*
- *Lakatos K, Nemoda Z, Birkás E, Rónai Z, Kovács E, Ney K, Tóth I, Sasvári-Székely M, Gervai J (2003) Association of D4 dopamine receptor gene and serotonin transporter promoter polymorphisms with infants' response to novelty. Mol Psychiatry 8: 90–97.*

GÁBOR ÉLŐ (2006)

End of life decisions in the intensive care—ethical and legal concerns of resuscitation

Supervisor: József Kovács

The former tipically paternalistic physician-patient relationship has changed gradually toward an autonomy based one in the second half of the 20th century. Patient's autonomy includes the right to refuse life-saving therapy in modern constitutional states. Hungarian law assures the right to refuse life-saving treatment as well. However, to my knowledge, no such threapy refusal has occurred since the law coming into force, likely because of the rather strict regulations. According to our results forgoing life-saving therapy is an everyday practice in Hungary. The decisions based on medical futility are regularly made by the physicians without involving patients violating their autonomy. Our results also concluded that patients claim more information and involvement in their own treatment. Forgoing resuscitation is basically determined by two factors: autonomy of the patient, and medical futility. The alteration of the deed's form can facilitate the lawful Do Not Attempt Resuscitation (DNAR) orders for the sake of emergence of patient's autonomy. Qualitative futility is characterized by quality of life, which only the patient has the right to judge. Resuscitation protocols based on results of controlled studies can significantly improve both the success rate of resuscitations and the quality of life. Education plays a prominent role in this process, as it was demonstrated in our prospective comparative study. According to our study Hungarian DNAR orders are paternalistic and patient autonomy plays a secondary role. It was also established that patient's autonomy significantly improved in the subgroup trained according to international standards. Hungarian results were compared to the results of a highly educated group in the second study. The results confirmed the presumption: the education of resustitation according to international standards improves the representation of the patient's autonomy in DNAR decisions, the survival rate and the quality of life.

- *Élő G, Diószeghy Cs, Dobos M, Andorka M (2005) Ethical considerations behind the limitation of cardiopulmonary resuscitation in Hungary—the role of education and training. Resuscitation 64: 71–77.*
- *Diószeghy Cs, Kiss D, Fritúz G, Székely G, Élő G (2005) Comparison of effects of different hand positions during cardiopulmonary resuscitation. Resuscitation 66: 297–301.*
- *Élő G, Pénzes I (2002) Etika és jog az intenzív terápiában. Orv Hetil 143: 1991–1995.*

GYÖRGY PUREBL (2006)

Gender differences in depressive symptoms and depression-related cardiovascular risk

Supervisor: Mária Kopp

Objectives: The aims of the recent study were the investigation of the following questions:
Does the elevation in the frequency of depression continue?
What psychological background factors are related to low socio-economic state concerning depression and cardiovascular morbidity?
What psychological background factors contribute in the lower gender ratio observed in depression in subsequent Hungarian community surveys?
Are the effect sizes of psychological risk factors comparable to the traditional risk factors in the case of cardiovascular disorder?
Vital exhaustion (VE), dysfunctional attitudes, coping strategies, anxiety and Type D Personality (TD) were investigated as psychological background factors.
Sample: 12653 persons were investigated representing the Hungarian adult population concerning age, gender and settlement.
Measurements: Type D Scale, shortened Beck Depression Inventory (BDI), shortened Vital Exhaustion Questionnaire and items measuring dysfunctional attitudes, coping strategies, anxiety and socio-economic state (SES) were filled out. History and treatment of cardiovascular disorders, hypertension, diabetes and depression were also detected.
Results: The prevalence of subtreshold and clinical depression were 15.3% and 13.5% indicating the stop in the increase of depressive symptomatology. SES, VE, TD and anxiety demonstrate important relationships with depression. Women have significantly higher risk for depression (OR 1.3), but the gender ratio were much lower than the expected 2:1. The separate examination of women and men demonstrates higher prevalence of males compared to other studies. Approximately 2:1 gender ratio was detected in the case of VE (OR:2.2), anxiety (OR:1.4) and in the utilisation of the treatment due to depression (OR:2.2). Dysfunctional attitudes and coping demonstrate lower risk and gender differences than expected.
Both VE and depression show significant relationship with cardiovascular disorders (OR: 2.7/2.0 me/wo and 1.9) and the strength of relationship does not differ significantly from the relationship between hypertension and cardiovascular disorders (OR:2.6/2.4 me/wo).
Conclusions: The lower gender ratio is possibly affected by the higher depression frequency of men, the lower specificity of the shortened version of BDI and the significantly higher health care utilisation of women. Vital exhaustion and depressive symptomatology demonstrate strong, important relationships with cardiovascular morbidity.

- *Purebl G, Birkás E, Csoboth C, Szumska I, Kopp MS (2006) The relationship of biological and psychological risk factors of cardiovascular disorders in a large scale national representative community survey. Behav Med 31: 133–139.*
- *Csoboth C, Birkás E, Purebl G (2005) Living in fear of experiencing physical and sexual abuse is associated with severe depressive symptomatology among young women. J Women Health 14: 441–448.*
- *Csoboth C, Szumska I, Purebl G (2006) Self-rated health and health damaging behaviour among young women in Hungary: The effect of offspring familiy characteristics. Soz praventivmed 51: 45–53.*

ZSUZSANNA SZÁNTÓ (2007)

Health related life style: Illness behavior and health behavior

Supervisor: László Molnár

The purpose of the dissertation is to study the health related life style of Hungarians, and to provide an insight into the understanding of the factors that direct health related behaviors.

The review of the literature exposes the roots of the concept of health related life style and describes the main theories about illness behavior and health behavior.

According to the survey results, lay self treatment choices are not influenced significantly by any background variables (gender, age, education, health status, type of settlement). However, between the two main types of lay self-care—self-medication and home remedies—there are significant differences along with some background factors. The odds of taking medications are about one and a half times more frequent among women and villagers than among men and the inhabitants of the capital. The higher the education, the lover the odds of pill-taking; but the difference is significant only between people who finished vocational education and elementary school. In the variation between the applications of home remedies, education provides the only social explanation. Comparing to the lower educated, both secondary and tertiary education are more likely to produce a higher frequency of usage. Members of the age group of 35–45 do not usually ignore their minor health complaints but instead of visiting a physician they apply various forms of self-treatment. However, they are likely to ignore or self-treat symptoms—for example signs associated with heart problems—which would better be visited a physician with.

The willingness to spend time, money, mental and physical efforts for preserving or enhancing one's health status is more prevalent among the socially advantageous than among those who live amongst various social disadvantages. People in better social positions in the present are more likely to enjoy better health and quality of life in the future than less fortunate members of the "threshold generation". Social advantages will turn into health advantages and a greater health capital will be preserved for longer. Social disadvantages do not support the increase of health capital; on the contrary, they probably lead to a faster exhaustion of health resources.

- *Szántó Z, Susánszky É, Kopp M (2005) Relationships between unfavourable health status and smoking cessation attempts in Hungary. Soz praventivmed 50: 324–333.*

- *Susánszky É, Szántó Z (2004) A lakosság nyitottsága a biotechnológia és az orvostudomány legújabb vívmányai iránt. LAM 14: 220–255.*
- *Szántó Z, Susánszky É, Rózsa S (2002) "Az egészségemért felelős vagyok, a betegségemért nem". Laikus betegségmagyarázatok. LAM 12: 584–588.*

SCHOOL OF PH. D. STUDIES

5. SPORT SCIENCES

Chairman:
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General overview

Doctoral courses embrace the whole field of sports science. Naturally, the specific topics reflect the orientation of the tutors, and respectively, the technical facilities of the laboratories. It is the Faculty of Physical Education and Sport Science that manages the doctoral courses, but other institutions, above all, the National Institute for Sports Medicine, extend the facilities available in the institutes and chairs, both in regard to educational staff and technical equipment. The other faculties and institutes of Semmelweis University are considered the basis of further development in this respect.

PROGRAM 5/1.

TRAINING AND ADAPTATION

Coordinator:
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Program overview

Different stress factors exert considerable impact on normal functions and pathological processes throughout the whole life span. An adequate intensity of regular physical training positively influences the whole metabolism, and thus presumably plays a beneficial role in compensating against the harmful effects of stress. In our earlier studies we extensively examined the effects of stress and stress hormones on the development of the brain and neuroendocrine system, as well as on adaptive behavior. Recently we aimed at examining the metabolic impact of prenatal stress and its role in the development of obesity. Regular training during pregnancy might counteract the negative effects of stress by influencing the development of the hypophysis-pituitary-adrenal (HPA) axis. Regular physical training in early postnatal life also could influence brain development by acting on trophic factor production, such as NGF, BDNF. Training might enhance the resistance against harmful

neonatal risk factors, such as hypoxia, NMD A and ethanol toxicity. Chronic stress produces disturbances in neuroendocrine regulation and in adaptive behavior. The possible protective effects of dietary factors and physical training are also examined. Stress has a cardinal role in the development and maintenance of drug addiction. The beneficial effects of regular training can be evaluated with behavioral studies (reinforcement, sensitization, anxiety) as well as with biochemical examinations (glucocorticoid hormones and receptors, neuropeptides: CRF, neurotensin). The projects use several different scientific techniques such as hormonal, immunocytochemical and behavioral examinations. Perinatal age requires special new methodology to develop with respect to surgical, immunocytochemical and behavioral procedures.

Titles of research projects	***Supervisors***
Unfolding the imperfections in lifestyle and fitness for the prevention and treatment of chronic internal diseases. Physical fitness and training programs for rehabilitation	Péter Apor
The role of physical training in the neurobiology of stress response	Klára Felszeghy
The impact of physical training and dietary factors on the neurobiology of stress response at different ages	Klára Felszeghy
Motion analysis in sport sciences; the effect of (professional) sport motion	Rita Kiss
The role of physical activity and nutrition in cardiovascular diseases	Éva Martos
The importance of non-invasive cardiovascular examinations in the establishment of performance ability	Gábor Pavlik
The genetic aspect of physical exercise	József Pucsok
Different exercise protocols and oxygen uptake kinetics. Relationships between biological status (maturation status), functional characteristics, and basic motor performances; The energy costs of different exercise protocols using different types of ergometers	Tamás Szabó

Ph. D. students		***Supervisors***
Judit G. Noé	pt	Gábor Pavlik
Álmos Gógl	ft	József Pucsok
Péter Horváth	pt	István Berkes
Éva Relle (Nyergesné)	pt	Gábor Pavlik
Magdolna Peresa	pt	Gábor Pavlik
Noémi Szakács	pt	Rita M. Kiss
Szabolcs Tóth	pt (a)	Gábor Pavlik
Barbara Varga-Pintér	ft	Gábor Pavlik

Ph. D. candidates		***Supervisors***
Viktória Anna Kovács	ft	Éva Martos
Anna Szamosi	pt	Éva Martos

Ph. D. graduates		***Supervisors***
Miklós Dékány	pt	József Pucsok
Zsuzsanna Fajcsák	pt	Éva Martos
Anita Gábor	ft	Éva Martos

Patrícia Horváth	ft	Gábor Pavlik
Zsuzsanna Kneffel	ft	Gábor Pavlik

a, absolutorium; ft, full-time; pt, part-time

Abstracts of Ph. D. theses successfully defended in 2006 and 2008

MIKLÓS DÉKÁNY (2006)

Relationship between oxidative stress and physical effort

Supervisor: József Pucsok

During a period of intensive physical effort, the oxygen uptake of the human organism increases considerably, leading to consumption levels several times greater than during a rest period. Reactive oxygen species or high reactivity compounds have been formed from 2–5 percent of the oxygen taken in. The human organism has a sophisticated defense mechanism against reactive oxygen components. The first defensive line of the human body is represented by the primary antioxidant system (superoxide-dismutase, catalase, glutathione-peroxidase). For different endurance sport disciplines, the ratio and fluctuation of the aerobic/anaerobic energy-supply were studied, as well as the changing of the primary antioxidant system, which depended on the intensity and the amount of the physical work load. The alteration of the primary antioxidant system is specific for certain sport disciplines and differs from the sedentary subjects. Glutathione-peroxidase eliminates hydrogen-peroxide more effectively as a result of oxidative stress for interval-trained athletes, while the primary eliminating enzyme for high endurance athletes is catalase. An indirect conclusion can be made that the production of hydrogen-peroxide is more significant in those athletes who mainly use the muscle groups of their lower limbs than those high-endurance athletes who mainly utilize the muscles of the arms. Consequently, physical exercise generates changes in the various compounds having an antioxidant effect, and these modifications are bound to different physiological parameters. Beyond the relative aerobic capacity and the duration of the work load, the physiological indices characterizing the intensity of respiratory-metabolism and aerobic/anaerobic metabolism can also be applied when evaluating the antioxidant status. By investigating the antioxidant capacity in different compartments, it has been indirectly proved that the level of performance during acute physical effort is mainly related to the antioxidant enzyme system of the resting venous red blood cell. The free radical eliminating capacity is an important factor in adaptation to training and exercise. An important aspect of the estimation of the actual training status is that the free radical eliminating enzyme systems and the antioxidant compounds correlate with each other, depending on the type of sport discipline, and based on the aforementioned relationship, the individual training status of an athlete can also be determined. According to our current knowledge, we recommend completing the individually-defined training amount and intensity with an antioxidant-rich diet so that the adequate functioning of the antioxidant system of physically active people can be ensured.

- *Dékány M, Harbula I, Berkes I, Györe I, Falus A, Pucsok J (2006) The role of insertion allele of angiotensin converting enzyme gene in higher endurance efficiency and some aspects of pathophysiological and drug effects. Curr Med Chem 13(18): 2119–2126.*
- *Dékány M, Nemeskéri V, Malomsoki J, Pucsok J (2001): Antioxidáns hatás változása különböző sportági csoportok esetén. Sportorvosi Szemle 4: 205–217.*
- *Dékány M, Nemeskéri V, Györe I, Harbula I, Malomsoki J, Pucsok J (2004) Antioxidant status of intervally trained athletes in various sports. Int J Sports Med 27: 105–111.*

ZSUZSANNA FAJCSÁK (2008)

The effects of low glycemic diet interventions and lifestyle changes in overweight/obese children

Supervisor: Éva Martos

The aim of the present study was to examine the effects of a twelve-week low-glycemic index/low glycemic load (low-GI/GL) diet, alone and combined, with six-weeks of lifestyle exercise on anthropometric and metabolic measures in overweight/obese children. The long-term effects of the initial treatment were followed up two and three years after the program. Following a pediatric examination, 21 overweight/obese children (ten girls and eleven boys; BMI=26.98±5.49 kg/m^2) participated in the study. The children were divided into two groups: (1) dietary changes alone and (2) dietary changes combined with lifestyle exercise. The diet intervention consisted of (1) replacement of at least 50% of the high-GI foods with low-GI foods and (2) weekly nutrition consultations. Lifestyle exercise included a daily 45-minute self-monitored walking program for six weeks. The daily walking distance was regularly monitored by a pedometer (OMRON, Japan). Body composition, fasting glucose, insulin, total cholesterol and its subfractions, and serum triglyceride were measured before and after the study in both groups. Dietary changes were assessed weekly with food diaries. For more accurate assessments, three-day food records were analyzed three times (at baseline and at weeks six and twelve) during the study period. Children also filled out questionnaires about their activity patterns and sense of hunger at baseline and weeks six and twelve. Hunger level was ranked via a five-point-scale (modified MIT medical hunger scale). Follow up telephone interviews were made to available parents of the children two and three years after the two studies. In line with our hypothesis, the twelve-week-long low-GI/GL intervention significantly reduced the daily hunger score, body fat, circumference measures and the number of cardiovascular risk factors in both groups. Surprisingly, the additional lifestyle exercise did not increase the beneficial effects of the program. The results of the follow-up showed that the children were able to continue the learned lifestyle changes at least at moderate levels. In addition, the BMI of the children was maintained after two to three years following the initial intervention. This means that the effect of the initial treatment was still apparent in the children's lifestyle and in their food selection. Based on the results of the present study, a low-GI/GL diet seems to be a useful tool for both preventing and treating overweight/obese prepubertal children. An exercise program, however, needs to be higher in intensity and longer in duration than the 45 minutes of daily walking. It seems that making changes in school meals, providing appropriate nutrition education for the children, and promoting

daily physical activity by parents and school staff may induce favorable changes in the obesity rate among Hungarian children.

- *Fajcsák Zs, Gábor A, Kovács VA, Martos É (2008) The effects of 6-week low glycemic load diet based on low glycemic index foods in overweight/obese children—pilot study. J Am Coll Nutr 27: 12–21.*
- *Martos É, Kovács VA, Fajcsák Zs, Pucsok J (2005) Bőrredő mérés és BIA összehasonlítása elhízott gyermekeknél. Obesitol Hung 5: S24.*
- *Gábor A, Kovács VA, Fajcsák Zs, Martos É (2005) Élvonalbeli tornászok testösszetételének meghatározása BIA-módszerrel. Sportorvosi Szemle 2005(2): 89–99.*

ANITA GÁBOR (2008)

Sport-specific aspects of nutrition—nutrition as an optimizing factor in sports performance

Supervisor: Éva Martos

The aim of our work was to analyze the dietary habits of elite athletes, while we examined the possible differences between various sports disciplines. Furthermore, our goal was to compare the nutritional practices of athletes and that of the age-matched general population. Considering the role of nutrition in sports performance, we were looking for the relationship between dietary intakes and certain performance components.

There were 615 elite athletes (306 men and 309 women) involved in the study. In 368 cases we completed a dietary analysis (three-day dietary record, NutriComp Calcul pro Sport software) only. An additional blood test (after twelve hours of fasting; venous blood; standard laboratory procedures) was performed in 347 athletes. Out of these 347 athletes, 257 subjects took part in an all out exercise test and a body composition analysis (eight-point skinfold measurements) in laboratory circumstances. For the comparison of the dietary assessment of athletes the age-matched non-athletic population data were gained from the First Hungarian Representative Dietary Survey (EMRTV) and from the Hungarian Population Health Survey (OLEF). At the 36th World Gymnastics Championship in Debrecen we examined the body composition (Bioimpedance method, Inbody 3.0) of 88 participants (56 men, 32 women) and compared those data to the participants' successfulness based on their placement in the competition.

Similar nutritional inadequacies were found both in elite athletes and in the non-athletic population. The nutritional practices of the examined athletes did not meet the sports-specific guidelines. The level of certain micro-nutrients—especially important from the point of view of performance—did not even reach the recommended dietary allowances (RDA) for non-athletes. The significantly different energy intake was responsible for the other nutritional differences found between the endurance and aesthetic types of sports in both genders. The remarkable differences in dietary intakes found within the same sports groups call our attention to the individual nutritional differences. We could not find any connections among dietary mineral intakes and the matched serum values in any micro-nutrients studied. We concluded that the measured serum ions did not serve as biomarkers characteristic of athletic micro-nutrient consumption. Examining the connection between certain performance indicators (aerobic capacity, body composition) and dietary compo-

nents, we did not find a strong direct correlation between the nutritional and performance parameters. For international level elite female gymnasts, we found a significant negative correlation between percentage of body fat and sports-specific successfulness. In male gymnasts, the high muscle mass and low body fat proved to be equally important for sports-specific efficiency.

Based on our findings, we have to make every effort to optimize the dietary intake habits of elite Hungarian athletes.

- *Gábor A, Martos É (2004) Táplálék-kiegészítés az élsportban. Magy Sporttud Szemle 2/3: 34–37.*
- *Gábor A, Kovács VA, Fajcsák Zs, Martos É (2005) Body composition analysis of elite gymnasts using bioelectrical impedance method. Sportorvosi Szemle 2: 89–99.*
- *Fajcsák Zs, Gábor A, Kovács VA, Martos É (2008) The effects of 6-week low glycemic load diet based on low glycemic index foods in overweight/obese children—pilot study. J Am Coll Nutr 27: 12–21.*

PATRÍCIA HORVÁTH (2008)

Influencing factors of hypertension and physical activity

Supervisor: Gábor Pavlik

Regarding influencing factors related to hypertension and physical activity, three main subjects were investigated. Two-dimensionally guided M-mode and Doppler echocardiographic data, spiroergometric data and the resting blood pressure values of different athlete and non-athlete subjects were compared with each other. The results were as follows. Regular physical activity decreases resting blood pressure; the degree to which depends on the type of the sport. Decreased cardiac output is the reason for decreased resting blood pressure. According to my results, dynamic exercises are recommended for the prevention of hypertension.

Some adverse, early cardiovascular signs of hypertension can be found in the normotensive offspring of hypertensive parents. From my present data, the diastolic function showed some modifications in the non-athlete offspring of hypertensive parents. Regular physical exercise seems to protect offspring from adverse cardiac signs which can be associated with hypertension when one has hypertensive parents.

The echocardiac features of top-level female water polo players were (as were male water polo players) similar to elite endurance female athletes. Their mean relative aerobic power, measured in an all-out treadmill run, was worse than that of other top-level athletes, such as male water polo players. This observation corroborated the validity of the assumption that swim tests were more suitable to check the physical fitness and condition of water polo players.

- *Pavlik G, Kemény D, Kneffel Zs, Petrekanits M, Horváth P, Sidó Z (2005) Echocardiographic data in Hungarian top-level water polo players. Med Sci Sports Exerc 37: 323–328.*
- *Horváth P, Petrekanits M, Györe I, Kneffel Zs, Németh H, Pavlik G (2006) Élvonalbeli női vízilabdázók echocardiográfiás és spiroergometriás adatai. Hung Rev Sports Med 47: 105–116.*

• *Kneffel Zs, Horváth P, Petrekanits M, Németh H, Sidó Z, Pavlik G (2007) Relationship between relative aerobic power and echocardiographic characteristics in male athletes. Echocardiography 24(9): 901–910.*

ZSUZSANNA KNEFFEL (2008)

Left ventricular morphological and functional characteristics in athletes of different levels

Supervisor: Gábor Pavlik

Regarding questions related to the "athlete's heart," three main subjects were investigated. Two-dimensionally guided M-mode and Doppler echocardiographic data of different athletic and non-athletic subjects were compared with each other, and differences between the data of different athletic groups were also analysed. The results were as follows.

The E/A quotient characterises left ventricular (LV) diastolic function, but it depends highly on the heart rate (HR). The higher E/A found in young athletes does not seem to be an independent effect of regular physical training, which can be a consequence of the training bradycardia. In elderly subjects, however, the higher E/A is independent of the HR, which suggests that regular physical training can diminish the age-associated impairment of diastolic function.

In investigating the data of different ball-game-players, characteristic differences were seen. In myocardial hypertrophy and resting HR, water polo players were the best and volleyball players the worst, but their values were also better than those of non athletes. E/A was, however, the highest in volleyball players. Differences between the different ball-game players can be explained by different movement patterns. The high level of the cardiac athletic characteristics of water polo players can be explained by their long sports career, their longer training sessions and the complexity of their movements; the relatively weaker cardiac results of the volleyball players can be explained by the fact that their movements are restricted to a smaller area.

In the summarized group of non-athletes and competitors of different kinds of sports (endurance athletes, ball-game-players, power-and-sprint-event athletes), body size-related LV wall thickness, internal diameter and muscle mass, and HR and E/A quotients correlated significantly with relative maximal oxygen consumption. Within the various athletic groups, cardiac data correlated differently: the higher the proportion of endurance activity in their training and competitive program, the stronger the correlations were. Differences can be explained by the supposed different muscular structure, i. e., fiber composition, of the different athletes. In the endurance athletes, due to the high oxidative capacity of their predominantly slow twitch muscles, aerobic power is mostly limited by the oxygen offered, which is highly dependent on heart condition. In power-and-sprint-events, athletes' muscular oxidative capacity is low, and performance is rather determined by the dynamic characteristics of the muscles.

• *Sidó Z, Jákó P, Kneffel Zs, Kispéter Zs, Pavlik G (2003) Cardiac hypertophy and diastolic function in physically well trained and in obese men. Int J Obes 27: 1347–1352.*
• *Pavlik G, Kemény D, Kneffel Zs, Petrekanits M, Horváth P, Sidó Z (2005) Echocardiographic data in Hungarian top-level water polo players. Med Sci Sport Exerc 37: 323–328.*

• *Kneffel Z, Horváth P, Petrekanits M, Németh H, Sidó Z, Pavlik G (2007) Relationship between relative aerobic power and echocardiographic characteristics in male athletes. Echocardiography 24(9): 901–910.*

PROGRAM 5/2.

PHYSICAL TRAINING, REGULATION AND METABOLIC TRANSPORT

Coordinator:
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Program overview

The current program focuses on the physiological, biomechanical, and biochemical effects of acute and regular exercise on humans and laboratory animals. In addition, sport-associated injuries and the science behind regeneration are also investigated. Human gait, motions, model and individual techniques in various sport events are studied in the laboratory of biomechanics with a close collaboration of the institute of biophysics. One of the key topics of this program is the complex mechanism of the effects of exercise on aging and the role of oxidative stress in adaptive response. The effects of exercise on the function, molecular physiology of skeletal muscle and brain serve as an exciting challenge to students and professors as well. The laboratories, animal house and the core facilities of Semmelweis University, along with the expertise of the program's professors provide unique support for excellent research in sport science.

Titles of research projects	***Supervisors***
Sports injuries of the knee	István Berkes
Hormonal and metabolic aspects of adaptation to physical training. Stress hormone responses to habitual physical exercise. Age- and event-dependent studies in ACTH, cortisol, STH and prolactin metabolism	Róbert Frenkl
Computer simulation of limb movements and the mathematical modeling of their neural control	József Laczkó
Somatic development of seven to eighteen-year-old school children	János Mészáros
Influence of physical activity and nutrition on cellular processes of normal and pathological brain aging	Csaba Nyakas
Interactions between physical activity, glucose and lipid metabolism. Movement therapy of obesity and diabetes	Csaba Nyakas
Exercise-induced adaptation to oxidative stress and aging	Zsolt Radák
In vivo biomechanical study of neuro-musculo-skeletal system and its mechanical, hormonal and genetic adaptation to strength exercises	József Tihanyi
The effect of lower education in somatic development	Márta Wilhelm

Ph. D. students		***Supervisors***
Zoltán Ács	pt (a)	Zsolt Radák
Rozália Apró	ft	Márta Wilhelm
Veronika Börzsei	pt	István Berkes
Anni Andrea Ember	ft	Márta Wilhelm
Réka Feszthammer	pt	István Berkes
Nikolett Hart	ft	Zsolt Radák
Anna Kacsándi	ft (a)	Róbert Frenkl
Irén Kalabiska	pt	Róbert Frenkl
Erika Koltai	ft	Zsolt Radák
Bence Kopper	pt	József Tihanyi
Zsófia Mészáros	ft	János Mészáros
Zsófia Mária Nagy-Erdei	pt	Zsolt Radák
Pampakas Polydoros	it	János Mészáros
Márta Zsuzsa Ránky	ft	Róbert Frenkl
Sándor Sáfár	ft	József Tihanyi
Linda Sárga	ft	Zsolt Radák
Zsófia Szabó	ft (a)	Zsolt Radák
Dóra Ureczky	ft	József Tihanyi
Szabolcs Zsigri	pt (a)	Csaba Nyakas

Ph. D. candidates		***Supervisors***
Andreas Costa	it	József Tihanyi
Riccardo di Giminiani	it	József Tihanyi
Izabella Jónás	ft	Csaba Nyakas
Zoltán Kereszténvi	pt	József Laczkó
Ágnes Andrea Mayer	pt	József Tihanyi
András Prókai	i	János Mészáros
Savvas Siamilis	it	Zsolt Radák
Márk Váczi	ft	József Tihanyi

Ph. D. graduates		***Supervisors***
Andreas Photiou	i	János Mészáros
Zsolt Károly Szakály	pt	János Mészáros
Tekla Kornélia Tihanyi	ft	József Tihanyi
Anna Toldy	ft	Zsolt Radák
Ildikó Vajda	i	János Mészáros
Eszter Völgyi	ft	János Mészáros
Petra Zsidegh	ft	Róbert Frenkl

a, absolutorium; ft, full-time; pt, part-time; it, international; i, individual

Abstracts of Ph.D. theses successfully defended in 2006, 2007 and 2008

ANDREAS PHOTIOU (2008)

Somatic development and body composition of six- to eighteen-year old boys —the first Cypriot growth

Supervisor: János Mészáros

Growth, development and age-dependency in body fat content of six- to eighteen-year-old Cypriot boys were determined in a nation-wide representative sample. The data collection was carried out in a cross-sectional data collection. The successive means of stature, body weight, BMI, body fat content relative to body mass, LBM, and growth type indices were evaluated. In addition, techniques for the estimation of morphological age and the prediction of young adult height were suggested. For the measurement of required body dimensions and the calculation of growth type indices, internationally accepted equipment and techniques were used. Descriptive statistics of distinctly overweight and obese subjects were compared to those of their counterparts of normal body composition.

The growth patterns in the height and body mass of Cypriot boys were in accordance with the normal growth patterns of healthy children and adolescents. The significantly taller stature and the non-proportionate body mass means of the overweight and obese boys between six and twelve years of age should be attributed to their advanced biological development, which is influenced by overfeeding. Consequently, the normal growth patterns of our subjects were and are markedly modified by environmental effects.

The exponential pattern of change with age in growth type indices of Cypriot boys did not differ from those of Hungarian and German children, adolescents, and young adults, but there were significant differences between the age groups. The Cypriot subjects can be characterised as having a slightly more picnomorphic body structure. This more or less expressed inter-group variability was associated with ethnic differences.

The observed prevalence of overweight and obese boys was unfavourably high. The prevalence increased between childhood and adolescence and did not change during the post-pubertal years. The high prevalence of overfed subjects should be related to a sedentary lifestyle and dietary habits. Taking into account the health consequences of long-term overweight and obese states, our predictions cannot be positive. Since overeating was characteristic in 25–28% of the sample, it can be interpreted as a real social problem.

The calculated references can be used for the estimation of morphological age in Cypriot boys. However, it cannot be excluded that depot fat corrected references result in more accurate age estimations in physically active children and adolescents. The reduction of relative depot fat down to 20% seems to be one of the most useful procedures.

- *Pampakas P, Mészáros Z, Photiou A, Sziva Á, Zsidegh P, Mészáros J (2008) Az emelt szintű testnevelés hatása 7–11 éves fiúk szomatikus és motorikus jellemzőire. Magy Sporttud Szemle 9(1): 3–8.*
- *Photiou A, Anning J, Mészáros J, Vajda I, Mészáros Z, Sziva Á, Prókai A, Ng, N (2008) Lifestyle, body composition and physical fitness changes in Hungarian school boys (1975–2005). Res Quart Exerc Sports 79(2): 168–173.*

• *Vajda I, Mészáros Z, Mészáros J, Photiou A, Nyakas ÉD, Prókai A, Sziva Á, Szakály Z, Kumagai S (2007) Activity-related changes of body fat and motor performance in obese seven-year-old boys. J Physiol Anthropol 26(3): 333–337.*

ZSOLT KÁROLY SZAKÁLY (2008)

The biomechanics of last strides and take-off of the long jump

Supervisor: János Mészáros

The candidate analysed by cross-sectional data collection the somatic and motor development, as well as the body composition of 7- to 14-year-old, non-athletic, volunteer boys of city GyŒr (West Hungary). A total of 3,621 boys took part in the investigation. The body build was described by the Conrad's (1963) metric and plastic indices. Nutritional status was assessed by the BMI and estimated weight-related body fat content. The technique was introduced by Parízková (1961). Physical capabilities were estimated by the scores in 30 m dash, standing long jump, fist ball throw, and 1200 m run test scores. Kinanthropometric characteristics were analysed in separate subgroups, created by the scores of nutritional status (normal body composition boys vs. overweight and obese). Mean heights and body masses of the boys investigated in 2006 and 2007 were significantly taller and heavier than those of previous means in the regional or nationwide samples, but they did not differ from the recent statistics. The growth type of the GyŒr boys was slightly leptomorphic and normplastic. Their mean physical performances were moderate according to the qualification of PE teacher. The prevalence of overweight and obese boys ranged between 8% and 25.5%. These are significantly greater than 1–2 decades earlier. The overfed children and adolescents were significantly taller than their normal body composition counterparts. Their growth type was consistently moderately picnomorph and normoplastic. The physical performances cannot be qualified by the point of view of PE teacher. The significantly taller means of stature can be attributed to the existing secular growth trend, however, the trend in height should be revised by the high prevalence of subjects with health risks and the combined functions of adipose tissue. The non-height proportional fraction of heavier body mass is the consequence of lifestyle. The distorting effects of high body fat are obvious in physique characteristics, too.

The depot fat decreases linearly the physical performances yet in the groups of normal body composition boys. The slopes of increases with age were small in both samples. The observed and dominantly negative qualifications of the studied boys have sourced from the dramatically changed lifestyle that developed in the region during the past 1–2 decades.

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• *Szakály Z, Lehmann T, Barthalos I, Konczos C, Liszkai Z (2007) Experience of bike training with ergometer in young triathlets. Kalokagathia 17(3/4): 149–154.*
• *Szakály Zs, Liszkai Zs, Lehmann T, Barthalos I (2008) Fiatal triatlon versenyzők téli kerékpáros felkészítésének tapasztalatai. Magy Sporttud Szemle 9(1): 20–23.*

TEKLA KORNÉLIA TIHANYI (2008)

Intra- and intermuscular control of hemiplegic patients and its alteration due to vibration exposure

Supervisor: József Tihanyi

Muscle contractility. The young, chronic hemiplege patients were able to generate greater torque than elderly acute stroke patients with both intact and effected knee extensor muscles. The difference between the two sides was significantly greater in the elderly than in young patients for all mechanical variables. In the rate of torque development and in muscle relaxation, young patients produced greater values. The bilateral deficit was similar for both groups. In both groups the decrease in torque production due to bilateral contraction was less for effected muscle than for intact muscle. The bilateral deficit depended upon the contraction velocity and joint angle. The ratio of eccentric and isometric torque was greater in elderly patients than in young hemiplegic people.
Control of arm movement. Visual control did not play a significant role in coordinating arm movement. The degree of freedom was reduced to the lowest possible level. However, the degree of freedom of the final hand position was significantly greater for patients than for healthy people without visual control. There was no significant difference between stroke patients and the healthy control in degree of freedom of arm position without visual control, but the degree of freedom was significantly less with visual control for stroke patients. The variability of the trajectory of arm movement and the relationship between hand and arm position did not differ in the two groups.
The effect of whole body vibration. Mechanical vibration increased the torque production during voluntary knee extension temporarily. The elevated torque and work production was due to acute neural adaptation indicated by the increased EMG activity of knee extensors. However, the co-activation of the antagonist muscle decreased significantly only under eccentric contraction.
The four week whole body vibration resulted in permanent muscle force growth, which can be attributed, first of all, to neural adaptation, that was supported by the increased EMG activity of the knee and the reduced EMG activity of antagonist muscles. The applied 20–25 Hz resonance frequency made a significant alteration in force generation only in the muscles of the effected side.

- *Tihanyi T (1999) Control of muscle contraction in young hemiplegic people. Pp 196–199 in: Zahálka F (ed) Pohybové aktivity. Praha.*
- *Eggert T, Tihanyi T, Straube A (2003) Solving the redundancy problem for unrestricted reaching movements: a comparison of patients with cerebral infarcts and healthy controls. Ann NY Acad Sci 1004: 511–515.*
- *Tihanyi T, Horváth M, Fazekas G, Hortobágyi T, Tihanyi J (2007) One session of whole body mechanical vibration increases voluntary muscle strength in patients with stroke. Clin Rehab 9: 782–793.*

ANNA TOLDY (2006)

The role of natural antioxidants and physical activity on the oxidative stress in neurodegenerative alterations

Supervisor: Zsolt Radák

Regular swimming training and phytotherapeutic supplementation are assumed to alleviate the severity of neurodegeneration leading to dementia through their antioxidant manner. The effect of regular swimming training and that of enriched lab chow containing 1% (w/w) dried nettle (*Urtica dioica*) leaf were investigated on the normal basis and in the prevention of the severity of brain injury caused by N-methyl-D-aspartate (NMDA) lesion in Wistar rats.

The rats were divided into groups and were subjected to swimming training (six weeks) or to nettle supplementation (eight weeks) or the combination of these two treatments. NMDA lesion was applied after the termination of treatments in the NBM. The oxidative stress, inflammation and neurotrophic markers in Wistar rat brains were investigated. The oxidative side of the experiment was tested by measuring electron spin resonance (ESR) in the frontal lobe and cerebellum, and photometry protein carbonyl residues were estimated in brain homogenates. Nuclear factor kappa B (NF-kB), activator protein 1 (AP-1) DNA binding activity were assayed by EMSA in cerebellum, while nerve growth factor (NGF) and brain derived nerve factor (BDNF) protein content were measured by E-max ImmunoAssay System from brain homogenates. The effects of lesion were tested with behavioral tests including learning paradigms (open-filed activity, passive avoidance tests). Regular swimming exercise was found to reduce free radical accumulation in the frontal lobe, however it was elevated in the cerebellum. In the frontal lobe, swimming was an effective preventing agent in NMDA lesion, supporting neuronal survival in brain tissues and improving the behavioural and cognitive functions in NMDA lesion.

Nettle is an important nutritional factor, and it has immense antioxidant capacity, reduces the activity of inflammatory transcriptor factors, and has an anti-inflammatoric effect, which influences antiapoptotic processes in favor of neuronal survival. The additive effect of swimming and nettle feeding was successful in the inflammatoric processes and in the cognitive functions to alleviate the neurodegeneration caused by NMDA lesion. Both treatments have an influence on brain lesion.

- *Toldy A, Stadler K, Sasvári M, Jakus J, Jung KJ, Chung HY, Berkes I, Nyakas C, Radák Z (2005) The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res Bull 65: 487–493.*
- *Radak Z, Tolvaj D, Ogonovszky H, Toldy A (2006) Exercise and cancer. Pp 168–190 in: Radak Z (ed) Exercise and diseases. Meyer & Meyer, Munich.*
- *Radak Z, Toldy A, Szabo Z, Siamilis S, Nyakas C, Silye G, Jakus J, Goto S (2006) The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem Int 49(4): 387–392.*

ILDIKÓ VAJDA (2008)

Relationship between motor performance and anthropometrical characteristics of non-athletic children aged seven to ten

Supervisor: János Mészáros

Somatic and motor development were analyzed in Nyíregyháza school-children during a 3.5-year observation period. Changes in somatic development and body composition were described by anthropometric methods accepted in the literature. Physical capabilities were characterised by the scores in the 30-meter dash, standing long jump, fist-ball throw, 400-meter run scores and by distance performed during the Cooper-test.

The results of the initial data collection show that the somatic development of Nyíregyháza children is similar to the characteristics published in a nation-wide representative study. The equalization of an earlier observed delay in somatic development can be supposed. The age-related increases in height and body mass were not proportionate in this sample, and there was a more marked increase in body mass. Consequently, the relative fat content of children was high, and its increase with age was faster in the group of boys.

There were many overweight and obese children at the end of the observation. This result is a logical contradiction of the linearity of the previously observed physique. This result cannot be attributed to genetic changes; this is obviously an environmental effect.

The physical performance of the children was moderate or weak throughout the whole observation period. The within-group variability was large in both sexes and every test score. Significant linear development was observed in the results of five motor-tests, but the developments in six months were not statistically significant in general. The very moderate level of physical capabilities and their increases can only be partly attributed to the higher than required body fat content relative to body mass. The physical performances of boys were initially better than those of the girls, and their slopes were also greater. Among the analyzed variables, only calendar age had a medium correlation with the physical performance scores. The very weak correlations between the motor performance scores indicate that the performances of a given child may represent very different levels of quality. By means of the observed somatic and physical characteristics, it is obvious that the habitual physical activity of our children is remarkably lower than required. Their normal physical activity level means hypoactivity. The high relative body fat content and the low level of cardio-respiratory endurance together create a health risk. The possibilities within school physical education are far from being one of the solutions.

- *Vajda I, Mészáros Z, Photiou A, Prókai A (2007) Testméret, testarány és teljesítménykülönbségek gyermekkorban. Pp 95–99 in: Mónus A (Szerk) V. Országos Sporttudományi Kongresszus. Válogatott tanulmányok. Magyar Sporttudományi Társaság, Budapest.*
- *Vajda I, Mészáros Z, Mészáros J, Photiou A, Nyakas ÉD, Prókai A, Sziva Á, Szakály Z, Kumagai S (2007) Activity-related changes of body fat and motor performance in obese seven-year-old boys. J Physiol Anthropol 26(3): 333–337.*
- *Vajda I, Mészáros J, Mészáros Z, Prókai A, Sziva Á, Photiou A, Zsidegh P (2007) Effects of 3 hours a week of physical activity on body fat and cardio-espiratoryparameters in obese boys. Acta Physiol Hung 94: 191–198.*

ESZTER VÖLGYI (2008)

Relationship between biological maturation, body composition and psychological funtion. Longitudinal study

Supervisor: János Mészáros

This dissertation investigated differences in anthropometric and psychological variables based on the onset of menarche among Hungarian teenage girls.
All together, 207 girls were included in the analysis (mean age of 11.04±0.39 at baseline). The subjects were divided into three groups by tertiles based on the onset of menarche (G1; n=69 early matured, G2; n=69 on-time matured, G3; n=69 late matured).
Anthropometric measurements were carried out ten times during the three-year observation period, every fourth month. Body mass-related body fat was estimated by the caliper metric method of Parízková. The physique patterns were described with plastic and metric indices. Self-administered questionnaires were used to describe state and trait anxiety and self-efficacy towards physical activity. Differences between the groups were analyzed by one-way ANOVA or Kruskal-Wallis ANOVA depending on respective distributions and measurement scales. Changes during the three years were tested with repeated measures of ANOVA or Friedman ANOVA according to the distributions and measurement scales. Relations between anthropometric variables and psychological variables by groups were analyzed by Kendall-τ correlation.
In summary, the principal results of the research were that girls who matured earlier were significantly heavier, having a higher value in BMI, body mass-related body fat percentage, plastic index, and metric index than on-time and late maturing girls. We did not find a significant difference in psychological variables among early, on-time and late developers. Nevertheless, there were no correlations between the biological and psychological variables.
The conclusion is that the differences among anthropometric characteristics are the consequences of the process of biological maturation, but the pace of their social maturation is not the same. Other factors (family background, type of settlement where they live) may influence social behavior.

- *Mészáros Z, Mészáros J, Völgyi E, Sziva A, Pampakas P, Prókai A, Szmodis M (2008) Body mass and body fat in Hungarian schoolboys: differences between 1980–2005. J Physiol Anthropol 27(5): 241–245.*
- *Völgyi E, Tylavsky FA, Lyytikäinen A, Suominen H, Alén M, Cheng S (2008) Assessing body composition with DXA and bioimpedance: effects of obesity, physical activity, and age. Obesity 16(3): 700–705.*
- *Mészáros Z, Mészáros J, Szmodish BM, Pampakas P, Osváth P, Völgyi, E (2008) Primary school child development—issues of socio-economic status. Kinesiology 40: 154–162.*

PETRA ZSIDEGH (2008)

An integrative diagnostic parameter used for variables of somatic, cardio-respiratory and physical performance

Supervisor: Róbert Frenkl

The positive effects of regular physical activity on growth and development are unquestionable, which was verified generally by the observations of various parameters.
The aim of the study was to describe the changes in somatic, cardio-respiratory and physical performance development with an integrative parameter in physically active, medium active and less active boys and girls aged between seven and fifteen years of age. The orderly and also the disorderly were estimated by statistical entropy. This parameter, as a joint one, also describes the whole biological system and the level of its regulation.
There were three arrangements in eleven- and fifteen-year-old athletic and non-athletic boys, and also seven- to fourteen-year-old pupils taking part in normal and elevated levels of physical education. A total of 778 subjects were investigated. Anthropometric techniques were used for the description of somatic development with age changes in body composition. For the assesment of the cardiorespiratory system, the standard spiroergometric variables were used. The differences in physical performances were evaluated based on suggestions and national references. Statistical entropy was calculated by the time-series of individually measured values of various characteristics. Thus, statistical entropy was determined through all the measured somatic, physical, and exercise physiological parameters. The calculated entropy in the athletic children was significantly lower throughout the whole period of analysis, especially during the exercise duration; however the recovery showed a less orderly regulation than their non-athletic counterparts. The decrease in entropy with age was attributed to training-induced adaptation. There were no differences between the somatic development in active and non-active pupils, but the development of cardio-respiratory functions was significantly more orderly in the active children. Somatic and physical developments were more orderly in the sample of normal PE children of both sexes. The possible explanations are that there were no training-induced increases, as indicated by the smaller number of significantly subsequent mean differences, and that the mean development in physical performances was not greater in the PE children. In spite of the significant morphological characteristics and physical performances, there were no differences between the age-related developments of orderliness in the pre-pubertal children. The DA has proved that the entropy gives qualitatively different characterisations of overall development. This parameter can only indicate the orderly versus disorderly functions of the system, which is the child alone.

- *Zsidegh P, Zsidegh M, Szmodis M, Szmodis I, Mészáros Z, Mészáros J. (2007) Kísérlet prepubertáskorú leányok komplex fejlődésének megközelítésére. Anthrop Közl 48: 91–104.*
- *Zsidegh P, Photiou A, Mészáros Z, Prókai A, Vajda I, Sziva Á, Mészáros J (2007) Body mass index, relative body fat and physical performance of Hungarian Roma boys. Kinesiology 39(1): 15–20.*
- *Vajda I, Mészáros J, Mészáros Z, Prókai A, Sziva Á, Photiou A, Zsidegh P (2007) Effects of 3 hours a week of physical activity on body fat and cardiorespiratory parameters in obese boys. Acta Physiol Hung 94: 191–198.*

PROGRAM 5/3.

SPORT AND SOCIAL SCIENCES

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Program overview

As in the political, economic, social, and cultural spheres, the tendency towards globalization has become more powerful in the sport subsystem. Although global growth has been beneficial for sport in many ways, its impact is regarded as contradictory. The fact that sport has become global in scope produced sweeping changes both on the international and national scenes, and sport institutions have had to face new challenges to which answers are expected on the basis of scientific results. The major objective of the program "Sport and Social Sciences" is to contribute to the understanding and explanation of the impact of globalization on sport as a social phenomenon, and of the mutual relationship between sport and society. The program embraces most areas of sport sciences dealing with various social issues related to sport from the perspectives of sport philosophy, sport history, sport politics, sport economy, sport sociology, sport psychology, sport pedagogy, sport management, sport marketing, and sport law. Theoretical backgrounds and methods used in the research of the different topics should satisfy the requirements of the individual disciplines, notwithstanding the promotion of a multidisciplinary approach. The program includes the study of physical education and all traditional fields of sport, that is, school sport, university sport, sport for all, elite sport, and sport for people with special needs. In addition, the investigation of new areas of contemporary sport (recreation, sport tourism, extreme sport, risk sport, etc.) as well as comparative and cross cultural studies from the aspect of social sciences is also welcomed.

Titles of research projects	*Supervisors*
Sport geography, sport tourism	Miklós Bánhidi
Examining the teaching-learning process in physical education and sport	Edit Bíróné Nagy
Value and practice orientation of adult and elderly people	József Bognár
Career, prestige, and reasons for leaving the profession of education	József Bognár
Exploration of the organic background of learning and behavioral difficulties from a neuropsychological perspective (Developmental neuropsychology)	Rita Fodorné Földi
Sport in modern society	Gyöngyi Földesiné Szabó
The situation and role of sport among transformed political, economic and social relations	Andrea Gáldiné Gál
Education work of sport coaches	János Gombocz

Theoretical and methodological fundamentals of physical education curricula	Pál Hamar
Scientific basics of living standards (recreation)	László Jakabházy
The application possibilities of new didactic methods on the different sport education	Katalin Keresztesi
The historical role and social background of the ancient and modern Olympic movement	István Kertész
Links between information, communication and sport	Ágnes Kokovay
Relationship between society and local state institutions; task orientations in sport	László Nádori
Personality characteristics of athletes, developmental effects on personality and applications in pedagogy	Csaba Nagykáldi
Content and organizational modernization of sport-professional training in Hungary with regards to EU integration and legal harmonization	András Nemes
Event management and marketing	Mihály Nyerges
Motor performance diagnosis and its methodological basis	Károly Ozsváth
Psychometric examination of anxiety, coping with stress, and self-efficacy	Kornél Sipos
Application of autogenic training in sports and school	Kornél Sipos
Comparison of the mood-improving effects of various sports and physical exercises	Attila Szabó
The role of musical styles in aerobic training	Attila Szabó
The impact of tai chi and yoga on the acute response to stress and electrocardiographic QT interval	Attila Szabó
The social impact of physical education and sport and their relation with the Hungarian society's special groups in the first half of the 20th century	Sándor Szakály
Relations of society, physical education and sport, from ancient times to the present—lessons in social history	Katalin Szikora
Philosophical problems in physical culture	Ferenc Takács
Effects of values and culture on lifestyle	Tímea Tibori
An examination of motor and psychological development during the sensitive period of personality development, motor learning and motor development	László Tóth
Problems of sports and social integration; the relationship between sports and deviant behavior	István Vingender

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Zsuzsa Kalmár	pt	Pál Hamar
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Antonis Alexopoulos	it	Gyöngyi Szabó (Földesiné)
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Csaba Ökrös	pt	Pál Hamar
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Szabolcs Török	pt	Teodóra Tomcsányi
Gabriella Trzaskoma-Bicsérdy	ft	József Bognár

t, full-time; pt, part-time; it, international; i. individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

ANTONIS ALEXOPOULOS (2008)

Sport in the European Union from a Cypriot historical perspective

Supervisor: Gyöngyi Szabó (Földesiné)

In theory as well as in practice, the people involved with a subsystem—the actors—have a critical role in the creation of policy. The most prominent example in sport in Europe is J. M. Bosman, whose case and subsequent ruling changed the world of professional sport in Europe. In this regard, and considering the important role played by sport actors in the policy chain, and due to the lack of studies dealing with Cypriot sport actors, this study aims to discover the opinions, knowledge and expectations of four groups of Cypriot sport actors regarding Cyprus' integration in the EU, EU sport policy, and the future of Cypriot sport in the context of EU. This study's major aims are to discover, through the responses of the Cypriot sport actors, what Cypriot sport actors' opinions are regarding the integration of Cyprus in the European Union, how much Cypriot sport actors know about the European Sport Policy, what their opinions are regarding the impact of Cyprus' EU integration on Cypriot sport, the expectations the European Union has of Cypriot sport actors in connection with sport in Cyprus, and what their personal expectations are as European citizens? This study is mainly based on a survey conducted with four groups of Cypriot sport actors with supplementary documentary analysis and in-depth interviews. The population of the study consisted of four groups of Cypriot sport actors: primary education teachers, physical education teachers, university sport students and football players (and it is n= 8813 sport actors). The stratified random method of sampling was used following certain grouping variables for each group. The sample size of the study was n=912 subjects, and it is 10.3% of the total population. The data was collected by means of four questionnaires, which consisted of four parts of open and closed questions, based on the study's variables, and were analysed with the SPSS 13.0 statistical program. The findings of the study reveal that Cyprus' integration in the EU has not been well received by the Cypriot sport actors. In addition, their knowledge about several issues regarding EU sport policy was low, insufficient and marked by false impressions and beliefs, which had led to their rather positive opinion about the future of Cypriot sport in the context of the EU, and their high expectations about the EU. However, the Cypriot sport actors were more reserved about their personal career expectations, which were attributed to the challenges in each field of the Cypriot labour market. From the findings it has been concluded that several factors in the wider Cypriot society and the wider Cypriot sport scene had been influencing the Cypriot actors' opinions and feelings about EU integration and their knowledge about EU sport policy. These factors had a subsequent impact on their expectations. Considering all the findings of the study, recommendations are made concerning the methods of informing the people involved in Cypriot sport and governing sport in the framework of the EU Law. Recommendations are also provided for future research dimensions and parameters related to the issue of the European Sport Policy.

• *Alexopoulos A (2004) Sports policy in the European Union versus sports policy in Cyprus. Pp 33–38 in: Kosiewicz J, Obodynski K (eds) Sports and society, University of Rzeszow, Rzeszow.*

- *Alexopoulos A (2005) Recent sport-political decisions of the Council of Europe and Cypriot sport: a preliminary study. Pp 50–70 in: Foldesi GS, Gal A (eds) New social conditions in sport. MSTT, Budapest.*
- *Alexopoulos A (2007) The Bosman case from the perspective of Cypriot football players. Hung Rev Sport Sci 8(29): 31–34.*

CSABA BARTHA (2006)

Professional performance and the role of referees in soccer

Supervisor: Pál Hamar

The research is of descriptive character and answers the question as to what factors are required to become a good referee (meeting every requirement). Besides the physical and mental preparation level, the psychic state of the referees (concentration, conflict handling, and anxiety) and the social differences which exists among them are also examined. The derivation of the purpose of the main role of the referees and the pedagogical methods used were also studied. It was supposed that referees with higher qualifications much better fulfil the requirements of football matches than those with lower qualifications. The tested persons are elite referees from seventeen countries, and the total number of the Hungarian referees. The "Stop Test" of FIFA was used to assess their conditional abilities and fitness level. The assessment of conflict handling, motivation, and the knowledge of the rules were carried out with the survey method. As far as attention and concentration are concerned, the Toulouse and Pierot barrier tests were applied. Verbal and non-verbal communication testing was carried out with a field study. The results were compared with parametric and non-parametric, one-way ANOVA, and Chi square methods. Post ad hoc tests were used to calculate the differences between the groups. The level of significance was $p<0.05$. Data processing was carried out with the Statistica for Windows 6.0, Stat-Soft Inc. (2001) software. The hypotheses were fully justified regarding men, and partially regarding women. It turned out that elite referees or assistant referees (with FIFA or national qualifications) working in higher divisions fulfilled the requirements and expectations much better than those with lower qualifications. The group of "reserves", with the aim of getting into the squad of higher-level referees, performed at an intermediate level. The results of the lower-level groups showed heterogeneity, thus those performing better can or may hope to qualify for higher-level divisions.

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MELINDA BÍRÓ (2008)

Teaching and learning strategies during primary school students' swimming education, with emphasis on interactions

Supervisor: Edit Nagy (Bíróné)

The specific features of swimming teaching, i.e., on the one hand, the motorical nature/character of giving and receiving information, and on the other, the particular characteristics of swimming itself, result in the altering of teaching methods, the selection of organizational forms and teaching equipments, and therefore the application of teaching strategies. Our results prove that with certain teaching methods, organizational formations alter during the teaching-learning process, not to mention the unique teaching equipment used in swimming teaching. All these components influence the choice as well as the application of teaching strategies, which are the methods, teaching aids and organizational forms together.

Student-teacher activities, teaching methods, organizational forms, and teaching equipment can be outlined mostly by the teaching location (shallow-deep water) and the subject being taught (water habituation, swimming styles). It follows that the teaching strategies and related methods, teaching aids and organizational tasks can be defined according to the teaching location and subject matter.

Our complex investigation required the application and development of specific methods. In our judgment surveying the different components (organizational forms, teaching equipment) of the problem separately would provide us with the possibility of executing a more detailed and thorough investigation. The results prove that the questionnaire and observational method confirmed and supplemented each other. Our study is not regarded as complete and finished. Its results raise many further questions and problems. On the basis of our results, the study provided useful information for the theory of swimming teaching, its efficiency, and was helpful to teachers and coaches working in this field.

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- *Révész L, Bognár J, Marina IS, Gita Sz, Bíró M (2007) Curriculum development as related to the teaching of swimming in Hungary. Int J Aquat Res Educ 1(2).*

ÁGOSTON DOSEK (2007)

The relationship between pursuing sports in nature and environment in favor of developing environment-conscious behavior

Supervisor: Katalin Szikora

The effect of sport activity on the environment is low compared to other intense activities. The role of sport in personality development and in the building of lifestyle is becoming more important. Sport activities can be well-exploited in forming an environmentally-

conscious lifestyle with proper pedagogical mediation. The training of PE teachers and their pedagogical work also serve this aim. Sports activities carried out in nature are very effective, in which the contradiction between the desire for recreational activity and protecting nature can be eliminated.
The opinions and attitudes of three different student groups (Hungarian university students studying to be PE teachers, secondary school students from a school in Budapest, and from a small town near Vienna, Austria) were examined with the help of questionnaires. The environmental awareness level of the university students was higher than the two secondary school student groups. There were no outstanding differences found between the secondary school students living in the different countries. Fewer students—those participating in hiking camps—were adhering to the comfort of civilization than secondary school students. Limiting hiking to restricted areas was much better received by the university students. More university students answered in an environmentally-conscious way in relation to damage caused by nature sports, from a sustainable development perspective. There were also differences observed in the motivations for protecting nature and the environment. It could be stated that the opinions of the Hungarian students are very similar to those of the university than those of the Austrian students. On the basis of the results, concrete recommendations were given as to how sports in nature can be organized in a more environmentally-conscious way.

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JÁNOS EGRESSY (2006)

Social inequalities in competitive sport: the case of swimming

Supervisor: Gyöngyi Szabó (Földesiné)

This thesis examines the equality of chances in competitive sport, through the example of swimming. Its aim is to offer both a descriptive and explanatory analysis of the difference in chances when engaging in competitive sport and during a sports career, as well as to examine in detail the social background of the future generation of swimmers. Complex research methods included survey method, document analysis and in-depth interviews. The sample of the survey research included those children and youngsters who took part in competitive swimming since the early 1980's (n=496). The results discuss the character and extent of social inequalities in competitive sport, one by one, according to its dimensions. Based on the research data, it can be stated that gender is an important dimension: there is a significant difference in the chances of becoming an adult athlete between boys and girls, in favour of boys. Swimming as a competitive sport is chosen mostly by children of middle-class families. The living standard of the families of young swimmers has constantly improved in the last twenty years. Further research points out the increasing degree of self-reproduction of swimming society. During the past one and a half decades the impact of the sporting past of parents on the engagement of their children with competitive sport has grown. It is first of all children of former swimmers and of elite athletes

from other individual sports who are participating in competitive swimming, mostly on the initiative of their parents. Sporting traditions of the family work as a double "filter"; they determine the circle of those choosing swimming and later of those who have more of a chance of becoming an elite athlete. Despite the outstanding economic development and infrastructural endowment of Budapest, and as a consequence, its over-representing the youngest generations, the regional distribution of future swimmers does not depend on the different levels of economic development and infrastructural endowment of the regions. However, inequalities caused by the type of settlement are notable, in favour of young athletes living in the capital and in other big cities. A growing tendency for adult elite athletes to study has characterized the last two decades. However, the aspiration to engage with university studies is often regarded as being in opposition to the career of the competitive swimmer, which in many cases leads their early departure from swimming and choosing other sports. This thesis points out guidelines for further research in the topic of social inequalities in sport.

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BALÁZS FÜGEDI (2007)

Examination of learning and reproducing choreographed calisthenics movement series

Supervisor: József Bognár

Our research was focused on choreographed exercise series and the complex in-depth study of the instruction and studying process of these forms of motion. The aim of our research was to become familiar with the attainment and the acquisition process of the study of motional action within the choreographed, artificial series of movements, which are in possession of definite space, time and dynamic features, like the movement material of the calisthenics exercise series. We approached the topic from two main directions. On the one hand is the study of movement, reproduction, and on the other, the experience, opinion and attitude with respect to these forms of motion. In the researches we analyzed the level and quality of movement reproduction, the number of faults and the level of movement retaining of primary school pupils, students taking the entrance examination to PE Teacher Education (PETE) and undergraduates. Moreover, we examined the experience, opinion and attitudes of the primary school pupils, their PE teachers, PETE students, and final-year PETE students in connection with the instruction and attainment of calisthenics exercise series forms of motion. We analyzed our results with the SPSS 13.0 for Windows statistical program, during which we used basic statistical calculations, non-parametric experiment (Chi2, K-independent), paired sample t-test and analysis of variance (ANOVA). The movement performances (examinations 1, 3, and 4) and experiences (examinations 2, 5, and 6) of both the primary school age-group and the PETE student

participants show that the choreographed forms of motion do not get enough emphasis in the education, though refusal of the activity is not experienced on either of the participants. According to our researches, it seems clear that there is no concrete negative attitude, based on experience, towards these forms of motion (examinations 2, 5, and 6). In the drafting of prospective PE teachers, the exercise series appears "only" as warming up and skill development, and occasionally as a disciplining device (examination 6). In accordance with our researches, we can say that the pupils expect the varied and colorful use of the calisthenics forms of motion, but the prospective PE teachers are not fit enough for its realization, since the exercises used "only"as warming up and skill development do not satisfy the pupils' claims (examinations 2, 5, and 6). In accordance with the integration of our finished researches we can use this dissertation within three policies, which are the following: the qualitative development of the study of movement; reproduction in the primary school; the modernization of (public) education with the content in connection with movement material of calisthenics; and the more efficient adaptation of knowledge on the primary school taught during physical education teacher education.

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- *Honfi L, Tóth L, Fügedi B (2005) Examination of the effect of the mental training in gymnastics. Kalokagathia 3: 81–84.*
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ANDREA GÁL (GÁLDINÉ) (2008)

Sport and gender in the mirror of the media in Hungary at the beginning of the 21st century

Supervisor: Gyöngyi Szabó (Földesiné)

According to international literature, women athletes are under-represented in the sports media from both quantitative and qualitative perspectives. This issue has never been studied in Hungary. The major aim of this thesis is to analyse the relationship between gender, sport and media as well as the representation of sportsmen and sportswomen in the sports media. In addition, the opinion of women athletes, sport journalists and newspaper readers on the same topics are examined. In the research the following methods were used: content analysis, survey method, and in-depth interviews. In the sample of the content analysis, two traditional Hungarian political daily newspapers and the most popular daily tabloid were chosen. In all cases, issues published prior to, during and following the 2004 Summer Olympic Games and issues from the same period in 2005 were selected. The survey sample consisted of regular newspaper readers (n=401). In-depth interviews were made with elite women athletes (n=34) and sports journalists (n=7). The hypotheses referred to the proportion of genders in the sport-related articles and photos of the daily papers; the changes in the above mentioned dimensions in Olympic periods, the interpretation of gender representation by sportswomen and newspaper readers, as well as the impact of male dominance in sports journalism on the selection of news. The theoretical framework of the research was based on the theory by Stuart Hall named "circuit of cul-

ture", which suggests five factors (consumption, representation, regulation, production and identity) in the shaping, continuation and development of the meanings circulating through the communication flows of society. The results of the thesis support the idea that these five factors lead together to the sportsman's over-representation in the media. News items are generally selected by men who stick to a traditional conception of masculinity and feminity and believe that men are much more interested in sports than women are. The most popular sports are practiced by men throughout the whole world, and the priority they are given regarding other sports also means a disadvantage for women sports representation in the media. Currently, Hungarian daily newspapers are not subject to any special regulation with the intention of promoting gender equity. Newspaper readers and sportswomen notice the inequality in gender representation in the media. However, they assume that the differences are much smaller than they are in reality. Their beliefs might be rooted in their experiences gained during Olympic periods. In conclusion, it is stated that the results are conform to international trends, revealing sportsmen's over-representation. Research data concerning the opinions of sportswomen and sport journalists about the same issues are new in the relevant literature.

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KATALIN KÄLBLI (2008)

Injury- and sport-specific training for athletes with disabilities —sitting volleyball players

Supervisor: László Nádori

The aim of my dissertation was to summarize and explore all of the knowledge which is indispensably important to a trainer who wishes to work efficiently with athletes with disabilities, sitting volleyball players. During my examinations I used three methods; questionnaire, document analysis and examination with observation (game-analyzing). My results include both the generic and sitting volleyball-specific knowledge regarding athletes with disabilities. The first results showed—and inspired in the meantime the need for further examinations—that the volleyball trainer's condemnation to be a trainer in a sport for people with a disability originates from the incompleteness of their knowledge. In my essay I tried to summarize the necessary cognitions. As a result of the comparative analysis of the Olympic and Paralympics movements, I recognized a similar style of development, but in certain areas (sports, sport events, continental distribution of the participating countries) there are significant discrepancies between the two movements. There was a difference among the expectations of athletes with disabilities towards their trainers compared with able-bodied athletes. The trainers tasks were wider in sports for disabled people.
Based on motivating factors of beginning sport activity, it may be noted that for those with congenital impairments, the roll of the parents is signficant, whereas for those with

acquisitive impairments, the roll of friends is significant. The examinations regarding sitting-volleyball attested to the need for special knowledge as well. The sport-selection of the sitting-volleyball players will be taken on an injury-specific basis, which is justified by the frequency of certain injury types. The analysis of different volleyball game elements indicates the use of special training tasks, which were summarized in my essay.

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- *Gita Sz, Kälbli K, Rigler E (2006) EU policies and legislations for social integration for people with disabilities. Kalokagathia 44: 137–145.*
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ÁGNES KOKOVAY (2007)

Multimedial possibilities in the methodology of teaching physical education

Supervisor: János Gombocz

We live in the age of the Information Society, where the acceleration of changes requires the reform of education and its adaptation to altered environmental conditions. Parallel with the appearance of informatics and the personal computer, the demand has emerged for the effective use of the computer in education. This is ensured by multimedia (e-learning) curricula and teaching materials. The basis of this dissertation is to study the effect of such an e-teaching material on high-school students. The aim was to compare the effectiveness of knowledge acquisition between the traditional and multimedial methods. The instrument used was the e-teaching material "Basics of Gymnastics"developed prior to the experiment. The experiment was carried out in the academic year 2002–2003 in three tertiary institutions of education in Baja, Eger and Nyíregyháza. The hypothesis to be substantiated by the experiment was that the new method was more effective than the traditional one in teaching the basics of gymnastics and in planning the exercises and exercise sequences of special effect which have an important part in physical education classes. Corollary expections included the increased activity of the students owing to the didactic conception of the material as well as enhanced learning motivation. Also to be verified was the assumption that the teacher's guiding-controlling role was indispensable in this new method of learning/teaching, a process which has been quite unfamiliar to the broad masses so far.

After the processing of the results, it was found that on the whole, e-learners achieved better results in acquiring the teaching material applied as the independent variable of the experiment than their fellow students studying in the traditional manner. The detailed analysis pinpointed a single area (factual knowledge) where our hypothesis concerning e-learning was not clearly verified. The performance of the experimental group in Eger called attention to the role of tutors. The performance of the students in this group was far below that of other groups learning under the supervision of a tutor. This is conclusive proof that e-learning cannot do without the teacher's participation, only this participation

is of a different nature. The positive experiences gained during the experiment emphasize that in our quickly changing world, the positive effects of this teaching methodology must be utilized and incorporated into the existing systems of education.

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KATALIN LAKATOS (2006)

The significance of the movement maturity examination in early identification of learning disorders

Supervisor: Rita Földi (Fodorné)

This thesis work comprises the comparison between the examinations of movements and statements in relation to organic disorder of maturation of the nervous system in childhood, as observed in pedagogy, psychology and medical science, and the examination of school maturation have been established in Hungary since 1972. It contains the demonstration of the mutual skill and ability profile, in addition to the results of the research of four groups involving 100 children on two occasions. The motoric psycho-cognitive social and psychomotoric, sociomotoric psycho-social syndromes of organic immaturity are recognizable for children of logopedic kindergarten, school-immature children in nursery school and children often diagnostized as "hyperactive". Three homogeneous study groups (25 children in each) were recruited from the population mentioned above, and 25 school-matured six-year-old children composed the control group. Two of these groups—those children recruited from logopedic kindergarten and hyperactive ones—had sensomotoric training between the yearly assessments. The groups of school-immature children and the control population have not had training like this at all.

Hypotheses:

(1) Groups of school-immature children with weak cognitive and/or partial ability achieved weak results in school maturity tests and also achieved lower scores in movements assays compared to the control group, thus cognitive and movement achievements correlate with each other.

(2) In groups where children were getting sensomotoric training, the improvements were more significant compared to those groups where the maturation occurred spontaneously. Indeed, sensomotoric training mediates the maturation of the nervous system, and helps in raising it up to a normal level.

Results:

The statistical analysis of the results of the survey with 375 subjects supports the first hypothesis; both groups achieved the same level relative to each other.

The second hypothesis is also proved; groups which had sensomotoric training showed improvement in organic maturation by 16.57% (logopedic kindergarten) and 29.29% (hyperactive population). The spontaneous improvement of groups without training is less significant: 0.28% (school-immature) and 7.14% (mature). There are sixteen common

items among basic skills and abilities in the examination of statement and movement and school maturation assessment. These items have strong predictive significance.

Significance:

The examination of statement and movement of five-year olds helps screen out the children with organic immaturation (they are referred to as risky children further on) and the sensomotoric training can be combined for making them close up.

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ÉVA LEIBINGER (2008)

Manifestation of psychosomatic developments and educational influences of girls' gymnastics in physical education

Supervisor: Pál Hamar

The aim of my survey is to show the role of girls' gymnastics curriculum within the education process featuring professional value-transmitting actions. The place and role of gymnastics and its specific skill-development influence possibilities will be determined in practice of school physical education. The effects of the PE teaching-learning process on students'cognitive, affective and psychomotor skills are examined, with an emphasis on the PE teachers'attitude toward gymnastics and its effect on students and students' performances. It is assumed that the orientation of the above mentioned effects are noticeable among female students, and the results of the gymnastics-specific psychomotor tests also show these impacts indirectly. In studying the lifestyle of female students it is assumed that sport as well as gymnastics-type activities are a decisive part of physical activities. Data from 210 girls were collected and processed from five different schools, and 51 subjects participated in a three-year-long longitudinal study as well. The survey consisted of a ten-piece psychomotor test-system and a weekly time-table and questionnaire measuring affective and cognitive level. Data processing was carried out with the software Statistics for Windows 7.1, Stat. Soft. The level of significance was $p<0.05$. The results were compared with parametric and non-parametric methods, just as with dependent and independent *t*-tests, with one-way ANOVA analysis or with Spearman's coefficient of rank correlation according to the type and the nature of the research. Differences were searched between the students' performance from different schools, the stance of PE teachers toward gymnastics and the results of the PE grade-groups of the students. The analysis also covered the examination of the control group's performance. In addition, there were attempts to compare the relationships between the cognitive, affective and psychomotor performance of the students. Significant differences among the students from the examined schools were manifested, supporting the fact that the schools' personnel and equipment casemaps are the main influencing factors regarding physical performance. The following conclusions can be drawn based on the survey results:

The results of gymnastics-specified psychomotor tests are reflected in physical education grades; It can be stated that the positive or negative attitude of the PE teachers toward gymnastics subject matter directly influences the students' approach and indirectly influences students' psychomotor performance; Those students who filled out the weekly timetable sheet prefer gymnastics-type activities.

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CSABA ÖKRÖS (2007)

Individual and collective pychomotor performances in male handball

Supervisor: Pál Hamar

The aim of our present study was to assist the practical work of trainers and teachers within the area of education and check how they are utilizing the results of our research. We consider our study as an initial research that takes a great step in the analysis of the sport's tactics. There are two methods applied in this Ph. D. thesis. The main outcomes of our double aspect empirical research were the following: (1) As regards match observations, the conformation of attacks in modern handball was investigated considering the completion of the attacks. Attack completions can be realized in several different ways, and they can be analysed according to the frequency of occurrence, the scored goals or their efficiency. Dominance of the center backs can be observed during the organized attack against a set-up defense. Long-range shots are the most frequent attack completion of game situations that come after negative and positive connection between the variables of other game situations. Other players such as wingers or pivot players have fewer number of scoring situations, thus the performance of the team claims better situation utilization from them. (2) A measuring method has been elaborated based on typical completions of the attacks. It models the most important features of live situations. The survey, carried out with 200 Hungarian players, has proved that the more time the player spends playing handball, the greater the development that can be observed in his general performance. The dominance of center backs are justified by on-site measures, as their performance was always at the highest level in all age-categories. Performance development of the wingers and pivots can be observed after the 5–6 age categories. In the model situations, long-range shots proved to be the most unsuccessful, even for specialists (center backs) as well. This moderate performance can be explained by the distance from the goal, and the difficult implementation of the shots which require special skills. Thus the close-range zone had a high standard, and those situations proved to be successful where teammates cooperated.

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TIBOR POLGÁR (2008)

Effect-mechanisms of sporting values in the sphere of secondary school and college students, Vas County

Supervisor: János Gombocz

The purpose of my study was to measure the relationship between a healthy lifestyle and regular physical activity. It was also our goal to find out what those values and activities are that occur in the lifestyle or in the daily routine of secondary school and college students in Vas County, Hungary. Based on a review of the relevant international literature, it can be stated that there are several empirical studies in the domain of the system of values and daily activities which deal with these aspects separately, but there is hardly any relevant literature in that contrasts and compares these two aspects. I have chosen the population of Vas County for my analyses. Through random sampling (n=608) I used a predesigned and valid questionnaire. The analysis started with frequency, which is followed by a non-parametric comparison analysis. So the data was analyzed and compared with the help of ChiÇ and Pearson ChiÇ on the basis of the non-parametric statistic. The level of significance was established at 5% of the level ($p<0.05$). Statistical analysis was conducted by the SPSS 15.0 for Windows programme. Based on the results, it is generally concluded that the vast majority of youth in Vas County do very little amount of sport. Their choice of sporting values differ, first of all, by the fact that unfortunately hardly any sporting activity become conscious and purposeful. Regarding sporting values, I discovered only average and unmeaningful values with mostly general information, passive and inconsiderable sporting activity. The degree of the hidden recall is beneath contempt, because the values hidden within the text are almost equal to zero. The situation is roughly the same concerning sport value definitions. Most participants were not able to define at least the basic values with their own words. The lack of the impulsive and home environment shows that the prior place of socialisation does not strengthen basic health and sporting values. The broader environment's contribution to the socialization process points out—roughly the same—that the confirmation of values happens through indirect and weak channels. The choice of values of those who do sports regularly seem to be a little more conscious and purposeful. Women tend to be more inactive regarding sporting activities than men, though they are more conscious in recognizing and defining sport value definitions. Secondary school students unequivocally do much more sport than college students, but they are little concerned with meaning and purpose.

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PIROSKA SZALAY (2008)

Some examinations of the psychological and somatic consequences of alcohol abuse

Supervisor: Gyöngyi Szabó (Földesiné)

We have to emphasize the importance of prevention, which should be not only the duty of the teachers of hygiene or PE, doctors, nurses and psychologists, but also that of experts who deal with young people. They should find ways of training them for a healthy way of life. We studied the effects of physical activity on the development of body image in alcohol-dependent patients. We used the Tennessee Self Concept Scale. Regular sports activity plays a major role in the rehabilitation of young alcohol-dependent adults, helping them to create a healthy way of life.

Smoking and sedentary lifestyles touch the lives of adolescents in high schools and students of universities. Self-efficacy towards the temptation to smoke and the taking of regular physical exercise (Schwarzer et al. 1993) are Hungarian standards for university students, teachers and hospitalized psychiatric patients with alcohol problems.

We started to examine different self-efficacy measures on secondary school pupils. Different secondary school samples were compared to each other here.

Analysis of social background-lifestyle data by regression model revealed that self-efficacy on temptation to smoke for high school students is determined by social factors (age, gender, type of school, and education level of the father), drug consuming habits, and self-efficacy towards physical exercise. The complex analysis of socioeconomic background, lifestyle data and the measures of self-efficacy (temptation to smoke, taking physical exercise) revealed the key points of the health education practice for secondary school boys and girls and medical staff members. The known consequence of alcohol abuse is the cerebrovascular and mental disorder. However, recent studies show a protective effect of moderate alcohol consumption against arteriosclerosis. Brain imaging and metabolic data supply evidence of the effect of alcohol abuse. However, such expensive procedures are unavailable in most routine clinical examinations. A potentially inexpensive diagnostic tool can be the pulsatile electrical impedance, rheoencephalogram – REG. REG has high sensitivity to the earliest cerebrovascular alteration during aging and arteriosclerosis: it is the decrease of the elasticity of the arteries, expressed as elongated anacrotic time on REG.

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HENRIETTE DANCS (SZEGENERNÉ) (2007)

Contribution of sport to regional sustainable development

Supervisor: Gyöngyi Szabó (Földesiné)

The aim of this thesis is to give answers to the following questions: (1) what role sport plays in the regional development plans based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourism experts; (2) which ways sports serve the quality of life of people based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourism experts; (3) how sport contributes to regional sustainable development based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourists experts; and (4) what role sport plays in Vas County and in Burgenland based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourism experts. Based on the above listed objectives, empirically tested hypotheses were as follows: (a) Political spheres and development policies of planning with respect to sport is not satisfactory based on the attitudes and opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourists experts. (b) Sport can serve the quality of life of people in many ways, but first of all its role in health protection is its most significant based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and tourists experts. (c) Sport can contribute significantly toward the regional sustainable development of all three—social, economical and environmental—dimensions of SD based on the opinions of Hungarian and Austrian coaches, PE teachers, development officers, mayors, sport journalists and touristic experts. (d) The judgement of the role of sport is significantly different in the two neighbouring regions due to the difference in the social and economical development level of the two countries. Sport's contribution to regional sustainable development may be determined by many factors, and it should be considered an extraordinarily complex and interdisciplinary topic, and because of this it requires several methods. These included the following: document analysis, Survey-Method (main method) and depth interviews.

The main aim of this dissertation was to focus attention—on a multidisciplinary or transdisciplinary basis—to the potential of sport and sustainable development, first of all, at a regional level. Sport has the potential to serve regional sustainable development in many ways: it might be a significant factor in the success of preservation development, connecting health and environmental protection, it might strengthen communication among people and different regions, it might be a supporting factor in social economical integration. But it can only fulfil these functions if the sphere of sport and the interests of its different levels will be stated and taken into consideration when decisions are made and in the designing of development projects.

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- *Dancs Szegner H (2005) Some theoretical questions of the sport and of the sustainable development. Pp 28–50 in: Szabo Földesi Gy, Gal A (eds) New social conditions in sport, 1995–2004. Semmelweis University, Budapest.*
- *Dancs Szegner H (2005) Sustainable quality of life through sport in the European Union. Pp 31–46 in: Kosiewicz J, Obodynski K (eds) Axiological dimensions of sport practical aspects. Warsaw.*

SZABOLCS TÖRÖK (2006)

The frequency and rehabilitation of malignant childhood diseases in Hungary

Supervisor: Teodóra Tomcsányi

Our research focused on the frequency of malignant childhood diseases in Hungary (incidence, prevalence) and the outcome effectiveness of special summer camps aimed at their psychosocial rehabilitation. The incidence of childhood malignant diseases in Hungary is 131–140 per million person-years, and its average annual increase is 1.1 %; the relative frequencies are: leukaemia 27.8%, central nervous system tumours 26.6%, lymphomas 11.3%, sympathetic nervous system tumours 10.0% (1993–2002; n=2436). The five-year overall survival rate is 65.2% (1988–1997; n=2146). The incidence of leukaemia in Hungary is 33.2–39.4 per million person-years, and the average annual increase is 0.71% (1973–2002; n=2204). The number of children in Hungary living with malignant diseases and are foremost in need of psychosocial rehabilitation can be estimated around one thousand. As an effect of participation in a rehabilitation camp, positive psychosocial changes were observed. Self-esteem increased significantly by the end of the camp, and for those whose initial self-esteem was low, a long-term increase was observed. Perceived self-efficacy increased significantly not only by the end of camp, but at the two-month follow up as well, in the subgroup of campers with initial low self-efficacy. The Hungarian data of epidemiology correlates well with other European data. Regarding the frequency of malignant childhood diseases in Hungary, our research is the first comprehensive analysis over an extended period of time. The novelties of our results regarding the outcome effectiveness of rehabilitation camping programmes are that they demonstrate a long-term positive effect on self-esteem and have a positive effect on self-efficacy.

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GABRIELLA TRZASKOMA-BICSÉRDY (2008)

Analysis of principal factors of success in wrestling

Supervisor: József Bognár

Three factors of success in sport were examined in this work. (1) The first step for later success in sport is to choose the discipline best suited to our abilities and interest, so how pupils get into a sport discipline was investigated. Our aim was to find out how often they pursue sports, what they choose, how they choose, who influences their choice and why they drop out. Upper elementary school pupils were surveyed through questionnaire (n=

1604). For data analysis basic statistics and Factor Analyses were used. Most of the upper elementary school students pursue sport, but the influence of experts (PE teachers, coaches) was very weak regarding their choice. In the opinion of a small group of wrestlers, PE teachers had no influnces on their choices at all, and coaches played no important role as well; their role seems to be related mostly in motivation, through emphasizing the importance of health care. Lots of pupils had already dropped out, and as regards the basis for this, mostly affective factors can be found. (2) Additionally, to achieve success we have to satisfy the needs of the chosen sport discipline, so we analyzed the anthropometrical parameters and physical abilities needed for wrestling and recognized as crucial for selection. The test results of thirteen- to fourteen-year-old wrestlers collected in 1984 (n=285) were analysed. We compared those who were proven to be successful later in their sports career with those who did not achieve outstanding results using basic statistics and Discriminant Analysis. In light- and medium-weight categories local muscle endurance, coordination and technical preparation show importance, while in the heavy-weight category anthropometrical parameters and power are significant. (3) The effective coach-athlete relationship is fundamental in the process, so the characteristics of cooperation were utilised. To get information about the coach-athlete relationship, the perspectives and experiences of well-known coaches were gained through in-depth, face-to-face individual interviews (n=5). Based upon the characteristics of the discipline, a strict leading style is used in trainings, as well as in competitions. The preparation is based upon trust, respect and love. In childhood parents are also involved in preparation. Coaches make sure to keep in contact with them as well.

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- *Bognár J, Trzaskoma-Bicsérdy G, Révész L, Géczi G (2006) A szülők szerepe a sporttehetség gondozásban. Kalokagathia 1/2: 86–95.*
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SCHOOL OF PH. D. STUDIES

6. JÁNOS SZENTÁGOTHAI NEUROSCIENCES

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General overview

The Neuroscience Graduate School blends the theoretical and clinical neuroscience research topics, treats the basic questions of the structure and function of the nervous system in a synthetic view as well as the normal and pathological functioning of the human brain as observed by the clinicians. The research topics as listed below and arranged in three basic science and three clinical research Programs witness the large array and variation of supply.

PROGRAM 6/1.

NEUROMORPHOLOGY AND CELL BIOLOGY

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Program overview

This basic research program of the Neuroscience Doctoral School covers a broad spectrum of the examination of the central nervous system—from the function and differentiation of the individual neurons to the higher cortical activity. The large variety of topics shares the methodology (neuromorphology, neurochemistry, molecular cell biology, synaptology), the functional view of the topics, and the use of the rich selection of functional neuromorphological methods. The program includes research areas intended to better understand the organization of neural tissues, neural differentiation, and the plasticity of the central nervous system. Within the program research areas cover molecular genetics, experimental neuromorphology, and the studies of normal and pathological (neurodegenerative diseases) human brains. Cytological, neuromorphological and neurochemical (immunohistochemical) areas of the program are tightly connected both with the regulatory mechanisms of the autonomous nervous system (stress, pain, food uptake, thermoregulation) and with topics evaluating the higher order functions of the central nervous system (information processing, learning, motivation, memory).

Titles of research projects	*Supervisors*
Localization of trigeminal pain-induced stress pathways (trigemino-hypothalamic pathway)	Miklós Palkovits
Neuroanatomical and neurochemical characterization of bidirectional neuronal pathways between the nucleus accumbens and the lateral hypothalamus	Miklós Palkovits
Morphological and *in vivo* physiological analysis of excitatory and inhibitory inputs in the thalamus	László Acsády
Subpallial systems in relation to avian learning	András Csillag
Comparative neuroanatomical basis of addictive behaviour in avian and mammalian model systems	András Csillag
Transforming growth factor beta proteins in the central nervous system	Árpád Dobolyi
Endocannabinoids in the brain: a novel communication channel between neurons	Tamás Freund
Study of synaptic plasticity in the spinal cord of normal, inflamed and nerve injured rats	Gábor Gerber
Functional characterisation of hippocampal inhibitory neurons using a combined anatomical and physiological approach	Attila Gulyás
Immunohistochemical monitoring of ependymal histogenesis	Mihály Kálmán
Position and function of G protein-coupled receptors in neuronal networks	István Katona
Neural cell differentiation. *In vitro* cell technology	Emília Madarász
Epileptic reorganization of the human hippocampus	Zsófia Maglóczky
Pathomechanism of the epileptic cell loss: regulation of inhibitory processes via receptors and membrane proteins	Zsófia Maglóczky
Synaptic information processing in the olfactory bulb	Zoltán Nusser
Neuronal and glial architecture of the conus medullaris and filum terminale	Miklós Réthelyi
Functional anatomy of pre- and postsynaptic receptors and their neurotransmitters in the hippocampus	Gábor Nyíri
Role of prolactin-releasing peptide in the central nervous system	Zsuzsanna Tóth

Ph. D. students		*Supervisors*
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Kristóf Giber	ft	László Acsády
János Hanics	pt	András Csillag
Mária Rita Karlócai	ft	Zsófia Maglóczky
Guillome Lourmet	pt	Miklós Palkovits
Nóra Ádinger	a	Emília Madarász
Barbara Orsolits	ft	Zsuzsa Környei
Pathak Anuj	it	Emília Madarász
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Éva Renner	ft	Miklós Palkovits
Kinga Szabó Meltzer	ft	Miklós Palkovits
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Ágota Ádám	pt	András Csillag
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Anita Zádori	ft	Emília Madarász
István Adorján	ft	Mihály Kálmán
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Tünde Molnár	pt	Miklós Palkovits
Rita Nyilas	i	István Katona
Adrienn Regina Szabó	pt	Mihály Kálmán

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András Bratincsák	ft	Miklós Palkovits
Márta Jelitai	ft	Emília Madarász
Natalya Zayats	pt	Teréz Tömböl

a, absolutorium; f, full-time; pt, part-time; it, international; i, individual

Abstracts of Ph.D. theses successfully defended in 2006, 2007 and 2008

CSILLA ARI (2008)

Correlation between the cerebralization, astroglial architecture and blood-brain barrier composition in Chondichthyes

Supervisor: Mihály Kálmán

Chondrichthyes display a wide range of cerebralization, differences in the glial architecture and in the composition of the blood-brain barrier. The present study supplements the former glial impregnation studies and GFAP immunohistochemistry with the immunohistochemical detections of glutamine synthetase and S-100 protein. The examination was extended to representatives of important groups of Chondrichthyes (myliobatiform rays, Holocephali), also to brain parts (rhombencephalon), on which no glial study has been done as yet. To reveal some characteristic features of the blood-brain barrier of cartilaginous fishes the dystroglycan complex (DG) and some of its associated proteins were also investigated, as well as aquaporin-4 and aquaporin-9.

Immunostaining to S-100, and mainly to glutamine synthetase revealed more astroglial elements, than did immunostaining to GFAP. By using glutamine synthetase astrocytes could be revealed in *Squalus acanthias* (Squalomorphii) and *Callorhinchus milii* (Holocephali), and GFAP immunonegative astrocytes and Bergmann glia were visualized in batoids. Myliobatiform rays, especially *Mobula japanica*, the representative of Mobulidae family, manifested advanced features to other batoids, e.g. appearance of astrocytes in cerebellum, lower brain stem, and spinal cord. The glial architecture of *C. milii,* a representative of Holocephali, proved to be similar to that of *S. acanthias,* but showed some advanced features: some telencephalic territories had individual glial pattern, and in the meningeal surface glial cells were inserted between the endfeet of radial glia. Immunostaining against DG and its associated proteins, but not aquaporins were detected in the vessels of every species investigated. Interspecific differences of blood-brain barrier, cerebralization, or astroglial architecture were not reflected in any differences of immunoreactivity.

There was no specific astroglial structure to distinguish the brains of galeomorph and squalomorph sharks. There was only difference between sharks and batoids (preponderance of astrocytes in latters), which confined to the prosencephalon and mesencephalon. It seems that the astroglial structures correspond to the local macroscopic structure of brain, rather than to the general cerebralization, and the laminar/elaborated categories. Astrocytes did not prevail in conservative brain regions as they did in the progressive brain regions in Elasmobranchii, in contrast to Amniota.

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PÉTER BARTHÓ (2008)

Anatomical and physiological characterization of a novel inhibitory thalamic pathway from the zona incerta

Supervisor: László Acsády

The thalamus is the major relay between subcortical structures and the cortex. It takes part in the genesis of cortical EEG, regulates the quality of information transfer according to the sleep-wake cycle, and its widespread lesion can lead to permanent loss of consciousness.

According to the traditional view, the inhibitory system of the thalamus is comprised by the thalamic reticular nucleus, and the local GABAergic interneurons. In the present study we described a novel GABAergic inhibitory system in the thalamus, and characterized the firing pattern of the cells of origin of the projection.

Following anterograde tract-tracing from the zona incerta we observed widespread axonal labeling in the thalamus. The incertothalamic tract showed dual selectivity. First, the fibers terminated exclusively in the so called higher-order nuclei (i. e. the ones that receive driver input from layer V. of the cortex). Second, the terminals selectively innervated the proximal dendrites of relay relay cells, forming symmetrical synapses with multiple release sites. We have used postembedding GABA immunostaining to verify the GABAergic nature of the tract. These properties suggest that the incertothalamic projection is an effective, new inhibitory system in the thalamus, which is fundamentally different from the GABAergic input originating from the nRt.

To get an insight on the activity of zona incerta neurons, individual cells were recorded and labeled by juxtacellular method. Unlike relay or nRt cells, zona incerta neurons fired exlcusively in a tonic manner, burst activity was not observed. In their relation to slow cortical oscillation, zona incerta cells could be divided into two groups. The majority of cells ("phasic cells") fired on the depth-negative phases of the slow oscillation, faithfully following cortical activity, while a minority ("tonic cells") fired with high frequency, independently of ongoing cortical activity. Phase locking of phasic cells was comparable 76 to, or exceeded the phase locking of thalamic relay cells. Anatomical study of the corticoincertal projection suggested strong and selective cortical control. These results suggest, that alongside the previously known peripheral effects, zona incerta is strongly influenced by cortical output signals. Integrating ascending and descending sensorimotor signals may have an important role in behavior dependent gating of higher order relays.

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ANDRÁS BRATINCSÁK (2008)

Integration of thermoregulation in the central nervous system

Supervisor: Miklós Palkovits

Thermoregulation is a basic physiological mechanism of the mammalian organism that is precisely regulated by the central nervous system. Environmental thermal stimuli trigger a coordinated physiologic response that is orchestrated by the thermoregulatory center located in the preoptic area of the hypothalamus. Although numerous data have been published about the physiology of thermoregulation, the neuroanatomical representation and topographical localization of these physiological reactions are incomplete, especially regarding the afferent network of thermoregulation. Our aim was to localize the activated cell groups and nuclei involved in different thermal exposures as well as hibernation, and to identify the mechanism of thermosensation and the afferent pathway to the hypothalamic thermoregulatory center. (1) We identified the nuclei, subdivisions and cell groups activated by warm and cold ambient exposure and compared their patterns of distribution. (2) We showed direct evidence that thermoregulatory responses in vivo are initiated by thermal stimuli originating in the peripheral and not intracranial thermoreceptors. (3) By tract tracing studies we identified a direct neuronal ascending pathway from a brainstem thermosensitive cell group located in the peritrigeminal area to the preoptic thermoregulatory center and thus identified a possible pathway for peripheral thermal signals to reach central thermoregulatory elements. (4) At last, we localized activated neurons during multiple phases of the hibernation bout and speculated over their physiologic importance in inducing, maintaining and ceasing torpor. Our studies complete previous experiments by identifying a possible mechanism and neuroanatomical pathway of temperature sensation and contribute to the overall understanding of thermoregulation.

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MÁRTA JELITAI (2006)

Physiological properties of developing cells in early stages of *in vitro* induced neurogenesis

Supervisor: Emília Madarász

The aim of my thesis work was to get insight into some early steps of neural cell fate determination and neuronal differentiation. I intended to find some physiological stem- and progenitor properties, which distinguish these cells from more mature neural tissue cells and enable them to function as stem cells in different phases of ontogenesis. I studied (1) the bioelectric properties of NE-4C neural stem cells and the changes of these charac-

teristics in the course of *in vitro* induced neuron formation; (2) the presence and role of GABA and glutamate in the early neuronal cell differentiation.
The stem/progenitor cell status, while it can be preserved for years, is characterized by high responsiveness to minimum inductive signals, which can evoke irreversible processes of differentiation. The main bioelectric characteristics of stem cells and progenitors are the multiple ionic coupling, the passive potassium and chloride conductance and the high intracellular chloride level. While these features may contribute to the maintenance of the non-committed stem-like phenotype, the passive movement of potassium and chloride ions may sensibly tune the excitability and responsiveness of progenitor cells. Important changes in the intracellular ion-milieu are, however, prevented by the formation of large clusters of ionically coupled cells and the rapid redistribution of ions in the enlarged (cross-coupled) cytoplasmic volume.
With the appearance of neuronal morphology, neighboring cells cease gap junction communication and voltage dependent ionic currents become detectable. The expression of voltage dependent ion channels is accompanied with the formation of complex current profiles. These profiles are changing with the advancement of neuronal maturation and characterize the process of neuronal development.
In early phases of neuron formation, GABA plays some more important roles than glutamate. Neural stem cells and progenitors contain and release a relatively high amount of GABA. The extracellular GABA can tonically depolarize the developing progenitors and early neural precursors through the early appearing GABA(A) receptors. Functional NMDA receptors, on the other hand, appear only in postmitotic neural precursors, but before the onset of synaptogenesis. Signalization by either GABA or glutamate effects the developing cells by regulating the actual level of $[Ca^{2+}]I$.

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NATALYA ZAYATS (2007)

The structure of the accessory optic nuclei in the chick

Supervisor: Teréz Tömböl

The accessory optic system (AOS) is readily identifiable in all classes of vertebrates with prominent visual systems. The AOS is characterized by a distinct retinal projection and a group neurons, the accessory optic nuclei. In birds, the AOS includes two nuclei: the nucleus of the basal optic root (nBOR), and nucleus lentiformis mesencephali magnocellularis (nLMmc). Physiological studies indicated a role for nBOR and nLMmc in the optokinetic nystagmus (OKN) that is the stabilization of eye movements, and the coordination of eye and neck movements. Despite numerous advances, however, many details of the morphology of accessory optic nuclei, which seem to be important in the understanding

of certain functions and in completing our knowledge of morphology are still unknown. Therefore, in this study the intrinsic organization (the morphology of the different types of neurons, and their relationship with the optic fibers) of two nuclei was investigated in chick (*Gallus domesticus*) by light and electron microscopy with different methods: Golgi impregnation, GABA immunostaining, biotinylated dextran-amine (BDA) tracing. In the Golgi analysis of nBOR a characteristic dendritic ramification pattern of two types of putative projection neurons was observed. The projection neurons form with their overlapping dendritic terminal sections dendritic nests that develop synaptic fields with the optic fiber terminals. GABA-immunopositive terminals which derives from small and/or elongated local circuit neurons, and from myelinated afferent fibers synapse with distinct loci of the dendritic trees of projection neurons; they may therefore play an important role in the inhibitory-modulatory system of the nBOR. In the nLMmc Golgi preparations neurons of large, medium-large, medium and small sizes were distinguished. The large and medium-large projection neurons are located in a tight topographical relationship. The dendrites of the large and medium-large cells are also observed to be in close proximity with each other, and also with retinal fiber terminals. The function of these cells modulated by the GABA-immunopositive terminals from various local circuit neurons, and most probably from GABAergic myelinated fibers as well, which may originate from the contralateral nLMmc and/or the visual Wulst.

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- *Tömböl T, Eyre M, Zayats N, Németh A (2003) The ramifications and terminals of optic fibres in layers 2 and 3 of the avian optic tectum: a Golgi and light and electron microscopic anterograde tracere study. Cells Tissues Organs 175(4): 202–222.*

PROGRAM 6/2.

NEUROENDOCRINOLOGY

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Program overview

The course gives a deep insight into the structural organization and functional properties of neuroendocrine brain centres controlling the operation of the endocrine system. It presents the classical breakthroughs and well-established results of the discipline and also provides information on contemporary research trends and discoveries in the field of neuroendocrinology. Special attention is paid to the most recently developed research methods exploring the frontline topics of the field at molecular, cellular and system levels. The course focuses on the effects of neuroendocrine brain centres upon the pituitary-endocrine axes and *vice versa*, the wide scale genomic and non-genomic actions of peripheral hormones modulating the performance of the nervous system. The main topics of the course include (1) The functional neuroanatomy of the hypothalamo-hypophyseal system; (2) The organization and functional characteristics of the magno- and parvicellular neurosecretory systems; (3) The physiology of reproduction; (4) The mechanisms of thyroid hormone actions; (5) The neurobiology of stress and adaptation; (6) The central regulation of feeding and energy homeostasis; (7) Regulation of neuroendocrine rhythms and (8) The behavioral neurobiology of endocrine events. The course also focuses on the translational aspects of endocrine/neuroendocrine research, highlights inventions and novelties in the diagnosis and therapy of endocrine diseases. The course is in harmony with the educational and research mission of the International Neuroendocrine Federation.Ph.D. stu

Titles of research projects	*Supervisors*
Neuronal and hormonal control of hypothalamic regulatory mechanisms	Zsolt Liposits
Examination of the central regulatory mechanisms involved in the development of the “low T3 syndrome”	Csaba Fekete
Investigation of the molecular regulation of thyroid hormone activation in the central nervous system	Balázs Gereben
Neural mechanisms underlying abnormal aggressive behavior	József Halász
Integrating role of cannabinoids in trauma-induced behavioral deficits	József Haller
Hypothalamic integration: relationship between regulation of stress and metabolism	Krisztina Kovács
Effect of perinatal events on sensitivity to stress and on stress related behavior	Gábor Makara

Neuroendocrine, paracrine and autocrine regulatory mechanisms in the regulation of adenohypophyseal hormone secretion	György M. Nagy
Investigation of the metabolism of dopamine and norepinephrine in sympatheticaly innervated peripheral organs (like liver, spleen or salivary gland).	György M. Nagy
The role of new signaling mechanism(s) in the regulation of pituitary function	György M. Nagy
The role of new signaling mechanism(s) in the dopaminerg regulation of pituitary prolactin secretion	György M. Nagy
Investigation of the interaction between salsolinol and different addictive drugs (like amphetamine)	György M. Nagy
The role of vasopressin in young and adults in connection with stress-related psychiatric disorders (anxiety and depression)	Dóra Zelena

Ph. D. students		***Supervisors***
Boglárka Barsy	ft	József Haller
Ágnes Judit Domokos	ft	Gábor Makara
Máté Tóth	a	József Haller

Ph. D. graduates		***Supervisors***
Balázs Bali	ft	Krisztina Kovács
Ádám Dénes	ft	Krisztina Kovács
Éva Mikics	ft	József Haller
Enikő Szabó	pt	Katalin Köves
Gergely Túri	ft	Zsolt Liposits
Viktória Vereczki	ft	Katalin Köves
Gábor Wittmann	ft	Csaba Fekete

a, absolutorium; ft, full-time; pt, part-time

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

BALÁZS BALI (2006)

The role of local neurocircuitry in the inhibitory control of the hypothalamic-pituitary-adrenocortical axis and stress

Supervisor: Krisztina Kovács

Coordinated neuroendocrine, autonomic and behavioral responses to stress critically depend on a specific cell population in the hypothalamic paraventricular nucleus (PVN). Parvocellular neurons in the PVN synthesize and release corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) to activate the hypothalamo-pituitary-adrenocortical (HPA) axis during stress. There are two major inhibitory mechanisms that constrain the activity of parvocellular neurons: the hormonal feedback inhibition provided by the glu-

cocorticoids and the neuronal inhibitory drive posed by GABAergic interneurons. Aim of our study was to characterize the local component of GABAergic inhibition of the PVN by means of immuncytochemical and in situ hybridization techniques in rats, in GAD65-eGFP transgenic mice, and in organotypic slice culture in vitro. Using hypothalamic slice cultures we have revealed that (1) CRH expression in the PVN is substantially influenced by local neuronal inputs, (2) CRH transcription is action-potential dependent and responsive to the inhibitory effect of glucocorticoids, (3) expression of CRH and AVP genes is under tonic GABAergic inhibition maintained by local GABAergic interneurons in the slice. *In vivo*, this local GABAergic circuit provides an inhibitory tone to the stress-related effector neurons, and also mediates effects of limbic efferents to the PVN. We have demonstrated that (4) suspension of GABAA-mediated inhibitory tone in the PVN effectively stimulates the HPA-axis *in vivo*. In addition, (5) acute ether stress activates inhibitory neuron populations in the hypothalamus and limbic structures, and (6) GABAergic system has a capacity to reduce stress-induced activation of parvocellular neurons in the PVN. Our results reveals that local GABAergic input to the parvocellular PVN exhibits distinct neuroanatomical and functional properties allowing effective control of CRH neurosecretion. Either maintaining or suspending the GABAergic tonic inhibition have a profound effect on the transcriptional and secretional activity of the parvocellular neuron population. We propose that reduction of the GABAergic inhibitory tone substantially contributes to stress initiation, while activation of local and extrahypothalamic GABAergic circuits, along with steroid negative feedback, is involved in termination of the central stress response.

- *Bali B, Kovács KJ (2003) GABAergic control of neuropeptide gene expression in parvocellular neurons of the hypothalamic paraventricular nucleus. Eur J Neurosci 18: 1518–1526.*
- *Bali B, Erdélyi F, Szabó G, Kovács KJ (2005) Visualization of stress-responsive brain circuits in the GAD65-EGFP transgenic mice. Neurosci Lett 380: 60–65.*
- *Kovács KJ, Miklós IH, Bali B (2004) GABAergic mechanisms constraining the activity of the hypothalamo-pituitary-adrenocortical axis. Stress: current neuroendocrine and genetic approaches. Ann NY Acad Sci 1018: 466–476.*

ÁDÁM DÉNES (2007)

Transsynaptic tracing with recombinant pseudorabies virus strains and examination of the viral infection evoked inflammation in the central nervous system

Supervisor: Krisztina Kovács

Pseudorabies virus (PRV), member of the α-herpesvirus family, is capable to transneuronal spread in the rodent peripheral and central nervous system and become suitable tool for tract-tracing experiments to reveal synaptically linked neuronal circuits. After peripheral administration, the virus is taken up by the local nerve terminals and following replication, viral particles reach the central nervous system through synaptically linked neurons. We have used attenuated PRV strains for transsynaptic tract-tracing. Ba-DupGreen (BDG), expressing green fluorescent protein (GFP) and Ba-DupLac (BDL) that expresses β-galactosidase allowed us to identify the infected cells and discriminate the two recombinant virus strains. Using these virus constructs three different topics have been investigated: (1) Neuronal circuits in the central nervous system, which are involved in the autonomic

innervation of the bone marrow have been identified. Following injection of BDG into the femoral bone marrow, virus infected neurons were revealed in the sympathetic ganglia and in several autonomic-related nuclei of the spinal chord as well as in various brain sites. Dual viral tracing and control experiments confirmed that the retrogradely labeled neuronal population is identical to the central autonomic cells, which innervate the bone marrow. (2) We used dual viral tracing to reveal the central autonomic neurons that are involved in the innervation of the epididymal white and the interscapular brown adipose tissues. Our results indicate that the majority of the retrogradely labeled brain areas innervate both fat depots, but in some nuclei of the spinal cord, brain stem and hypothalamus, the innervation pattern of the two different adipose tissues is different. (3) We have investigated the inflammatory processes seen during central PRV infection. Simultaneous visualization of the immediate early expressed viral reporter protein GFP and the viral structural proteins allowed us to identify different stages of virus infection in correlation with the ongoing local inflammatory response. Microglia become rapidly activated upon virus infection, isolated infected neurons and, together with exogenous infiltrating leukocytes, prevented contact neuronal infection in the compromised brain regions. Our results contribute to understanding innate immune mechanisms during herpesvirus infections in the central nervous system.

- *Boldogköi Z, Sík A, Dénes Á, Reichart A, Toldi J, Gerendai I, Kovács KJ, Palkovits M (2004) Novel tracing paradigms—genetically engineered herpesviruses as tools for mapping functional circuits within the CNS: Present status and future prospects. Progr Neurobiol 72: 417–445.*
- *Dénes Á, Boldogköi Z, Uhereczky G, Hornyák Á, Rusvai M, Palkovits M, Kovács KJ (2005) Central autonomic control of the bone marrow: multisynaptic tract tracing by pseudorabies virus. Neuroscience 134: 947–963.*
- *Dénes Á, Boldogkői Z, Hornyák Á, Palkovits M, Kovács KJ (2006) Attenuated pseudorabies virus-evoked rapid innate immune response in the rat brain. J Neuroimmunol 180: 88– 103.*

ÉVA MIKICS (2007)

Rapid, non-genomic effects of glucocorticoids in behavior

Supervisor: József Haller

Glucocorticoids modulate brain function and subsequent behavioural responses through different mechanisms. The "classical" mechanism of action of glucocorticoids implicates an interaction with the genome, inducing slowly developing and more persistent changes in neural functions. However, it has been shown that these hormones also exert rapid non-genomic effects that are independent of protein synthesis. Data show that these rapid effects of glucocorticoids have a high impact on behavioural processes. In our experiments, the acute inhibition of glucocorticoid synthesis inhibited aggressive behaviour, while an acute corticosterone injection rapidly stimulated offensive behaviour in resident rats in a territorial setting. This effect was very rapid (it occurred within 2–7 min) and centrally mediated since it could also be elicited when corticosterone was administered directly into the brain, and cardiovascular activation during social interaction was independent of glucocorticoid background. The rapid effects on aggression were resistant to protein synthesis inhibition, suggesting the non-genomic way of action. These rapid effects

of glucocorticoids lasted less than 25 min after which a genomic way of action "overtook" the control of aggressive behaviour with the same behavioural outcome at the beginning. Under non-social, novelty related behavioural challenges (the elevated plus-maze and open-field tests) corticosterone facilitated risk-assessment behaviour rapidly, within 2–7 min. This rapid effect was also mediated by non-genomic mechanisms and was followed by a rapid genomic action (within 25 min) with the same behavioural effects. These data suggest that rapid glucocorticoid effects are strongly context dependent, i. e. in different challenging situations only specific behaviours are activated while others remain unaffected. The interaction between challenge exposure and glucocorticoid effects was studied in established colonies of rats that were not exposed to an acute social challenge. Acute corticosterone treatment without behavioural challenge induced no specific behavioural changes, suggesting that the rapid behavioural effects of glucocorticoids develop in conjunction with challenge-induced neuronal activation. Taken together, our results suggest that the very rapid effects of glucocorticoids play a crucial role in the regulation of immediate adequate behavioural responses in challenging situations.

- *Mikics E, Barsy B, Haller J (2007) The effects of glucocorticoids on aggressiveness in established colonies of rats. Psychoneuroendocrinol 32: 160–170.*
- *Mikics E, Barsy B, Barsvári B, Haller J (2005) Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and open field. Horm Behav 48: 152–162.*
- *Mikics E, Kruk MR, Haller J (2004) Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. Psychoneuroendocrinol 29: 618–635.*

ENIKŐ SZABÓ (2007)

The role of PACAP in the regulation of gonadotroph hormone secretion

Supervisor: Katalin Köves

The pituitary adenylate cyclase activating polypeptide (PACAP) was isolated from ovine hypothalamus based on its ability to activate adenylate cyclase. It is present in a number of places in the body, but it is synthesized in the greatest concentration in thehypothalamus. PACAP mRNA can be demonstrated in the anterior pituitary by PCR tecnique, but the amount of PACAP for immunostaining is enough on the day of proestrus. Numerous data reveal that PACAP plays a role in the regulation of gonadotroph hormone secretion.

In our experiments we used the cell immunoblot assay (CIBA) to show thatPACAP is secreted by anterior pituitary cells and released in vitro not only by pituitaries taken out on the day of proestrus, but also on other days of the estrous cycle *(Szabó et al. 2002, 2004)*, however the number of secreting cells in the latter stages is very low.

The amount of PACAP released by the cells is less than the amount of LH released. This is shown by the diameter of the blots formed around the cells and visualized by immunstaining. LH synthesized in a greater amount affects organs distant from the pituitary gland through the general circulation. Supposedly PACAP synthesized in a lesser amount has an auto- or paracrine effect in the anterior pituitary *(Szabó et al. 2001, Köves et al. 2003)*. The anterior pituitary cells release PACAP in a daily rhythm which is similar in male and female rats; however, the number of PACAP blots was much higher in proestrus than in other stage of estrous cyle. On the night of proestrus the number of blots increases

12–13 times. Under *in vitro* conditions PACAP added to the medium changes the LH release of gonadotroph cells. The changes depend on the time of sacrifice when the pituitaries were removed, that is on the time of day, on the gender of the animal and in females on the stage of estrous cycle. In proestrous females in the morning PACAP inhibited LH release compared to untreated cells, in the afternoon when LH surge happens *in vivo* it stimulated LH. In diestrous stage of females and in males PACAP did not significantly affect LH release of gonadotroph cells compared to untreated cells. Because the number of PACAP blots was highest in the night in cell cultures of proestrous female rats, it lets us suppose that PACAP plays a role in the terminating of the LH surge *(Szabó et al. 2004)*.

- *Szabó E, Nemeskéri Á, Heinzlmann A, Suzuki N, Arimura A, Köves K (2002) Cell immunoblot assay study demonstrating the release of PACAP from individual anterior pituitary cells of rats and the effect of PACAP on LH release. Regul Peptides 109: 75–81.*
- *Szabó E, Nemeskéri Á, Arimura A, Köves K (2004) Effect of PACAP on LH release studied by cell immunoblot assay depends on the gender, on the time of day and in female rats on the day of the estrous cycle. Regul Peptides 123: 139–145.*
- *Köves K, Vereczki V, Kausz M, Kántor O, Molnár J, Nemeskéri Á, Heinzlmann A, Szabó E, Szabó F, Fógel K, Lakatos A, Szeiffert G, Arimura A (2002) PACAP and VIP in the photoneuroendocrine system (PNES). Med Sci Monitor 8: SR5–SR20.*

GERGELY TÚRI (2008)

Implications of novel neurotransmitter systems in the regulation of gonadotropin-releasing hormone neurons

Supervisor: Zsolt Liposits

The hypothalamo-pituitary-gonadal (HPG) axis regulates the reproductive functions. The upper pole of the axis is the gonadotropin-releasing hormone (GnRH) producing neuron population located in the preoptic area (POA). The neurosecretory activity of GnRH neurons is under the influence of humoral factors and several neurotransmitter systems. The aim of this dissertation is the identification of novel neurotransmitter systems in the neuronal regulation of the GnRH neurons. (1) Acetylcholine (ACh) is able to modulate the activity of GnRH neurosecretion and sexual behavior. We have provided neuroanatomical evidence for the direct innervation of GnRH neurons by cholinergic axons in male rats *(Turi et al. 2007. Neurochem Int)*. Our results indicate that the cholinergic axons often formed direct contacts but rarely established classical synapses with the GnRH neurons. Therefore, similarly to other brain areas we propose nonsynaptic mechanisms whereby ACh modulates the functions of GnRH neurons in the POA. (2) Despite the profound central effect of neuropeptide Y (NPY) on the HPG axis, the sources of NPY afferent to GnRH neurons were unidentified. Therefore, we addressed the sources of origin of NPY-containing afferents to GnRH neurons in male GnRH-GFP transgenic mice. Our results indicate that NPY/AGRP neurons of the Arc gave rise to 49–64% of the NPY-immunoreactive axonal contacts on the somata and proximal dendrites of GnRH neurons (depending on the calculation approach we used), and an additional 25% of juxtapositions originated in adrenergic/noradrenergic cell groups of the brainstem *(Turi et al. 2003. Endocrinology 144(11): 4967–4974)*. (3) Moreover, we have revealed with electron microscopy that the AGRP-immunoreactive terminals establish symmetric synapses with GnRH neurons *(Turi*

et al. 2003. Endocrinology 144(11): 4967–4974). (4) The neurotransmitter glutamate is packed into synaptic vesicles by the three recently discovered vesicular glutamate transporters (Vglut1-3). Using the Vglut2 as a marker of glutamatergic neuronal phenotype, we have provided conclusive evidence for a marked glutamatergic phenotype of GnRH neurons in the adult male rat by demonstrating Vglut2 mRNA expression in the perikarya and Vglut2-immunoreactivity in the axons of these cells (*Hrabovszky et al. 2004. Endocrinology 145(9): 4018–4021)*. Using immuno-electron microscopy, we have demonstrated that the Vglut2-immunoreactivity is localized to small clear vesicles in the neurosecretory endings of the ME *(Hrabovszky et al. 2007. Neuroscience 144(4): 1383–1392).* The physiological significance of endogenous glutamate in the regulation of GnRH secretion requires clarification.

- *Turi GF, Liposits Z, Hrabovszky E (2008) Cholinergic afferents to gonadotropin-releasing hormone neurons of the rat. Neurochem Int 52: 723–728.*
- *Hrabovszky E, Deli L, Turi GF, Kallo I, Liposits Z (2007) Glutamatergic innervation of the hypothalamic median eminence and posterior pituitary of the rat. Neurosci 144: 1383–1392.*
- *Hrabovszky E, Turi GF, Kallo I, Liposits Z (2004) Expression of vesicular glutamate transporter-2 in gonadotropin-releasing hormone neurons of the adult male rat. Endocrinol 145: 4018–4021.*

VIKTÓRIA VERECZKI (2006)

The centrifugal visual system of rat

Supervisor: Katalin Köves

The sensory organs transmit the stimuli of the outside world towards the central nervous system. Presumably, pathways exist in the opposite direction: from the central nervous system towards the sensory organs. In bird and lower vertebrates the origin of the centrifugal visual (retinopetal) fibers are well described now, but in mammals data on the existence and the origin of the centrifugal visual fibers is contradictory. In rat, the centrifugal visual fibers may arise from the mesencephalic area; in dog and monkey the hypothalamic area was published as the origin of retinopetal fibers. Some authors query these results, claiming, that the retinopetal neurons previously reported could be the product of technical artifact due to transneuronal labeling or the leakage of some tracers. My present work utilizing the retrograde or anterograde transport of tracers (biotinylated dextran amine (BDA) and Fluorogold (FG), respectively), and light microscopic approach, I have provided direct evidence for the of origin of a novel centrifugal pathway in rat and its termination in the retina. Intravitreous injection of BDA resulted in retrogradely labeled cells in several structures: the supraoptic and paraventricular nuclei, the hippocampus (CA1, CA3), the dentate gyrus, the indusium griseum, the olfactory tubercle, and the medial habenula, all of which belong to the limbic system. I estimated that the total number of the retrogradely labeled cells was 1495±516. I have seen fiber labeling in the retinorecipient suprachiasmatic nucleus and in the primary visual center, the lateral geniculate body. However, I have never seen labeled nerve cell bodies in these structures. Iontophoretic application of FG into the hippocampal formation, where the major part of the BDA labeled cell bodies were observed, resulted in labeled fibers in the optic nerve and in the retina indicating that the retrogradely labeled cells in the hippocampus and the dentate

gyrus, among others, are the origin of the centrifugal visual fibers. Using double labeling immunohistochemistry I found that some BDA labeled cells are also VIP, PACAP or LHRH immunoreactive. I conclude that a distinct and partially VIP, PACAP or LHRH immunoreactive centrifugal pathway exists in rat and that it originates from limbic structures. I propose to call it the limboretinal pathway of rat *(Vereczki et al. 2003. Endocrine 22(3): 225–237; Vereczki et al. 2006. Neuroscience, in press)*.

- *Vereczki V, Köves K, Csáki Á, Grósz K, Hoffman GE, Fiskum G (2006) Distribution of hypothalamic, hippocampal and other limbic peptidergic neuronal cell bodies giving rise to retinopetal fibers: anterograde and retrograde tracing and neuropeptide immunohistochemical studies. Neurosci 140: 1089–1100.*
- *Vereczki V, Köves K, Tóth ZE, Baba A, Hashimoto H, Fógel K, Arimura A, Kausz M (2003) Pituitary adenylate cyclase-activating polypeptide does not colocalize with vasoactive intestinal polypeptide in the hypothalamic magnocellular nuclei and posterior pituitary of cats and rats. Endocrine 22: 225–237.*
- *Köves K, Vereczki V, Kausz M, Kántor O, Molnár J, Nemeskéri A, Heinzlmann A, Szabó E, Szabó F, Fógel K, Lakatos A, Szeiffert G, Arimura A (2002) PACAP and VIP in the photoneuroendocrine system (PNES). Med Sci Monit 8: SR5–SR20.*

GÁBOR WITTMANN (2008)

Elucidation of feeding-related neuronal networks involved in the regulation of the hypophysiotropic thyrotropin-releasing hormone- and corticotropin-releasing hormone-synthesizing neurons in the rat

Supervisor: Csaba Fekete

Hypophysiotropic thyrotropin-releasing hormone (TRH)- and corticotropin-releasing hormone (CRH)-synthesizing neurons in the hypothalamic paraventricular nucleus (PVN) play important roles in the regulation of energy homeostasis through the regulation of the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenocortical axes, respectively. During the period of fasting, both TRH and CRH syntheses are inhibited, mainly through neural inputs arising from orexigenic neuropeptide Y (NPY)- and agouti-related protein (AGRP)-synthesizing neurons and anorexigenic α-melanocyte-stimulating hormone (α-MSH)- and cocaine-and-amphetamine-regulated transcript (CART)-producing neurons of the arcuate nucleus. Previous morphological observations suggested that the arcuate nucleus is not the exclusive source of the dense NPY and CART innervation of the TRH and CRH neurons. We conducted a series of experiments to reveal the sources of NPY and CART innervation of TRH and CRH neurons, using retrograde tract-tracing, multiple-labeling immunocytochemistry and combined fluorescent in situ hybridization and immunofluorescence. We revealed that about 27% of the NPY innervation of TRH neurons arises from medullary adrenergic neurons. We identified the CART-producing cell groups that innervate the PVN: these included CART neurons of the arcuate nucleus, the zona incerta and the lateral hypothalamus, medullary adrenergic neurons in the C1–3 regions, and the nucleus of the solitary tract *(Fekete et al. 2004. J Comp Neurol 469: 340–350)*. TRH neurons were found to receive 44% of their CART-immunoreactive (IR) innervation from adrenergic neurons *(Wittmann et al. 2004. Brain Res 1006: 1–7)*. The CART innervation of CRH neurons consisted of adrenergic CART fibers (60%), α-MSH/

CART fibers (18%) and CART-IR axons from other sources (22%) *(Wittmann et al. 2005. Endocrinol 146: 2985–2991)*. To reveal whether NPY or CART expression in adrenergic neurons is altered in fasting and may contribute to the suppression of TRH and CRH synthesis, we performed quantitative *in situ* hybridization for NPY and CART in the medulla. Except for a small reduction in CART mRNA level in the C1 cell group, we did not detect any changes in NPY and CART mRNA in the C1–3 regions, suggesting that these peptides mediate other stimuli on TRH and CRH neurons. We also revealed that axons containing the orexigenic peptide galanin densely innervate TRH neurons, while only a very small fraction of TRH neurons receives innervation from galanin-like peptide (GALP)-containing axons. Our data indicate that TRH and CRH neurons are innervated by axons containing feeding-related peptides that arise from multiple sources and diferentially regulate these hypophysiotropic neurons.

- *Wittmann G, Liposits Z, Lechan RM, Fekete C (2002) Medullary adrenergic neurons contribute to the neuropeptide Y-ergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the rat. Neurosci Lett 324: 690–732.*
- *Fekete C, Wittmann G, Liposits Z, Lechan RM (2004) Origin of cocaine- and amphetamine-regulated transcript (CART)- immunoreactive innervation of the hypothalamic paraventricular nucleus. J Comp Neurol 469: 340–350.*
- *Sarkar S, Wittmann G, Fekete C, Lechan RM (2004) Central administration of cocaine- and amphetamine-regulated transcript increases phosphorylation of cAMP response element binding protein in corticotropin-releasing hormone-producing neurons but not in prothyrotropin releasing hormone-producing neurons in the hypothalamic paraventricular nucleus. Brain Res 999: 181–192.*

PROGRAM 6/3.

FUNCTIONAL NEUROSCIENCE

Coordinator:
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Program overview

The doctoral program mainly covers training and research in the application of neuropharmacological, neurochemical and neurophysiological methods to approach the function of neuronal networks mainly from the functional point of view. The major direction of neuropharmacological research is the study of the non-synaptic model of the brain, which not only defines a new mechanism of chemical transmission of nerve impulses but also explains the mechanism of action of some medications with effect on the central nervous system, and may also suggest new targets for the treatment of neu-

ropsychiatric disorders. A further research priority is the study of the less well known connection between the nervous system and the immune system, and the identification of new neurotransmitters and modulators in the central nervous system. The neurochemical research focuses on pathological processes following hypoxia and oxidative stress in the neurons of the central nervous system, with major interest in Na^+ and Ca^{2+} homeostasis, in *in situ* mitochondrial function and in changes of excitability. Neurophysiological studies are performed mostly in the area of cognitive psychophysiology, and aim to unsderstand the central nervous system mechanisms of higher level neuronal functions with the analysis of event related cerebral potential changes.

Titles of research projects	***Supervisors***
Function of ion channels and ionotropic receptors as revealed by nonlinear optical imaging and electrophysiological methods	Szilveszter E. Vizi
Nonsynaptic action mechanisms of neuroactive substances involved in chemical transmission in animal and in isolated human (obtained from surgical biopsy) tissues	Szilveszter E. Vizi
Models of brain diseases established by drug-induced alterations in neural networks of the peripherial and central nervous system	Szilveszter E. Vizi
Mechanisms in neurodegeneration and neuroprotection	Veronika Ádám
Investigation of synchronous neuronal activities using *in vitro* electrophysiological techniques	Norbert Hájos
Non-conventional effects of monoamine uptake blocker-type antidepressants in the central nervous system	János Kiss
A possible role of nitric oxide in the regulation of neurotransmission: study of the glutamate-monoamine interaction	János Kiss
Role of receptors and ion-channels in the integrative functions of neurons: spatial and temporal patterns of calcium signaling and membrane potential changes	Balázs Lendvai
Drugs affecting ion channels: Molecular action mechanisms	Árpád Mike
The role of ATP- and adenosine-mediated signalling in the nervous system	Beáta Sperlágh
Role of GABA signaling in the regulation of embryonic development	Gábor Szabó
Impact of local microanatomy on the morphology and hemodynamics of aneurysms on the circle of Willis	István Szikora
Electrophysiological analysis of evoked, spontaneous oscillations and pathological signs	István Ulbert
Mechanisms of cellular damage in the central nervous- and sensory systems; potential pharmacological targets	Tivadar Zelles
Development of 3 dimensional (3D), real-time, two-photon microscopes and new methods to measure 3 dimensional signal integration in neurons and dendrites	Balázs Rózsa

Ph. D. students		***Supervisors***
Richard Csercsa	ft	István Ulbert
Cecília Csölle	ft	Beáta Sperlágh
Balázs György Hangya	ft	Zsolt Borhegyi
Attila Heinrich	pt	Beáta Sperlágh

Tamás Horváth	pt	Szilveszter E. Vizi
Attila Kaszás	ft	Szilveszter E. Vizi
Zsófia Komáry	pt	Veronika Ádám
Ildikó Kondorosi	pt	Szilveszter E. Vizi
Nóra Lenkey	ft	Árpád Mike
Péter Lukács	ft	Árpád Mike
Miklós Mándi	pt	Veronika Ádám
Orsolya Papp	ft	Norbert Hájos
Gergely Szalay	ft	Balázs Rózsa

Ph. D. candidates		***Supervisors***
Marija Svirtlich		Gábor Szabó
Tamás Füzesi	ft	Csaba Fekete
Andrea Heinzlmann	pt	Katalin Köves
Judit Menyhért	ft	Csaba Fekete
Márk Oláh	ft	György Nagy
Flóra Szabó	pt	Katalin Köves
Dániel Székács	ft	György Nagy

Ph. D. graduates		***Supervisors***
Albert Miklós Barth	ft	Balázs Lendvai
Péter Lakatos	pt	György Karmos
Péter Mandl	pt	Szilveszter E. Vizi
Csaba Rajkai	i	György Karmos
Balázs Rózsa	i	Szilveszter E. Vizi
Zsolt Selmeczy	pt	Szilveszter E. Vizi
Zoltán Somogyvári	i	Péter Érdi
Bernadett Szász		Szilveszter E. Vizi, János Kiss

f, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2005, 2007 and 2008

ALBERT MIKLÓS BARTH (2008)

Noradrenergetic influence on pyramidal cells in the prefrontal cortex: A two-photon laser scanning microscopy study

Supervisor: Balázs Lendvai

There is increasing number of evidence in the literature that noradrenaline as well as dopamine are key elements of prefrontal cortical function. In behavioural experiments moderate levels of noradrenaline improve working memory related to prefrontal cortex via actions at post-synaptic α_{2A}-adrenoceptors, while high levels of noradrenaline release

during stress impair prefrontal cortical functions via α_1- and possibly β1-adrenoceptors with lower affinity for noradrenaline. In our experiments we investigated the possible cellular mechanism underlying the noradrenergic action on working memory.
We used two-photon microscopy and patch clamp technique and demonstrated that high-frequency trains of backpropagating action potentials avoided filtering in the apical dendrite and initiated dendritic spikes in layer 5 pyramidal neurons of the prefrontal cortex. The block of hyperpolarization-activated currents by ZD 7288 could shift the frequency threshold and decreased the number of action potentials required to produce dendritic spikes. Activation of α_2-adrenergic receptors could also shift the frequency domain of spike induction to lower frequencies. Our data suggest that noradrenergic activity in the prefrontal cortex influences dendritic Ih and lowers the threshold of dendritic spikes in the apical dendrite via α_2-adrenergic receptors.
Dendritic spikes are key factors in the generation of action potential bursts which may represent a reliable neural signal through unreliable yet facilitating synapses thereby contributing to the synaptic reverberation in the prefrontal circuits. This mechanism might be one cellular correlate of the α_2-receptors-mediated improving actions on working memory.
We also investigated the noradrenergic effect on the somatobasal compartment of layer 5 pyramidal cells in the prefrontal cortex. Noradrenaline, applied in the bath, increased the number of evoked action potentials following current injection to the soma. In the basal dendrites the evoked Ca^{2+} responses were also markedly enhanced. Noradrenaline-induced effects could be blocked by the β-adrenergic blocker propranolol.

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PÉTER LAKATOS (2005)

Behaviour dependent changes of auditory event related potentials and gamma activity in the auditory cortex of cat

Supervisor: György Karmos

Our everyday environment contains numerous objects that would be impossible to process simultaneously. Selective attention and arousal enables us to reduce the complexity of this environment by extracting behaviorally relevant details. The purpose of the present study was to separate the differential effects of attention and arousal on components of the auditory event related potential and gamma activity, recorded with epidural electrodes placed over the auditory cortex of cat.
The animals performed a simple instrumental alimentary conditioning task with different modality (visual and auditory) conditioned stimuli (CS). Even though there is no selective attention without arousal, the opposite can be achieved in sensory systems by directing attention towards a different modality. Using a given modality CS in an experimental ses-

sion, the animal's attention was selectively directed towards either auditory or visual modality cues, which yields "attend auditory" and "do not attend auditory" conditions.
The amplitude of the auditory evoked components increased only when the cats performed auditory conditioning, while the latency of the evoked components during conditioning decreased compared to the resting state independent of the modality of the conditioned stimulus.
One of the most important findings of this study is that spontaneous gamma activity is not a stationary phenomenon, but rather consists of short, periodic, spindle-shaped oscillations that we call gamma bursts. The power of the gamma oscillations showed a uniform increase in the case when the cats performed auditory conditioning, and no change when the cats had to attend visual conditioned stimuli. The frequency of gamma oscillations increased during conditioning when compared to the resting state.
Our results show that the amplitude of the event related potential components and the power of gamma activity increased in a modality specific manner, thus they are related to attention. The non-modality specific latency changes of the evoked components and frequency changes of gamma activity are related to arousal level changes.

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PÉTER MANDL (2007)

The effects of antidepressants on nicotinic acetylcholine receptors of the central and peripheral nervous system

Supervisor: Szilveszter E. Vizi

Most of the commonly used antidepressants achieve their antidepressant effects by increasing the levels of monoamine neurotransmitters. In recent years several studies on antidepressants have highlighted certain effects produced by these drugs that are unrelated or indirectly related to monoamine neurotransmission. This work focuses on the effects of antidepressants on the nicotinic acetylcholine receptors (nAChRs) located in the central, and peripheral nervous system, more specifically in the enteric nervous system.
Following its morphologic confirmation, we were succesful at providing functional evidence of the existence of the presynaptic nAChR on the terminal region of myenteric motoneurons in the enteric nervous system. Based on our experiments we assume that, through its participation in a positive feedback cycle, the presynaptic nAChR enhances the release of acetylcholine (ACh) in the enteric nervous system.
We recognized and confirmed that hemicholinium-3 (HC-3), a choline-transporter blocker routinely used in ACh release experiments is capable of inhibiting the activation of the presynaptic nAChR, by directly blocking the receptor. In accordance with our earlier findings in the central nervous system, the three investigated antidepressants fluoxetine, citalopram and desipramine had a similar, significant and clinically relevant attenuating

effect on ACh release through their inhibitory effect on the presynaptic nAChRs of the enteric nervous system. Through the parallel measurement of nicotine-evoked 5-HT and ACh release in *in vivo* microdialysis experiments, we affirmed the inhibitory effect of citalopram on nAChR in the central nervous system that was shown in previous *in vitro* experiments performed by our workgroup. Our results indicate that while the nAChR-inhibiting effect of antidepressants in the central nervous system could play a role in their net therapeutic effect, however the same effect in the preiphery may be responsible for the development of a certain common, gastrointestinal adverse effects, obstipation.

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- *Döme P, Rihmer Z, Gonda X, Pestality P, Kovács G, Teleki Z, Mandl P (2005) Cigarette smoking and psychiatric disorders in Hungary. Int J Psychiat Clin 9: 145–148.*
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CSABA RAJKAI (2007)

Electrophysiological study of the role of visual fixation in the modulation of visual cortex activity

Supervisor: György Karmos

In natural vision, humans and primates actively examine the visual world by rapidly shifting their gaze (fixation) from element-to-element in a scene. Nevertheless, the regular approach of studying visual system of primates is presenting visual stimuli in front of the animals with fixed gaze, hereby might ignoring important modulation effects associated with eye movements. To better understand the dynamics of natural vision, we examined the effects of changes in visual fixation on ongoing activity.

We recorded field potentials and concomitant multiunit action potentials (MUA) with a linear array multicontact electrode from five visual areas both subcortical (LGN) and cortical (V1, V2, MT, IT) from 4 macaques. Recordings were made both in total dark and during red strobe flash stimulation. One-dimensional current source density (CSD) profiles were calculated from local field potentials and data averaging based on both the onset and offset of saccade and the onset of stimulus. MUA and CSD measurements address neuronal firing patterns and underlying synaptic processes, respectively.

We found that in all areas both MUA and CSD show suppression before the onset of eye movement and enhancement after the fixation of gaze in the dark, furthermore the suppression in current flow is nonphase-locked to the eye movement. The onset and peak latencies of the positive peak (enhancement) of saccade evoked CSD through visual areas are between 25–65 ms and 100–180 ms, respectively and show similar pattern than the stimulus evoked CSD (i.e. earliest in LGN and latest in IT). Furthermore, the post-saccadic CSD shows a phase concentration compared to the pre-saccadic CSD. The oscillatory phase associated with the onset of fixation (in the absence of stimulation) is not discriminably different from the ideal phase, that at which maximal visual evoked responses occur. The ideal phase does appear to reflect a high excitability state in local cortical neu-

rons. Overall, these findings are consistent with the hypothesis that the onset of fixation is associated with an increase in cortical excitability (Fixation Amplifier Hypothesis). Fixation effects reflect the ability of the brain's gaze control systems to predictively prepare the visual system for a temporal pattern of visual input that is a straightforward consequence of the way in which the eyes are used to actively sample the visual environment.

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- *Lakatos P, Szilágyi N, Pincze Z, Rajkai C, Ulbert I, Karmos (2004) Attention and arousal related modulation of spontaneous gamma-activity in the auditory cortex of the cat. Cog Brain Res 19: 1–7.*
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BALÁZS RÓZSA (2007)

Two-photon measurement of dendritic Ca^{2+} signaling in stratum radiatum interneurons

Supervisor: Szilveszter E. Vizi

We transferred and improved the technology of modular nonlinear microscopes. Among other innovations, we invented a real-time, non-linear microscope system applicable—both in *in vivo* and in *in vitro* applications—to make measurements and/or to perform photochemical stimulations in a three-dimensional space (in a maximal volume of at least 800 μm x 800 μm x 200 μm), at high speed (even with a resolution time of >1 kHz), providing a high spatial resolution characteristic of the scanning two-photon absorption fluorescence microscopy, wherein the number of the measuring points can reach 100 *(Rozsa et al. 2007)*.

Although interactions between backpropagating action potentials and synaptic stimulations have been extensively studied in pyramidal neurons, dendritic propagation and summation of these signals in interneurons is not as well known. We used two-photon imaging to explore the basic properties of dendritic Ca^{2+} signaling in CA1 stratum radiatum interneurons. In contrast to hippocampal pyramidal neurons, backpropagating action potential-evoked Ca^{2+} transients in dendrites of interneurons underwent distance-dependent increment. Ca^{2+} responses in interneurons had smaller amplitude, slower rise time and decay than in pyramidal neurons. To explore the factors underlying the difference, we compared the Ca^{2+} binding capacity in interneurons and in pyramidal neurons. Our finding that endogenous Ca^{2+} buffers proved to have a higher level in interneurons may largely explain the different kinetics and amplitudes of Ca^{2+} transients. Synaptic stimulation-evoked Ca^{2+} transients were also larger at distant dendritic locations. Spread of these signals was restricted to 6–7 μm-long dendritic compartments *(Rozsa et al. 2004)*.

In contrast to previous physiological studies, we found that functional a7-nAChR are abundant on dendrites of CA1 stratum radiatum interneurons, and that Ca^{2+} transients elicited by these receptors underwent distance-dependent increment. These nonsynaptic receptors, which are localized (>93 %) at glutamatergic and GABAergic synapses, can facilitate or depress synaptic transmission and bAPs, depending on the timing of nAChR activation, and may modulate the recently described plasticity of interneurons *(Vizi et al.*

2004). In fact, we provide a potential alternative for muscarinic modulation of theta activity, which is slow, suggesting a new mechanism for a cholinergic switch in memory encoding and retrieval on the time scale of seconds, or even with theta frequency.

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- *Vizi ES, Rozsa B, Mayer A, Kiss JP, Zelles T, Lendvai B (2004) Further evidence for the functional role of nonsynaptic nicotinic acetylcholine receptors. Eur J Pharmacol 500: 499–508.*

ZSOLT SELMECZY (2007)

The effect of the sympathetic nervous system on the production of mediators regulating inflammatory processes

Supervisor: Szilveszter E. Vizi

During the *in vivo* study of the effect of the sympathetic nervous system on the production of mediators regulating inflammatory processes, we showed that either genetical absence or pharmacological blocking of the noradrenaline (NA) transporter (NET) by desipramine enhanced LPS-induced production of TNF-α, while LPS-induced production of IL-10 decreased in NET-KO mice, in contrast with the increase found in desipramine-treated wild type animals. Moreover, desipramine proved to be effective in the modulation of the production of both cytokines in NET-KO animals, in a similar manner as in wild type animals. However, β_2- and β-adrenoceptors (βAR), both essential in the regulation of the effect of NA, retained their integrity in NET-KO mice.

In *in vitro* experiments, non-differentiated (ND), granulocytic-differentiated (by dimethylformamide), and macrophagic-differentiated (by D3-vitamin) cells of PLB-985 cell line showed a differentiation-dependent reactivity for inductors applied during the experiments, which was in connection with the expression of the cell surface receptors instrumental in the recognition of these inductors. Besides, we found that the direction of sympathetic modulation can also change during differentiation, since βAR agonist isoporterenol blocked PMA-induced production of TNF-α in ND cells, while increased it in differentiated ones. This alteration was in parallel with the changes of amount of pERK taking part in the regulation of TNF-α production.

Based on our results we may conclude that the biophase concentration of NA and the duration of its biophase presence are important regulators of LPS-induced cytokine response, therefore monoamine transporters also have an important immunregulating role. Our further important finding was that the sensitivity of cells for different inductors and modulating effects changed during differentiation, which could result in complete change of direction in the case of the latter one. This is in connection with changing of the expression pattern of receptors having a crucial role in the recognition of inductors and/or changing of intracellular signalling pathways. These observations may have important effects on the therapy of immunological diseases.

- *Szelényi J, Kiss PJ, Puskás É, Selmeczy Zs, Szelényi M, Vizi ES (2000) Opposite role of α_2- and β-adrenoceptors in the modulation of interleukin-10 production in endotoxaemic mice. Neuroreport 11: 3565–3568.*
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ZOLTÁN SOMOGYVÁRI (2007)

Model based analysis of the cortical dynamics: from single cells to networks

Supervisor: Péter Érdi

A new model-based analysis method was set up for revealing information encrypted in extracellular spatial potential patterns of neocortical action potentials. Spikes were measured by extracellular linear multiple microelectrode in vivo cat's primary auditory cortex and were analyzed based on current source density (CSD) distribution models. Validity of the monopole and other point source approximations were tested on the measured potential patterns by numerical fitting.

We have found, that point source models could not provide accurate description of the measured patterns. We introduced a new model of the CSD distribution on a spiking cell, called countercurrent model. This new model was shown to provide better description of the spatial current CSD of the cell during the initial negative deflection of the extracellular action potential. The new model was tested on simulated extracellular potentials. We proved numerically, that all the parameters of the model could be determined accurately based on measurements. Due to model fitting, CSD could be calculated with much higher accuracy as done with the traditional method because distance dependence of the spatial potential patterns was explicitly taken into consideration in our method. Average CSD distribution of the neocortical action potentials was calculated and spatial decay constant of the dendritic trees was determined by applying our new method.

The slow dynamics of epileptic seizures is studied by combining *in vivo* electrophysiology, data analysis by using wavelet transformation and neural network modeling. Even a skeleton model with simple neurodynamics was able to reproduce many characteristics of the epileptic seizure.

Then, *in vitro* experiments, interictal epileptiform activity elicited by 4-aminopiridine, bicuculline or Mg^{2+}-free solution was recorded with a 16-channel multielectrode assembly in different layers of the somatosensory cortex, and classical CSDs were calculated. The characteristics of the CSD activation patterns were determined. Finally, an approximate calculation of the length of state cycles in random Boolean networks, as a function of the size and connectivity of the network was presented. Using an annealed approximation we derive a recursive formula for the length of steps of the system. We have compared the analytical results with Monte Carlo simulations.

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BERNADETT SZÁSZ (2007)

Non-conventional modulation of monoamine transporters and ion channels

Supervisor: Szilveszter Vizi E., János Kiss

This thesis focuses on the pharmacological similarities between monoamine transporters and ion channels. Our results suggest that certain nicotinic agonists (DMPP, lobeline) are able to influence the function of monoamine transporters, unlike classical nicotinic agonists. Lobeline increased the noradrenalin release through the noradrenalin transporter in the hippocampus, although in the process of the transmitter release the nAChR-mediated part was not detectable. In addition to the stimulation of nAChR, DMPP also had a significant effect on the monoamine transporters, and evoked a sustained release of monoamines through the reversal of the monoamine transporters.
Conversely, drugs modulating the function of monoamine transporters, also influence the function of ionotropic receptors and ion channels. This idea is also supported by that, the selective dopamine transporter blocker GBR-12909, besides the selective noradrenaline and serotonin transporter blockers, also inhibited the function of nAChRs. On the other hand, the atypical antidepressant tianeptine, which enhances the uptake of serotonin also showed nAChR antagonism, confirming the nAChRtheory of depression.
Our results, that the tricyclic desipramin and the SSRI fluoxetine, besides inhibiting nAChRs, are also able to inhibit NMDA receptors and Na^+ channels, and that the nicotinic agonist DMPP also influence the mechanism of monoamine transporters, illustrate the pharmacological similarity between monoamine transporters, nAChRs, NMDA receptors and Na^+ channels. The direct inhibition of ionotopic receptors and ion channels by antidepressants with different type, chemical stucture and selectivity, highlights the possible role of ion channel inhibition in the mechanism of action of antidepressants, leading to a better understanding of the neurochemical background of depression.

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PROGRAM 6/4.

CLINICAL NEUROSCIENCE

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Program overview

The Clinical Neuroscience Program within the Neuroscience Doctoral School focuses on the research of mechanisms of neuropsychiatric disorders. Laboratories in the program apply cell biological, molecular biological, pharmacological and electrophysiological methods involving Ph. D. students. Of the three research groups the vascular neurological group (head Prof. Dr. Zoltán Nagy) performs studies using permanent or transient ischemic animal models and PC12 and endothelial cell cultures applying cell biological and neuronal apoptotical studies. The epilepsy group (head: Prof. Dr. Péter Halász) studies the mechanism of spike-and-wave epilepsy, the relationship of sleep and epilepsy, and the relationship between epileptic mechanisms and cognitive function, the temporal spike activity and memory. The pharmacological group (head: Prof. Dr. György Bagdy) studies the pharmacology of serotonine, and the relationship between serotonine and sleep disorders. Research groups from the 2nd Department of Pediatrics and the National Therapeutic Center (professors Rozália Kálmánchey and Csaba Nyakas) also participate in the program.

Titles of research projects	***Supervisors***
Regulation of sleep and circadian rhythm by neurotransmitters, neuropeptides and their receptors	György Bagdy
Video-EEG analysis of epilepsies in infancy	Rozália Kálmánchey
Functional outcome of brain tumours in childhood	Rozália Kálmánchey
Neurophysiological and functional neuroimaging approach of speech disturbances in childhood	Rozália Kálmánchey
The introduction of genome systems biology research into the diagnostic, prevention and therapy of neurological and psychiatric disorders	Mária Judit Molnár
Physical activity and brain aging: effects of vascular and neurotrophic factors (animal studies)	Csaba Nyakas
GAP-43 signal and/or regulator protein in post-ischemic brain plasticity	Zoltán Nagy
Brain monitoring by 128 channels EEG after ischemic stroke	Zoltán Nagy

Ph. D. students		***Supervisors***
László Entz	ft	Péter Halász
Gabriella Inczédy-Farkas	ft	Mária Judit Molnár
István Kapás	pt	Gábor Kovács
Zita Klára Kátai	ft	György Bagdy
Tamás Kitka	ft	György Bagdy
Klára Pentelényi Roszkos	ft	Mária Judit Molnár
Léna Szabó	ft	Rozália Kálmánchey

Ph. D. candidates		***Supervisors***
Endre Pongrácz	i	Zoltán Nagy
Zsanett Tárnok	ft	Péter Halász
Rómeó Andó	pt	György Bagdy
Lóránd Erőss	i	Péter Halász
Anikó Gál	ft	Zoltán Nagy
Eszter Kirilly	ft	György Bagdy
Csaba Óváry	i	Zoltán Nagy
György Rásonyi	i	Péter Halász
Nóra Sziray	pt	György Lévay
Edina Amália Wappler	ft	Zoltán Nagy

Ph. D. graduates		***Supervisors***
Brigitta Balogh	pt	György Bagdy
Tamás Benjamin Bereznai	i	Mária Judit Molnár
Dániel Fabó	ft	Péter Halász
Rita Jakus	ft	György Bagdy
Márton Graf	pt	György Bagdy
László Simon	ft	Zoltán Nagy
Géza Szilágyi	pt	Zoltán Nagy

f, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

BRIGITTA BALOGH (2008)

Effects of MDMA on vigilance and pharmacological consequences of MDMA neurotoxicity

Supervisor: György Bagdy

The recreational drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) has become increasingly used by young people all over the world. Despite the well documented acute neurochemical and long-term serotonergic neurotoxical actions of MDMA, short- and long-term effects on sleep-wake cycle have not been extensively explored. No information

is available on the acute sleep effects of a second MDMA dose on MDMA-pretreated animals and the effect of the selective serotonin reuptake inhibitor citalopram on sleep patterns has not been explored under these conditions either.

Our aim was to assess possible functional changes in the 5-HT system following the administration of a single dose of MDMA. In our experiments we used Dark Agouti rats which provide a model for the genetically-defined, poor metabolizer human sub-population in which clinical complications of MDMA may be more likely to occur. We examined the effects of MDMA on motor activity and sleep-wake cycle up to 4 weeks after treatment in drug-naive rats and studied the acute effects on rats exposed to the drug 3 weeks earlier. Additionally we compared the acute effects of citalopram on sleep patterns in drug-naive rats and in rats previously exposed to MDMA. Furthermore, in parallel groups of animals, we quantified axonal damage by measuring (3H)-paroxetine binding in motor areas and the occipital cortex 3 weeks after MDMA treatment.

In drug-naive rats, MDMA initially increased motor activity and the awake period and caused longer inhibition of REM (rapid eye movement) sleep than that of slow wave sleep. 10–12 hours later adverse subacute effects such as decrease in motor activity and increase in sleep followed the acute effects. Three days after MDMA treatment motor activity was reduced mainly during dark (active) phase. Furthermore, MDMA increased wakefulness and reduced REM-sleep 5 days after treatment. Circadian patterns of motor activity and sleep/vigilance parameters were also disturbed 14 and 28 days after treatment. An increase in the length of REM was observed early in the sleep cycle 3 weeks after MDMA treatment. Significant reductions in [3H]paroxetine binding in the occipital cortex of MDMA-treated animals provided evidence for axonal damage, although no significant change was observed in motor areas. In rats exposed to MDMA 3 weeks earlier, acute effects induced by MDMA on motor activity and vigilance had a shorter duration. Both groups of animals exhibited a decrease in REM following citalopram treatment, although the effects seen in rats previously exposed to MDMA were attenuated compared to drug-naive animals. Furthermore citalopram did not increase passive wakefulness in rats exposed to MDMA 3 weeks earlier.

In conclusion, our findings provide evidence that even a single dose of MDMA can result in long-term changes in the regulation of circadian rhythms, motor activity and sleep generation, highlighting the potential dangers of human ecstasy-use.

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- *Kirilly E, Molnar E, Balogh B, Kantor S, Hansson SR, Palkovits M, Bagdy G (2008) Decrease in REM latency and changes in sleep quality parallel serotonergic damage after MDMA: a longitudinal study over 180 days. Int J Neuropsychopharmacol 8: 1–15.*

TAMÁS BENJAMIN BEREZNAI (2008)

New results on the fields of genetics and therapy of neurodegenerative disorders

Supervisor: Mária Judit Molnár

We report about our results in the most expanding discipline of neuroscience, from the field of neurogenetics and from interventional neurosurgery, exacting from deep brain stimulation used for the treatment of dystonia. The purpose of our scientific work was to determine the genetic background of neurodegenerative diseases with heredity transmission, Parkinson, dystonia and ALS, furthermore to investigate the correlation between phenotype and genotype of these entities and to investigate the therapeutic effect of deep brain stimulation in different types of dystonia.
(1) Mutations at the PARK1 locus in the European Caucasoid population are probably a rare cause of autosomal-dominant parkinsonism, therefore it is not recommended to look for this gene in the first-line routine diagnostics.
(2) We describe a new genetic locus that appears to be involved in the development of parkinsonism. This locus, PARK3, was detected in a group of families of European origin.
(3) We performed mutation analysis on Caucasoid families with at least two affected sibs. As we did not detect any mutation in the UCH-L1 (PARK5) and NR4A2 gene we conclude that this genes are not responsible for familial PD.
(4) A large family with "myoclonic dystonia with lightning jerks responsive to alcohol" was identified. By linkage analysis the candidate genes DYT1, GABA A receptor subunits and glycine receptor subunits could be excluded as the disease gene in this family.
(5) We describe a family with 5 clinically affected individuals carrying the DYT1 mutation presented as a dystonic writers cramp without progression to a generalised form of dystonia. In contrast to this phenotype we also describe two brothers with the same genotype (3 bp deletion in exon 5 of DYT1 gene) and a progressive course starting with focal dystonia and generalisation over a period of 6 to 10 years.
(6) The results of deep brain stimulation (DBS) of the Gpi in six patients with generalised, focal and segmental dystonia are presented. Clinical symptoms were evaluated before and after surgery using rating scales and SF-36 Health Survey for health status. We conclude that chronic high-frequency Gpi stimulation in different types of dystonia is an effective and safe treatment.
(7) We report on a pedigree with autosomal dominant ALS and a novel T to A missense mutation in exon 1 of the SOD1 gene. This novel mutation can contribute for understanding the disease mechanism in the future.

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DÁNIEL FABÓ (2008)

Properties of spontaneous and evoked discharges in the human subiculum

Supervisor: Péter Halász

Abundant data are available on the properties of neuronal microcircuits of mesial temporal lobe epilepsy (mTLE) from examinations of animal models including several studies using laminar multi-electrodes (ME). The activity of neuronal networks underlying human mTLE is however poorly understood. Recently emerging evidences support the idea that the subiculum (Sub) plays an important role in the epileptic human hippocampal formation. Currently high resolution MEs had been developed for local field potential (LFP), current source density (CSD), and multiple unit activity (MUA) measurements in humans.

Our aims were to adapt the existing ME system with which we were able to do LFP, CSD and MUA measurements in the Sub of mTLE patients during the temporal lobectomy in general anaesthesia. Using the adopted system we examined the spontaneous interictal spikes (IIS), electrically evoked potentials (EP) and after-discharges (AD), with special attention to its laminar organization, spectral properties and synchrony.

Based on our results we concluded that the co-registration of local electrical activity and the cell layers visualized by the histological processing of the tissue can be carried out in the Sub. The Sub was active under general anaesthesia and reflected in IISs with high intra-regional synchrony. We distinguished between two major types of IIS that agreed the well known spike and wave morphology. The more common type 1 originated in the somatic, the less frequent type 2 in the dendritic layer of the Sub.

The EPs and ADs in the Sub were generated in the somatic layer also, but reflected more complex spatio-temporal pattern than the IISs.

Occasionally the EPs appeared with less than 5 ms delay after the stimulus in the Sub that suggested mono-synaptic link between the temporo-basal neocortical areas and the Sub. The potentials fitting the activation of the perforant pathways occurred later with peak latency between 9 and 22 ms. Not only the amplitude, onset latency of the EPs depended on the site and strength of the stimulus, but the laminar organization also does. This suggests multiple functional pathways to the Sub.

Prominent ripple and fast ripple activity was attached to the IIS, EP and AD with increasing power in this order. Both type of ripples had intrasubicular generators.

- *Fabó D, Maglóczky Zs, Wittner L, Pék Á, Erőss L, Czirják S, Vajda J, Sólyom A, Rásonyi Gy, Szűcs A, Kelemen A, Juhos V, Grand L, Dombovári B, Halász P, Freund TF, Halgren E, Karmos Gy, Ulbert I (2008) Properties of in vivo interictal spike generation in the human subiculum. Brain 131: 485–499.*
- *Ulbert I, Magloczky Z, Eross L, Czirjak S, Vajda J, Bognar L, Toth S, Szabo Z, Halasz P, Fabo D, Halgren E, Freund TF, Karmos G (2004) In vivo laminar electrophysiology co-registered with histology in the hippocampus of patients with temporal lobe epilepsy. Exp Neurol 187: 310–318.*
- *Kelemen A, Rasonyi G, Szucs A, Fabo D, Halasz P (2006) A temporalis epilepszia műtétek kimenetelét előre jelző tényezők vizsgálata. Ideggyógy Sz 59: 353–359.*

RITA JAKUS (2006)

Generation of spike wave discharges in absence epilepsy: modulatory effects of serotonin, glutamate and glycine

Supervisor: György Bagdy

Idiopathic generalized epilepsies are a developmentally, neurophysiologically and pharmacologically unique group of epilepsy syndromes. Evidence suggests that the basic mechanisms underlying the bilaterally synchronous spike-wave discharge (SWD) burst, a common electrophysiological (EEG) marker that characterizes these seizure types, are related to the thalamo-cortical (TCR) network. In the last decade, the thalamic rhythmogenic mechanisms responsible for sleep spindles and SWDs have been intensively investigated leading to a better understanding of their anatomical-physiological substrate. It has been shown that the aberrant developmental shift in the balance of NMDA-mediated excitation and/or GABAB-mediated inhibition turns the TCR circuitry in favour of synchronization, "burst firing" mode and consequently to SWD. Further studies investigating the neurotransmitters and their receptors that modulate excitation and inhibition in TCR pathways may help to understand the control mechanisms of the SWD expression.

In the thesis we examined the role of two 5-HT receptors, 5-HT2C and 5-HT7, AMPA receptors and glycin-transporter-1 in the generation of SWDs in the accepted rat model of human absence epilepsy. The results provide further evidence that in addition to the known GABA-ergic, glutamatergic and dopaminergic control, serotonergic mechanisms also play an important role in the triggering and maintenance of epileptic activity, demonstrating that serotonin play a dual effect in absence epilepsy: activation of 5-HT2C receptors by receptor agonists or by increase in endogenous 5-HT concentration inhibit SWD, although this inhibitory effect is not significant at basal 5-HT tone. In contrast, activation of 5-HT1A receptors by receptor agonists or increase in endogenous 5-HT concentration promotes SWD. Changes caused in the SWD activity were independent of the effects on vigilance and sleep. Since the selective 5-HT7 receptor antagonist reduced the cumulative duration of SWDs, as well as the number and average duration of paroxysms compared to vehicle in the WAG/Rij rat model of absence epilepsy, our results strengthened the hypothesis that 5-HT7 receptors, which density is relatively high within the thalamus, has influence on SWD in WAG/Rij rats.

Studies with selective AMPA receptor antagonists in animal seizure models have indicated that AMPA receptors are potentially promising anticonvulsant drug targets.

However, our results may raise doubts about the strong involvement of AMPA receptors in generating SWD, and may indicate that part of the effect of AMPA receptor antagonists in regulation of epileptic activity occur through vigilance effects in this model. Similarly, the examined glycin-transporter-1 inhibitors caused a transient antiepileptic activity only at the highest dose, what was clearly a result of their effects on vigilance. Our results suggest that AMPA modulators may have a therapeutic action in other types of epilepsies and glycin-transporter-1 inhibitors have no significant effects in absence seizures.

• *Kantor S, Jakus R, Bodizs R, Halasz P, Bagdy G (2002) Acute and long-term effects of the 5-HT2 receptor antagonist ritanserin on EEG power spectra, motor activity, and sleep: changes at the lightdark phase shift. Brain Res 943: 105–111.*

- *Jakus R, Graf M, Juhasz G, Gerber K, Levay G, Halasz P, Bagdy G (2003) 5-HT2C receptors inhibit and 5-HT1A receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. Exp Neurol 184: 964–972.*
- *Jakus R, Graf M, Ando RD, Balogh B, Gacsalyi I, Levay G, Kantor S, Bagdy G (2004) Effects of two non-competitive AMPA receptor antagonists GYKI 52466 and GYKI 53405 on spike-wave discharges, vigilance and behavior in a genetic rat model of absence epilepsy. Brain Res 1008: 236–244.*

MÁRTON GRAF (2006)

The effects of selective serotonin reuptake inhibitors on the function of distinct serotonin receptor subtypes in the rat

Supervisor: György Bagdy

Selective serotonin reuptake inhibitors (SSRIs) have well-documented efficacy in depression, dysthymic disorder, panic disorder, obsessive-compulsive disorder, social phobia, bulimia nervosa and several other psychiatric and neurological conditions.

Although inhibition of serotonin reuptake occurs immediately after administration of an SSRI, the clinical effect is characterized by delayed onset and it is generally only the side effects of these agents, which are manifest immediately. Anxiety is one of the most common early adverse effects of SSRI treatment, and SSRIs were reported to have an anxiogenic-like profile after acute administration in animal experiments. In our present work, we provide evidence that acute fluoxetine treatment dose-dependently increases anxiety in rats in the social interaction test. Furthermore, using subtype selective receptor antagonists, we prove that this anxiogenic effect is mediated by activation of 5-HT2C receptors. Our studies demonstrate that 5-HT2C receptor activation also causes self-grooming, a stereotype behaviour which is associated with increased anxiety and is characteristic for animal models of obsessive-compulsive disorder.

Desensitisation of 5-HT receptors associated with chronic SSRI treatment is a leading theory explaining the late therapeutic effects and the development of tolerance to side effects. In our experiments, physiological and behavioural responses mediated by 5-HT1A and 5-HT2C receptors were attenuated after chronic fluoxetine treatment, which supports the hypothesis of 5-HT receptor desensitisation.

Our studies proved that chemically induced, lasting depletion of brain 5-HT resulted in enhanced responses to m-CPP, which indicates an increase in 5-HT2C receptor sensitivity. This result supports the assumption that decreased brain 5-HT transmission is associated with increased sensitivity of 5-HT2 receptors, a mechanism believed to play a role in depressive disorders.

Previous studies in the WAG/Rij rat strain suggested a modulatory role of 5-HT on epileptic activity in this an animal model of human absence epilepsy. In our experiments, fluoxetine-induced increase in brain 5-HT neurotransmission produced an upsurge in epileptic activity, however simultaneous inhibitory and excitatory effects were detected, which are exerted through activation of different 5-HT receptor subtypes. Stimulation of 5-HT2C receptors appear to inhibit spike-wave discharges (SWDs), although this inhibitory effect is not significant at basal 5-HT tone. In addition, our studies further confirm that activation of 5-HT1A receptors promotes the generation of SWDs.

In conclusion we can state that acute and chronic effects of SSRIs are mediated by a variety of 5-HT receptor subtypes. Our studies, focusing primarily on the role of 5-HT1A and 5-HT2C receptors provide evidence for their role in modulating anxiety, stereotype behaviour, thermoregulation, penile erection and spike-wave discharges. The results presented in the thesis may facilitate the understanding of serotonergic mechanisms underlying certain psychiatric as well as neurological conditions in humans (e.g. depression, anxiety disorders and epilepsy) and help to clarify the complex and diverse effects of SSRI pharmacotherapy. Furthermore, our studies may promote the development of future treatments targeting the serotonergic system in a wide range of psychiatric and neurological disorders.

- *Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S (2001) Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. Int J Neuropsychopharmacol 4: 399–408.*
- *Graf M, Kantor S, Anheuer ZE, Modos EA, Bagdy G (2003) m-CPPinduced self-grooming is mediated by 5-HT2C receptors, Behav Brain Res 142: 175–179.*
- *Jakus R, Graf M, Juhasz G, Gerber K, Levay G, Halasz P, Bagdy G (2003) 5-HT2C receptors inhibit and 5-HT1A receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. Exp Neurol 184: 964–972.*

LÁSZLÓ SIMON (2007)

The neuroprotective effects of (-)deprenyl in an *in vivo* stroke model and in an *in vitro* hypoxia model

Supervisor: Zoltán Nagy

Blockage of a nutritive artery, energy crisis, cell necrosis in the infarct's core and adjacent to the core, in the penumbra region, reperfusion injury, delayed neuronal death and/or apoptosis are processes modeling the infarcts' volume.

(-)Deprenyl protects neurons from oxidative damage and it helps to maintain mitochondrial membrane potential by influencing intracellular anti-apoptotic oncoproteins, like Bcl-2. The cellular rescue in the penumbra region by (-)deprenyl administration has been examined in a permanent MCA occlusion (pMCAO) model in rat. (-)Deprenyl was given continuously following pMCAO, at a dose of 0.2 mg/kg/day continuously, for two days. At the end of the treatment period, the rats were sacrificed and the volumes of their infarct determined. TUNEL-caspase-3 and TUNEL-NeuN double labelled cells were counted. Neural plasticity was characterized by GAP-43 immunohistochemistry.

The lesion size decreased after 48 hours of continuous treatment with (-)deprenyl by 50% on average ($p<0.05$). The average lesion size in treated animals was 36.5 mm^3, while it was 65.8 mm^3 in control rats. The number of TUNEL labelled cells averaged in 60 samples was 17+12 in treated rats, while 28+25 in control rats ($p=0.002$). The number of TUNEL-caspase-3 labelled cells averaged in 30 samples was 3+3 in treated rats, while 8+6 in control rats ($p=0.0003$). (-)Deprenyl treatment increased the number of GAP-43-positive cells.

(-)Deprenyl reduced the number of affected cells and induced plasticity in the lesioned cortex. (-)Deprenyl, the Bcl-2 activator had a significant neuroprotective effect in our permanent MCA occlusion stroke model. It reduced the infarct-volume as well as the number

of TUNEL-positive cells. These effects were statistically significant. GAP-43 and synapsin expression were significant using *in situ* hybridisation technique in the (-)deprenyl treated group.

The main goal, to reduce the infarct volume, could be achieved by the (-)deprenyl treatment. The upregulated brain plasticity suggests a close relationship between the apoptosis cascade and the induction/expression of plasticity proteins. The exact link between these two phenomena, however, is far from being well characterized.

Hypoxia leads to a collapse in mitochondrial transmembrane potential ($\Delta\Psi_m$), a fall in the ATP/ADP ratio, and finally cell death. Since (-)deprenyl directly modulates $\Delta\Psi_m$ and production of reactive oxygen species (ROS) by altering the respiratory function of mitochondria, we were interested in the dose-response relations of these effects. The changes in JC-1 red/green signal ratios ($\Delta\Psi_m$), and the changes in the cerium staining (intracellular ROS) in hypoxic and normoxic PC12 cell cultures were measured following 1 h of Argon hypoxia and 24 h of re-oxygenation in the absence and in the presence of various concentrations of (-)deprenyl. $\Delta\Psi_m$ shifted to lower values following hypoxia/re-oxygenation and all cells had decreased and uniform $\Delta\Psi_m$ levels. The amount of ROS increased. Following 24 hours of treatment with various concentrations of (-)deprenyl during the re-oxygenation period, survival increased, the $\Delta\Psi_m$ shift caused by oxygen deprivation was reversed and the peroxy radical levels decreased except for at 10^{-3} M.

Our JC-1-Cerium double staining is able to characterize the lesioned cell in a quantitative manner. JC-1 is a non-toxic fluorescence probe for monitoring mitochondrial transmembrane potential. This dye exerts no effect on living cells. JC-1 monomers accumulate selectively in mitochondria and aggregate. The result of this aggregation is the J aggregate. The aggregation of JC-1 is due to the action of the electrochemical gradient. JC-1 at high levels of transmembrane potentials is highly concentrated in mitochondria, and J aggregates are formed in high quantities. JC-1 monomers in the cytosol are emitting green fluorescence, while J aggregates in mitochondria are emitting red fluorescence. The red/green ratio is an indicator of mitochondrial transmembrane potential. It is independent of dye-loading, lightscattering, and other optical factors. Following re-oxygenation the mitochondrial transmembrane potential decreases significantly. Using the cerium method, we could visualize ROS at the cellular level. This semi-quantitative method is based on a change in the level of cerium reaction products resulting from the activity of oxidases and phosphatases. The highest reflectance intensities are resulting from the presence of cerium perhydroxide ($Ce^{III}[OH]_2OOH$ or $Ce^{IV}[OH]_3OOH$). Cerium also reacts with phosphate derivatives. Cerium phosphate derivatives, however, do not have a relevant level of reflectance detectable using a confocal laser scanning microscope. The quantitative analysis of cerium distribution present in the cells subjected to hypoxia/reoxygenation and in normoxic control cells showed a significant increase in cerium signals present in hypoxic/reoxygenated cells as compared to controls. In our experiments we were able to simultaneously measure these two modalities in the same cells. Their relationship has an inverse feature described with this equation: $y=7.41x\pm0.47$, where y is the cerium signal and x is the JC-1 ratio. This novel double staining method is easily reproducible. It is also a good tool for screening neuroprotective, ROS blocking, drug-candidate molecules.

- *Simon L, Szilágyi G, Bori Z, Orbay P, Nagy Z (2001) (-)-d-Deprenyl attenuates apoptosis in experimental brain ischaemia. Eur J Pharmacol 430(2/3): 235–241.*
- *Simon L, Szilagyi G, Bori Z, Telek G, Magyar K, Nagy Z (2005) Low dose (-)deprenyl is cytoprotective: it maintains mitochondrial membrane potential and eliminates oxygen radicals. Life Sci 78(3): 225–231.*

• *Szilagyi G, Simon L, Koska P, Telek G, Nagy Z (2006) Visualization of mitochondrial membrane potential and reactive oxygen species via double staining. Neurosci Lett 399(3): 206–209.*

GÉZA SZILÁGYI (2008)

Mitochondrial neuroprotection

Supervisor: Zoltán Nagy

In my Ph.D. work I have investigated the cell protective effect of vinpocetin, deprenyl and deprenyl-N-oxide. The present investigations focused on these drugs' cellular effects, with special regard to their mitochondrial effects. In first part of the PhD period I established a new method by combining two well known methods: JC-1 staining (a mitochondrial transmembrane potential dye) and CeCl3 staining (which could assess the free radical production) *(Szilágyi et al. 2006. Visualization of mitochondrial membrane potential and reactive oxygen species via double staining. Neurosci Lett 399(3): 206–209)*. Furthermore, in our laboratory we established fast and well reproducible methods for assessing cell death, measuring mitochondrial membrane potential, and free radical staining. The cell biological effects of vinpocetin, deprenyl and deprenyl-N-oxide were investigated in PC-12 cell culture. We proved that all molecules have a concentration dependent protective effect against hypoxia *(Simon et al. 2005. Low dose (-)deprenyl is cytoprotective: it maintains mitochondrial membrane potential and eliminates oxygen radicals. Life Sci 78(3): 225–231)*. We suggested and proved a new mechanism of vinpocetin: that vinpocetin is a PBR ligand.

With this new mechanism we could explain the cellular effects as well as the changes in aerobic/anaerobic metabolism in the brain after the treatment with vinpocetin *(Szilágyi et al. 2005. Effects of vinpocetin on the redistribution of cerebral blood flow and glucose metabolism in chronic ischaemic stroke patients: a PET study. J Neurol Sci 229/230: 275–284)*. We have demonstrated a concentration dependent protective effect of deprenyl-N-oxide, a newly synthesized metabolite of deprenyl. This effect was MAO-B independent. On the basis of our result we suggest that the active metabolite of deprenyl is DNO. Furthermore we hypothesise that DNO has an affect on the mitochondrial transition permeability pore complex.

• *Simon L, Szilágyi G, Bori Z, Orbay P, Nagy Z (2001) A (-)deprenyl hatása kísérletes agyi ischaemiában. Agyérbetegségek 7: 6–11.*
• *Szilágyi G, Nagy Z (2006) Új módszerek a hypoxia kutatásban. Ideggy Szemle 59: 411–415*
• *Simon L, Szilágyi G, Bori Z, Orbay P, Nagy Z (2001) (-)-d-Deprenyl attenuates apoptosis in experimental brain ischemia. Eur J Pharm 430: 235–241.*

PROGRAM 6/5.

CLINICAL NEUROLOGICAL INVESTIGATIONS

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Program overview

This doctoral (Ph. D.) program includes clinical research projects in neurology. Full time participation in clinical research can be applied by medical students from the Hungarian, English and German faculties who accomplished their fifth year training, furthermore young doctors with certain research experience in neuroscience. Part-time participation is possible for young neurologists before their neurology special exam. Participants who are not affiliated, working on any subspecialties of neuroscience, could join to the subprograms if their topic may be suited to the clinical neurology. The clinical research program includes partially laboratory work, the research activity of participants may concern to investigate clinical patients and statistical evaluation of clinical and laboratory information. The advertised programs contain mostly unsolved questions of the clinical neurology.
Research of cerebro-vascular diseases includes clinico-pathological evaluation and classification of leukoaraiosis comparing clinical symptoms and brain imaging findings. Analysis of clinical picture and outcome of neurological deficits of elderly patients who suffered ischemic insults caused by lacunar infarctions is available. By the help of the clinical register clinical and epidemiological investigations of cerebrovascular disorders is advertised. The pathomechanism of the primary headaches is unknown. To study the biochemical and physiological causes of headaches in a clinical working group is in progress. Physiological studies involve the nonivasive measurement of blood flow velocity in the intracerebral arteries. With the help of statistical software EEG activity and Doppler flow data can be analyzed simultaneously. Neuropsychologic tests, and electrophysiological measurements can be used in patients with degenerative dementias and aphasia. Research on the movement disorders especially Parkinson disease is a prominent scientific field of the department. Projects for the research of movement and coordination will start in 2012. Many aspects of phenomenology, medical and surgical treatment are under investigation; furthermore neurosurgical research field was opened in 2011. Additionally other neurosurgical programs are also available. Psychometric methods, tremorometry and electrophysiology help the differential diagnosis of movement disorders. New program for the investigation of the basic mechanism of epilepsy was just opened. Topographic aspect and mechanism of human tremor is unknown. Subprograms provide to join to the tremor-research group. Subprogram for neuropathology is ready for the investigation of dementias and cerebrovascular diseases. Learning the instrumental diagnostic technics by the aim to improve the topography of lesions of roots and nerves and scientific analysis of the underlying diseases of peripheral nerves is possible. Research topic for the investigation of optokinetic nystagmus was planned for the approach of brainstem's pathology.

Titles of research projects	*Supervisors*
Disturbance of cognition, behaviour and speech in cerebrovascular disorders	Imre Szirmai
Clinicopathology of leukoaraiosis	Imre Szirmai
Investigation of optokinetic nystagmus in cortical and subcortical lesions	Imre Szirmai
Examination of the characteristics of lacunar cerebral infarcts	Dániel Bereczki
Clinical and epidemiological investigations of cerebrovascular disorders	Dániel Bereczki
Examination of the pathomechanism and clinical characteristics of primary headaches	Dániel Berecki
Examination of event related desynchronisation, tremor and co-ordination in Parkinson's disease	Anita Kamondi
Investigation of normal and pathologic movement regulation with the help of EEG and trascranial magnetic stimulation	Anita Kamondi
Clinico-morphological correlations in degenerative diseases of the central nervous system	Tibor Kovács
Neuropathological investigations in vascular diseases of the central nervous system	Tibor Kovács
Polygraphic investigation of the blood flow regulation in healthy subjects and patients with cognitive deficit	Róbert Debreczeni
Examination of neuropathies with high-resolution ultrasonography and comparison with electrophysiological findings	Zsuzsanna Arányi
3D motion analysis in movement disorders	Gertrúd Tamás
Analysis of efficacy and mode of action of deep brain stimulation in movement disorders	Loránd Erőss
Clinical characteristics, diagnosis, pathomechanism and therapy of headache disorders	Csaba Ertsey
Modern neurosurgical treatment opportunities in central nervous system pathologies	Péter Banczerowski
Electrophysiological analysis of optokinetic nystagmus	Szilvia Gulyás
Spontaneous and electrically evoked high frequency oscillations (ripples) in epilepsy	Daniel Fabo

Ph. D. students		*Supervisors*
Bence Barna Gunda	ft	Dániel Bereczki
Nóra Manhalter	pt	Dániel Bereczki
István Szaniszló	pt	Anita Kamondi

Ph. D. candidates		*Supervisors*
Tamás Patkó	pt	Zsuzsanna Arányi
Róbert Debreczeni	i	Imre Szirmai
Zsuzsanna Farkas	ft	Anita Kamondi
Magdolna Simó	i	Zsuzsanna Arányi

f, full-time; pt, part-time; i, individual

PROGRAM 6/6.

BIOLOGICAL PSYCHIATRY

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Program overview

The aim of the "Biological Psychiatry" program is to study the theoretical and practical aspects of brain and mental sciences, utilising and integrating knowledge from different disciplines in understanding pathopsychological functions and therapeutic response, and to contribute with its results to everyday practice in mentalhygiene and psychiatry. One challenge for biological psychiatry is to integrate our psychopathological knowledge about brain functional changes with our present knowledge about the relationship between brain and behavior and brain structure. Research in the Biological Psychiatry program targets psychiatric disorders from neurobiological, neurochemical, genetic and neurocognitive aspects, building on knowledge from cooperation with other disciplines and experience from clinical observation and effective treatment of patients. Presently the program includes 7 Ph. D. themes offering the study of neurobiological, clinical and therapeutic aspects of adult and geriatric psychiatric disorders. The tutors are well-known and internationally acknowledged theoretical and clinical experts.

Titles of research projects	***Supervisors***
Biochemical background of mood and anxiety disorders	Kornélia Tekes
Interrelationship between biogenic amines and nociceptin/nocistatin system	Kornélia Tekes
Genetic correlats of behavioural phenotypes in major depression and their relationship to response to antidepressant medication	Gábor Faludi

Ph. D. student		***Supervisor***
Gabriella Balogh	ft	Gábor Faludi

Ph. D. candidates		***Supervisors***
Zoltán Makkos	i	Gábor Faludi
Annamária Rihmer	pt	Gábor Faludi
Andrea Sárosi	i	Andrea Sárosi

Ph. D. graduate		***Supervisor***
Gábor Vincze	i	Gábor Faludi

f, full-time; pt, part-time; i, individual

Abstract of Ph.D. thesis successfully defended in 2008

GÁBOR VINCZE (2008)

Epidemiological surveys in hospitals and elderly homes with special respect to consultation-liaison psychiatry and dementia

Supervisor: Gábor Faludi

The consultation-liaison psychiatry (CLP) is an important field of psychiatry, although the research of CLP is still in an initial phase in Hungary. The present work overviews the international literature of history, application, and research of CLP. In this study a questionnaire screening of 2485 patients treated in nine hospitals was performed. The response rate was 53.4%. The patients were treated in different departments of general hospitals. The questionnaire evaluated certain psychiatric characteristics of the patients, involving demographic data, addictions (CAGE-questionnaire), depression (Beck Depression Inventory) and measurement of level of anxiety (STAI). The application of pharmacotherapy, especially the use of antidepressants and anxiolytics was also analyzed.
The other research analyzed the risk factors of dementia in an elderly home population of 2142 people, aged above 50 years. The main results of the studies can be summarized as follows:
(1) This study was the first such a survey in Central-Eastern Europe. It would be useful to compare our data with those of the neighbouring countries to get a real picture about the regional features of hygiene culture. This research was the first comprehensive survey of psychiatric disorders of general hospital population in Hungary as well.
(2) The ratio of smoking and alcohol dependence of the studied sample is identical with the data of the average Hungarian population. The highest averages of alcoholism as a risk factor were found in the pulmonology, surgical, traumatological and orthopaedic departments.
(3) In the general hospital population a high rate of depression and anxiety was found. About half of the patients suffer from depression of different level severity. The depression occurs mainly in the departments curing people suffering from chronic diseases.
(4) In spite of high frequency of depression the adequate treatment is missing in general. Only a low percent of patients suffering from depression get antidepressants in the different departments. The anxiolitics are more widely used for the treatment of patients suffering from depression. About one third of these patients is cured with anxiolitics.
(5) The frequency of anxiety is very similar to that of depression. The situation is also similar in the pharmacotherapy: one third of patients gets anxiolitics and only a very low percentage of patients are treated with antidepressants.
(6) According to the data of the first dementia survey carried out in elderly homes the well-known dementia risk and protective factors demonstrated that the Hungarian cohort is similar to the other European ethnic groups.

- *Vincze G, Török IA, Túry F (2000) A pszichiátriai konzultáció. Alternatív fejlődési modell a háziorvosi ellátásban, pszichiátriai-pszichológiai szolgáltatások bevezetése a csoportpraxisba. Magy Alapell Arch 3: 117–122.*
- *Vincze G, Túry F (2001) A konzultációs-kapcsolati (liaison) pszichiátria aktualitása – újabb irodalmi adatok. Mentálhig Pszichoszom 3: 5–8.*
- *Török I, Vincze G, Papp T, Oláh Sz (2002) Az SMS használat addiktív és funkcionális viselkedéses elemeinek azonosítása a serdülő korosztályban. Az SMS-kommunikáció minőségi specifikumai, és az SMS-chat viszonylat. Psych Hung 17: 585–598.*

SCHOOL OF PH. D. STUDIES

7. MOLECULAR MEDICINE

Chairman:
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General overview

The postgraduate school of molecular medical sciences serves for both biomedical basic research and primary training of researchers starting their careers in the fields of clinical research. One of the major drawbacks of biomedical research is that there is no efficient connection between the basic and clinical research. Therefore, five main Programs involve applied theoretical knowledge together with clinical research.

PROGRAM 7/1.

CELLULAR AND MOLECULAR PHYSIOLOGY

Coordinator:
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Program overview

The Program provides opportunity to receive training in the field of physiology. The professors of the Program offer courses and individual training for the Ph. D. students on their respective scientific research areas. Training courses include continuous basic methodical and scientific training for small groups of students. Individual training focuses on research under the supervision of training advisors aimed at understanding physiological regulatory mechanisms at the cellular level using electrophysiological, molecular biological, biochemical, cell biological and physiological methods.

Titles of research projects	***Supervisors***
Investigation of two-pore domain potassium channels	Gábor Czirják
Investigation of the structure-function relationship of TrpM2 cation channels	László Csanády
Molecular chaperones and biological networks	Péter Csermely
Receptor mediated regulation of type 2P potassium channels	Péter Enyedi
Investigation of reactive oxygen producing enzymes in mammalian cells	Miklós Geiszt
From stem cells to liver: ABC transporters during hepatic differentiation	László Homolya
ABC half transporters participating in cholesterol metabolism	László Homolya
Regulation of G protein-coupled receptors	László Hunyadi
Molecular basis of regulation of the circadian rhythm	Krisztina Káldi
Role and regulation of Rho family GTPase activating proteins (GAPs)	Erzsébet Ligeti
The role of positional information (position along the body-axes) in the early differentiation of neural cells	Emília Madarász
Investigation of proteins involved in the differentiation and function of osteoclasts	Attila Mócsai
Role of oxygen radicals in the physiological effect of angiotensin II and other Ca^{2+} mobilizing hormones	András Spät
The molecular and physiological role of inositol lipids	Péter Várnai

Ph. D. students		***Supervisors***
Roland Csépányi-Kömi	pt	Erzsébet Ligeti
Ágnes Donkó	ft (a)	Miklós Geiszt
Balázs Enyedi	ft	Miklós Geiszt
Pál Gyombolai	ft	László Hunyady
Norbert Gyöngyösi	ft	Krisztina Káldi
Dávid Sándor Győri	ft	Attila Mócsai
Zsuzsanna Kertész	ft (a)	Attila Mócsai
Miklós Kovács	ft*	Attila Mócsai
Magdolna Krisztina Lévay	ft	Erzsébet Ligeti
Tamás Németh	ft	Attila Mócsai
Robin Dezső Palotai	ft*	Péter Csermely
Zalán Péterfi	ft	Miklós Geiszt
Bence Szalai	ft	László Hunyady
Gergő Szanda	ft	András Spät
Balázs Tóth	ft	László Csanády
Dániel Tóth	ft*	Péter Várnai

Ph. D. candidates		***Supervisors***
Péter Koncz	pt	András Spät
Gábor Turu	pt	László Hunyady

Ph. D. graduates		***Supervisors***
Kornél Demeter	ft	Emília Madarász
Zoltán Jakus	ft	Attila Mócsai

Gábor Sirokmány	ft	Erzsébet Ligeti
László Szidonya	ft	László Hunyady

*Semmelweis Doctoral Fellowship; a, absolutorium; ft, full-time; pt, part-time

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

KORNÉL DEMETER (2007)

Integration of embryonic neuroectodermal stem cells into brain tissue: studies on implanted neural stem cells

Supervisor: Emília Madarász

Pheno- and genotypically identical NE-4C neural stem cells were implanted into the brain tissue in order to approach the question: why inherent neural stem cells have a limited capacity to replace neurons decaying in neural diseases or brain injuries.
Non-induced NE-4C cells proliferate continuously, but differentiate to neurons and astrocytes if induced by all-trans retionic acid (RA) *in vitro*. These cells can be transfected by desired gene constructs and stable sub-clones can be established. For implantation GFP or PLAP histological marker gene expressing clones were used.
GFP- or PLAP-4C stem cells were implanted into the forebrains of early embryonic chicken, fetal, newborn or adult mice, as well as into lesioned cortical areas or tumourous brains. In the adult mouse brain the implanted cells survived a maximum of 4 weeks, displayed limited proliferation, and the rate of differentiations was very low. Long-term survival, however, was observed in neurogenetic areas of the adult brain. In the young postnatal brain, the cells proliferated and survived long (more than 6 weeks) periods, but did not differentiate. In the early embryonic brain, NE-4C cells migrated long distances from the graft, differentiated into neurons and integrated into the host tissue.
In cryogenic cortical lesions, the implanted stem cells survived for a long time, but neural tissue-type differentiation was not detected. The intrinsic clonal features of the implanted cells were not shifted upon long-term intracerebral survival.
For investigating whether NE-4C cells can be used for targeting brain tumors, in vitro tests were elaborated to study the cellular interactions between stem- and tumour cells. The preferences of cell-to-cell adhesion and the potential proliferation-enhancing interactions were tested. The data indicated that stem cells can increase the rate of proliferation of some tumour cells. On the basis of the tests, Gl261 mouse-derived glioma cells were selected for eliciting tumours in adult mice. NE-4C cells survived in intracranial Gl261 tumours, but did not migrate towards the tumour through the intact brain parenchyma. The data showed that the intracerebral fate of implanted neural stem cells is determined by the host environment. Further experiments are in progress to determine the intracerebral fate of *in vitro* pre-differentiated neural progenitors in various brain environments.

• *Demeter K, Herberth B, Duda E, Domonkos A, Jaffredo T, Herman JP, Madarász E (2004) Fate of cloned embryonic neuroectodermal cells implanted into the adult, newborn and embryonic forebrain. Exp Neurol 188: 254–267.*

- *Demeter K, Zádori A, Ágoston VA, Madarász E (2005) Studies on the use of NE-4C embryonic neuroectodermal stem cells for targeting brain tumour. Neurosci Res 53: 331–342.*
- *Ágoston VA, Zádori A, Demeter K, Nagy Z, Madarász E (2007) Altered behaviour of neural stem cells in intact and lesioned brain areas. Neuropathol Appl Neurobiol 33: 510–522.*

ZOLTÁN JAKUS (2006)

Signaling mechanisms of integrins, Fc receptors and G-protein coupled receptors in neutrophils

Supervisor: Attila Mócsai

Neutrophils play an essential role in the destruction of invading microorganisms and in the pathogenesis of several autoimmune diseases. In these processes, activation of the cells is mediated by several different neutrophil cell surface receptors. In my Ph.D. work, the signaling mechanisms utilized by integrins, Fc receptors and G protein coupled receptors were investigated in neutrophils.

Both integrin and nonintegrin receptors are required for neutrophil activation in the inflamed environment. Prior findings suggested that the nonintegrin signals are only required for increasing the binding capacity of integrins, while the eventual cellular responses are mediated by integrin crosslinking upon their ligand binding (i.e., suggesting a serial hierarchical relationship between the two signals). This hypothesis was based on the fact that neutrophil activation by anti-integrin antibodies results in full activation of the cells without any additional stimulus, indicating that integrin crosslinking by itself is sufficient for full activation of neutrophils. In the first part of my Ph.D. work, using gene deficient (knockout) mice, blocking antibodies and antibody Fab fragments we showed that low affinity Fcγ receptors are indispensable for neutrophil responses induced by anti-integrin antibodies. These findings challenge the earlier hypothesis that crosslinking of integrins is sufficient for full activation of neutrophils, and suggest that integrin and nonintegrin signals function in a parallel fashion. Furthermore, we showed that the different nonintegrin signals (TNF, Fc receptor) are interchangeable and likely converge on the p38 MAP kinase.

In the second part of my experiments, the mechanism of Fc receptor signaling was investigated in neutrophils. Using gene deficient (knockout) mice and a newly set up activation system, we found that Src family tyrosine kinases, the Fc receptor γ chain, Syk, as well as the ERK and p38 MAP kinases are indispensable for neutrophil responses induced by immune complexes that act through Fc-receptors. These proteins could be placed into a signaling pathway where Src family tyrosine kinases phosphorylate the Fc receptor γ chain, which then recruits Syk, eventually leading to activation of the ERK and p38 MAP kinases.

In the third part of my Ph.D. work, the contribution of tyrosine kinase pathways to G protein coupled receptor signaling was investigated. Using pharmacological inhibitors and gene deficient (knockout) mice, we showed that the p38 MAP kinase activated via Src-family kinases is indispensable for the degranulation induced by the bacterial tripeptide fMLP. Using Syk deficient neutrophils, we also disproved prior assumptions that Syk is crucial for G protein coupled receptor (e.g. fMLP, chemokine) signaling.

These results help us to understand the working paradigms of the immune system, and they may point to possible novel targets for pharmacological control of autoimmune diseases.

- *Jakus Z, Berton G, Ligeti E, Lowell CA, Mócsai A (2004) Responses of neutrophils to anti-integrin antibodies depends on costimulation through low affinity FcγRs: full activation requires both integrin and nonintegrin signals. J Immunol 173: 2068–2077.*
- *Mócsai A, Jakus Z, Vantus T, Berton G, Lowell CA, Ligeti E (2000) Kinase pathways in chemoattractant-induced degranulation of neutrophils: the role of p38 mitogen-activated protein kinase activated by Src family kinases. J Immunol 164: 4321–4331.*
- *Mócsai A, Zhang H, Jakus Z, Kitaura J, Kawakami T, Lowell CA (2003) G-protein-coupled receptor signaling in Syk-deficient neutrophils and mast cells. Blood 101: 4155–4163.*

GÁBOR SIROKMÁNY (2006)

Signaling role of the protein domains of p50 Rho GTPase activating protein in intracellular membrane traffic

Supervisor: Erzsébet Ligeti

Rho GTPase proteins take part in many diverse cellular processes, like cytokinesis, gene transcription, membrane traffic, superoxide production. According to human genome data, the number of Rho GTPases have been estimated to be between 20 and 25, however the number of their regulatory proteins such as exchange factors and GTPase activating proteins (GAPs) is at least threefold higher. This difference implies that each intracellular function of a Rho GTPase might be governed by a distinct set of regulatory proteins in a special subcellular environment. The formation of such regulatory molecular complexes is driven by protein-protein and protein-lipid interactions.

We decided to study the ubiquitously expressed p50RhoGAP protein that contains an interesting Sec14 homology domain with unknown function. We raised a polyclonal antibody against the protein and made GFP constructs to study its subcellular localization. We found that it showed strong colocalization with markers of the early endosome and recycling endosome compartments (transferrin-receptor and Rab11) and that this localization was clearly determined by the Sec14 homology domain. Measuring bioluminescence resonance energy transfer between p50RhoGAP and Rab11 it seems to form molecular complexes with the Rab11 small GTPase on endosomal membranes and this also depends on the Sec14 domain. According to our *in vitro* GTPase measurements p50 is clearly not a GAP of the Rab family proteins. Using quantitative fluorescent transferrin assays we showed that overexpression of p50RhoGAP inhibits transferrin uptake and recycling which effect is dependent on the intact Sec14 domain of the protein.

In summary we can presume that p50RhoGAP is part of a molecular complex where it forms a link between Rab and Rho small GTPases. Our results emphasize the importance of functionally diverse protein domains of GAP proteins that might appoint the place and time of the GAP activity.

- *Sirokmány G, Szidonya L, Káldi K, Gáborik Z, Ligeti E, Geiszt M (2006) Sec14 homology domain targets p50RhoGAP to endosomes and provides a link between Rab- and Rho GTPases. J Biol Chem 281: 6096–6105.*
- *Szászi K, Sirokmány G, Di Ciano-Oliveira C, Rotstein OD, Kapus A (2005) Depolarization induces Rho-Rho kinase-mediated myosin light chain phosphorylation in kidney tubular cells. Am J Physiol Cell Physiol 289: C673–C685.*

• *Di Ciano-Oliveira C, Sirokmány G, Szászi K, Arthur WT, Masszi A, Peterson M, Rotstein OD, Kapus A (2003) Hyperosmotic stress activates Rho: differential involvement in Rho kinase-dependent MLC phosphorylation and NKCC activation. Am J Physiol Cell Physiol 285: C555–C566.*

LÁSZLÓ SZIDONYA (2008)

G protein-independent signaling of the AT1A-angiotensin receptor in C9 cells

Supervisor: László Hunyady

Angiotensin II (Ang II) is a major regulator of the fluid homeostasis, blood pressure, thirst and aldosterone secretion. In addition to its physiological roles, Ang II is an important factor in pathological conditions, such as hypertension, renal fibrosis and cardiac hypertrophy. Most of the known effects of Ang II are mediated by the AT_1 angiotensin receptor, which activates a multitude of signaling pathways, including G protein-dependent and -independent responses, the latter of which being the subject of intensive studies in the recent years.

Although mutant receptors are highly useful to dissect the signal transduction pathways of receptors, they are difficult to study in physiological target tissues, due to the presence of endogenous receptors. To study AT_1 angiotensin receptors in their physiological environment, we tried to construct a mutant receptor, which differs from the AT_{1A} receptor only in its reduced affinity for candesartan, a biphenylimidazole antagonist. We have determined that the conserved S109Y substitution of the rat AT_{1A} receptor abrogates its candesartan binding, without exerting any major effects on its Ang II and peptide angiotensin receptor antagonist binding, internalization kinetics, β-arrestin binding, and potency or efficacy of the inositol phosphate response. The recruitment of β-arrestin to the receptor was followed by bioluminescence resonance energy transfer, which confirmed the stable association of this molecule with the receptor.

To demonstrate the usefulness of the S109Y mutant receptor in signal transduction studies, we combined it with substitution of the highly conserved DRY sequence with AAY, which abolishes G protein activation without affecting agonist-induced β-arrestin binding to the receptor. In rat C9 hepatocytes the S109Y receptor caused ERK activation with the same mechanism as the endogenous AT_1 receptor.

After combination with the DRY/AAY mutation, G protein-independent ERK activation was detected demonstrating that this approach can be used to study the Ang II-stimulated signaling pathways in cells endogenously expressing AT1 receptors.

• *Szidonya L, Süpeki K, Karip E, Turu G, Várnai P, Clark AJL, Hunyady L (2007) AT1 receptor blocker-insensitive mutant AT1A angiotensin receptors reveal the presence of G protein-independent signaling in C9 cells. Biochem Pharmacol 73: 1582–1592.*
• *Turu G, Szidonya L, Gáborik Z, Buday L, Spät A, Clark AJL, Hunyady L (2006) Differential β-arrestin binding of AT1 and AT2 angiotensin receptors. FEBS Lett 580: 41–45.*
• *Turu G, Simon A, Gyombolai P, Szidonya L, Bagdy G, Lenkei Z, Hunyady L (2007) The role of diacylglycerol lipase in constitutive and angiotensin AT1 receptor stimulated cannabinoid CB1 receptor activity. J Biol Chem 282: 7753–7757.*

PROGRAM 7/2.

PATHOBIOCHEMISTRY

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Program overview

Pathobiochemistry showed a remarkably dynamic progress in the past decades. The Program has two roles: (1) it outlines the etiology and pathogenesis of different pathological conditions, (2) it aims to help the detailed knowledge of certain important fields of pathology. In planning the program the following viewpoints were considered: in diseases different mechanisms of pathological regulation can develop, reflecting changes in extracellular signals or signal transduction.

Titles of research projects	***Supervisors***
Comparative examination of vascular gene polymorphisms in clinical pictures	György Acsády
Transport systems in the endoplasmic reticulum	Gábor Bánhegyi
Neuronal scaffold proteins	László Buday
Metabolic fate of glucuronides in the endoplasmic reticulum	Miklós Csala
Characteristics of calcium transporters	Ágnes Enyedi
Separation technique methods with big efficiency in the proteomic basis of biomarker research	András Guttman
Application of the specialities of separation techniques in QSAR studies	Miklós Idei
Study of transporter-drug interactions in human and rat hepatocytes	Katalin Jemnitz
The genetic background of gynaecological clinical pictures	József Gábor Joó
Changes of extracellular and adhesic molecules in the human uterus	Anna Kádár
Effects of anti-cancer and anti-inflammatory peptides—signal transduction therapy	György Kéri
Rational drug design of kinase inhibitor agents	György Kéri
Cellular signalling therapy with kinase inhibitors	György Kéri
Cell dependent thrombolysis	Krasimir Kolev
Study of calcium transport systems in various cancer cells	Tünde Kovács
The role of pathobiochemical factors in the development and progression of inflammatory bowel diseases	Péter László Lakatos
Role of leucocytes in fibrinolysis	Rajmund Machovich
Molecular mechanisms of endoplasmic reticulum stress	József Mandl

Determination of hydrophobicity of the new selective tyrosine kinase inhibitor molecules. Modelling the relationship between structure and biological activity	György Mészáros
Selection and application of protein specific aptamers	Tamás Mészáros
Genetic polymorphisms in monoamine neurotransmitter systems: association analyses and functional study	Zsófia Nemoda
Design, synthesis and structure—biological activity correlation studies of anticancer and antimicrobial agents	László Őrfi
Molecular pharmacology of the signal transmission therapies affecting the regulation of cell death following molecular farmaco-diagnostics	István Peták
Analysis of small and large-scale copy number variations	Zsolt Rónai
Pathobiochemistry of pancreatic digestive enzymes	Miklós Sahin-Tóth
Membrane transporter proteins of human stem cells and their changes during cell differentiation	Balázs Sarkadi
Association between the structure and function of human ABC transporter proteins	Balázs Sarkadi
Genetical risk factors in complex hereditary diseases	Mária Sasvári
Cross-talk between signaling pathways regulating proliferation, differentiation and cell death of B-lymphocytes	Gabriella Sármay
The role of protein denaturation and stress response in aging	Csaba Sőti
Simultaneous application of quantitative molecular genetic measurements and high capacity cell sorting in malignant disorders of the myeloid system	Attila Tordai
Biosynthesis of nitric oxide, its relation to oxidative stress and their roles in the pathobiochemistry of human placenta	Miklós Tóth
Characterization of ABCG-type transporters	András Váradi
The role of Na-K ATPase in the pathomechanism of diabetes mellitus	Ágota Vér
UV-induced tumorigenesis in skin: Molecular biological mechanisms, its regulation and pathobiochemical events	Norbert Wikonkál

Ph. D. students		***Supervisors***
Mehmet Alper Arslan	it	Csaba Sőti
Zsófia Balogh	ft	Tamás Mészáros
Melinda Bence	ft	Mária Sasvári
Gábor Bőgel	pt	László Buday
Zsuzsanna Literáti-Nagy	pt	József Mandl
Éva Magyar	pt (a)	Miklós Csala
Nóra Meggyesi	ft	Attila Tordai
Nguyen Minh Tu	ft	Csaba Sőti
Réka Gabriella Nagy	pt	Zsolt Rónai
Abdul Rahman Omar	ft	Mária Sasvári
Diána Papp	ft	Csaba Sőti
Klára Rosta	pt	Ágota Vér
Zsolt Rottenberger	pt	Krasimir Kolev
László Seres	crc	László Homolya

Boglárka Sonkoly	ft	Gábor Bánhegyi
Gábor Viktor Szabó	pt (a)	György Acsády
Péter Szelényi	crc	József Mandl
Anikó Szilvási	crc	Balázs Sarkadi
Roopesh Udupa	it	László Buday
Zsóka Weiszhár	pt (a)	László Romics
Nikolett Wohner	pt	Krasimir Kolev

Ph. D. candidates		***Supervisors***
Annamária Balázs	ft	László Buday
Balázs Hallgas	crc	Miklós Idei
Ildikó Kasza	crc	Balázs Sarkadi
Ákos Kertész	crc	Gabriella Sármány
Edina M. Komlódi-Pásztor	ft	András Váradi
György Paragh	ft	Norbert Wikonkál
Ferenc Pintér	pt	István Peták
Gyöngyi Rábai	ft	Rajmund Machovich
Polett Ribiczey (Szentirmainé)	pt	Tünde Kovács
Gábor Sobel	pt	Anna Kádár
Flóra Mária Szeri	ft	András Váradi
Anna Tanka-Salamon	ft	Rajmund Machovich
Dávid Varga-Szabó	pt	Ágnes Enyedi
Edit Várkondi	crc	István Peták
Zsuzsanna Eszter Wolf	pt	László Romics

Ph. D. graduates		***Supervisors***
Ibolya Anett Czegle	pt	Gábor Bánhegyi
Judit Cserepes	ft	Balázs Sarkadi
Levente Deák	pt	Ottó Ribári
Dániel Erős	ft	György Kéri
Simon Fischer	i	Péter László Lakatos
Judit Gombás	i	Krasimir Kolev
András Illés	pt	László Buday
Éva Kereszturi	ft	Mária Sasvári
Orsolya Király	ft	Miklós Sahin-Tóth
Imre Kovács	pt	Albert Császár
Zoltán Kukor	i	Miklós Tóth
Rozália Laczkó	crc	Balázs Sarkadi
Beáta Lizák	pt	Gábor Bánhegyi
Marianne Varsányi (Lótosné)	pt	Gábor Bánhegyi
Éva Margittai	pt	Miklós Csala
Attila Molvarec	pt	István Karádi
Gábor Nagy	ft	József Mandl
Melinda Oroszlán	crc	László Romics
Eszter Papp	ft	Péter Csermely

Orsolya Polgár	i	András Váradi
Richárd Szmola	pt	Miklós Sahin-Tóth
Balázs Váradi	ft	Krasimir Kolev

a, absolutorium; ft, full-time; pt, part-time; it, international; i, individual; crc, Cooperation Research Centre

Abstracts of Ph.D. theses successfully defended in 2006, 2007 and 2008

IBOLYA ANETT CZEGLE (2007)

The redox systems of the endoplasmic reticulum and the metabolic syndrome

Supervisor: József Mandl

Metabolic syndrome is one of the most common diseases with a prevalence of about 20–25%. Its main symptoms are insulin resistance, obesity, hypertension and dyslipidemia. 11β-hydroxysteroid dehydrogenase type 1 plays an important role in the pathogenesis of the disease.

11β-hydroxysteroid dehydrogenase type 1 is expressed in many tissues: in the pathogenesis of the metabolic syndrome enzyme in the liver and adipose tissue is the most important. Its role is the local regulation of corticosteroid effect. The active site of the 11β-hydroxysteroid dehydrogenase type 1 is located in the lumen of the endoplasmic reticulum. The enzyme uses NADPH as a cofactor, the activity can be regulated via its cofactor supply. The cooperation between 11β-hydroxsteroid type 1 and hexose-6-phosphate dehydrogenase has been proved in a biochemical, as well as in a genetical way. The basis of their cooperation is the mutual cofactor generation for each other. Many other reactions are also known that use NADPH in the lumen of the endoplasmic reticulum, supposing the existence of a reduced-state pyridine nucleotide pool.

We have proved that the cooperation of the two enzymes is based on an isolated, common pyridine nucleotide pool. Both the substrates of 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase can influence the redox state of this intraluminal pyridine nucleotide pool. The calculated redox potential of the luminal $NADPH/NADP^+$ system is –376 mV.

We have also examined the role of 11β-hydroxysteroid dehydrogenase type 1 in the phenotype of the metabolic syndrome. We have proved in obese Zucker rat and lean Goto-Kakizaki type 2 diabetes model rat the increased and decreased protein expression and activity of the adipose tissue enzyme, respectively. The increased expression and activity of the hepatic 11β-hydroxysteroid dehydrogenase type 1 in the lean Goto-Kakizaki rat may be important in the pathogenesis of type 2 diabetes, while the decreased expression and activity of the enzyme in the liver of the obese Zucker rat may be a compensatory effect to the developed metabolic syndrome.

• *Czegle I, Piccirella S, Senesi S, Csala M, Mandl J, Banhegyi G, Fulceri R, Benedetti A (2006) Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool in the lumen of the endoplasmic reticulum. Mol Cell Endocrinol 248: 24–25.*

• *Piccirella S, Czegle I, Lizak B, Margittai E, Senesi S, Papp E, Csala M, Fulceri R, Csermely P, Mandl J, Benedetti A, Banhegyi G (2006) Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. J Biol Chem 281: 4671–4677.*

JUDIT CSEREPES (2008)

Expression and functional investigation of two less characterized human ABCG proteins: the ABCG1 and ABCG4 transporters

Supervisor: Balázs Sarkadi

The aim of my work was to characterize two recently described ABC proteins, the human ABCG1 and ABCG4. Most of the ABC proteins are active membrane transporters, they use the energy of ATP hydrolysis for the transmembrane transport. The members of the family share a common structure, an active transporter consists of at least two transmembrane domains and two nucleotide binding domains. On the basis of sequence homology ABC proteins are classified into seven subfamilies ranging from ABCA to ABCG. Members of the ABCG subfamily are so-called half transporters, they consist of a single transmembrane domain and a single nucleotide binding domain only. These proteins are thought to dimerize to form functional transporters. Some members of the ABCG subfamily are known to function as homodimers (ABCG2) whereas others as heterodimers (ABCG5/ABCG8). At the beginning of our work there were no data available on the dimerization, structure and function of human ABCG1 and ABCG4 proteins. Therefore, we expressed and functionally characterized the human ABCG1 and ABCG4 proteins. The approach we used for studying these transporters was similar to that previously used for other members of ABC proteins. Additionally we investigated if these proteins form homo- or heterodimers.

We have expressed the wild type proteins as well as their catalytic site mutant variants in insect cells, generated specific antibodies, and analyzed their function in isolated membrane preparations or in intact cells. Measuring the vanadate sensitive ATPase activity made it possible to detect the basal activity of the transporters as well as to screen for potential substrates and inhibitors. We also studied the cell physiological changes resulted by the expression of these two proteins. We investigated the dimerization of these proteins by measuring the ATPase activity of membranes coexpressing ABCG1 and ABCG4. Our results revealed that these proteins are active ATPases when expressed alone, and the ATPase activity of ABCG1 was stimulated by potential substrates and blocked by identified inhibitors. However on the basis of our experiments with co-expressed proteins we suggest these proteis can also be active as heterodimers. Taken together, we suggest that they differ essentially in their function and physiological role from ABC transporters investigated before.

• *Cserepes J, Szentpetery Z, Seres L, Ozvegy-Laczka C, Langmann T, Schmitz G, Glavinas H, Klein I, Homolya L, Varadi A, Sarkadi B, Elkind NB (2004) Functional expression and characterization of the human ABCG1 and ABCG4 proteins: indications for heterodimerization. Biochem Biophys Res Commun 320: 860–867.*
• *Seres L, Cserepes J, Elkind NB, Töröcsik D, Nagy L, Sarkadi B, Homolya L (2008) Functional ABCG1 expression induces apoptosis in macrophages and other cell types. Biochim Biophys Acta 1778: 2378–2387.*

LEVENTE DEÁK (2006)

Influence of the second messenger system on the motor protein of the outer hair cell

Supervisor: József Mandl

Outer hair cells in the mammalian organ of Corti express a unique feature called "electromotility," which is thought to provide the local active mechanical amplification of the cochlear response to sound. A widely accepted explanation for the cellular mechanism of electromotility is that a unique motor protein (prestin) in the OHC changes conformation in response to the OHC's receptor potential, leading to a change in OHC length and stiffness. In the past, it had been shown that the acetylcholine and the second messenger system can influence the OHC electromotility.

The objective of this dissertation was to investigate whether the second messenger cascade (cAMP and cGMP) acts on prestin directly or on other membrane-bound cytoskeletal proteins which secondary affect electromotility. Also we identified two positions on the prestin molecule that are potential cAMP/cGMP–dependent protein kinase G (PKG) phosphorylation sites. Whether these sites are involved in cGMP-dependent reactions is as yet unknown. To address this question, we have established a heterologous system to study prestin function and its potential modification. In this system, prestin cDNA is transiently transfected into a human embryonic kidney cell line, TSA 201. Cells that express prestin are selected to measure nonlinear capacitance (NLC), a signature of outer hair cell motility. Different chemicals, including a cAMP and cGMP analog and a protein kinase G blocker are applied to the transfected cells.

Taken together, this dissertation presents further evidence that (1) prestin plays an active role in the second messenger cascade regulation, (2) two active potential PKG phosphorylation sites had been identified and (3) prestin might be working as a direct cyclic gated transducer.

- *Deák L, Zheng J, Orem A, Du GG, Aguinaga S, Matsuda K, Dallos P (2005) Effects of cyclic nucleotides on the function of prestin. J Physiol 1563: 483–496.*
- *Zheng J, Du G, Matsuda G, Orem A, Aguinaga S, Deák L, Navarrete E, Madison L, Dallos P (2005) The C terminus of prestin influences nonlinear capacitance and plasma membrane targeting. J Cell Sci 118: 2987–2996.*

DÁNIEL ERŐS (2006)

Structure-activity/property relationships of kinase inhibitors based on calculated and measured parameters, and applications in drug design and development

Supervisor: György Kéri

In my thesis I demonstrated the necessity of the application of QSAR/QSAPR which is one of the most important branches of rational drug design. The theoretical and pactical aspects of QSAR/QSPR was reviewed. The second part of the thesis dealed with concrete QSAR and QSPR methods. Development of these models aimed at the finding of potential tyrosine-kinase inhibitors from large molecular libraries applying virtual screening.

In the second chapter I reviewed the main branches of rational drug design, I briefly showed the main aspects of structure based design and ligand based design (2D, 3D QSAR, CoMFA, etc.).
In the third chapter I demonstrated the theory and the methods of QSAR and QSPR in detail.
In the fourth chapter I showed the most important predictive models that were prepared during my Ph. D. work. Two of them were QSPR (logP, logS) and three of them were QSAR (EGF receptor inhibition, kinase inhibitor-likeness and mutagenicity) models.

- *Erős D, Kövesdi I, Őrfi L, Takács-Novák K, Acsády G, Kéri G (2002) Reliability of logP predictions based on calculated molecular descriptors: A critical review. Curr Med Chem 9: 1819–1829.*
- *Erős D, Kéri G, Kövesdi I, Szántai-Kis Cs, Mészáros G, Őrfi L (2004) Comparison of predictive ability of water solubility QSPR models generated by MLR, PLS and ANN Methods. Mini Rev Med Chem 4: 167–177.*
- *Erős D, Őrfi L, Kéri G (2004) Rational drug design and signal transduction therapy. Pharmacem 1/2: 27–30.*

SIMON FISCHER (2008)

Inflammatory bowel diseases: genetic markers and their use in predicting disease course, response to treatment and need for surgery

Supervisor: Péter László Lakatos

Inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are well described medical conditions; however, the pathogenetic mechanisms behind them are still obscure. The three tenets of their development are environmental factors, luminal gut flora, and genetic susceptibility. While much research has been invested in deciphering the exact cause, no specific environmental factors have been found, variations in luminal gut flora have been found, and some genes have been positively correlated with IBD. Certainly, much research is still needed.
This thesis consisted of two separate, yet contextually related studies that investigated the association between five genetic polymorphisms and disease behavior, response to therapy, and need for surgery, in a Hungarian IBD cohort. The specific gene variants included: DLG5 R30Q, MDR1 C3435T and G2677T/A, and ABCG2 G34A and C421A. In the first study, focusing on DLG5 R30Q, we also tested for any association with other polymorphisms in IBD susceptibility genes, NOD2/CARD15 and TLR4.
In the first study, a cohort of 773 unrelated IBD patients (CD: 639, UC: 134) and 150 healthy controls were genotyped for DLG5 R30Q, TLR4 D299G, and NOD2/CARD15 SNP8, SNP12, and SNP13. DLG5 and TLR4 variants were detected using polymerase chain reaction/restriction fragment length polymorphism, while NOD2/CARD15 mutations were detected using denaturing high-performance liquid chromatography. In the second study, MDR1 and ABCG2 polymorphisms were detected using real-time polymerase chain reaction with the LightCycler equipment. The protocol was developed specifically for this study. The study's population consisted of 414 unrelated IBD patients (CD: 265, UC: 149) and 149 healthy control subjects.
There were no significant differences in carriage frequencies of DLG5, ABCG2 or MDR1 variants. However, a trend was observed for the MDR1 G2677 allele to be associated with

disease susceptibility in CD patients. The two MDR1 variants were linked to each other in IBD, CD, UC and controls. The MDR1 2677TT was associated with 3435CC, 2677GT with 3435CT, and 2677GG with 3435TT. In ulcerative colitis, carriage of the ABCG2 allele appeared to be protective against arthritis. The DLG5 R30Q variant was significantly associated with steroid resistance. Perianal disease and frequent relapses were independently associated with steroid resistance. While no genotype-phenotype associations could be made, a trend for the DLG5 variant was observed in extensive UC disease.

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JUDIT GOMBÁS (2008)

Blood plasma proteins and cellular components as modulators of fibrinolysis

Supervisor: Krasimir Kolev

In recent years it has become clear that thrombolysis is not identical with fibrinolysis. The models of thrombolysis take into consideration the composition of *in vivo* thrombi, the concentration of various molecules in the thrombus compartment and the flow conditions. We investigated the effects of different macromolecules present at high molar concentration in the blood plasma or in thrombi on the fibrinolytic process. The contribution of neutrophil leukocyte elastase (NE) and α_1-protease inhibitor (α_1-PI) to in vivo thrombolysis was also examined.

Immunoglobulin G isolated from normal plasma (N-IgG) stabilizes the structure of fibrin clot and prolongs the time for its dissolution under static and flow conditions. The IgG from patients suffering in antiphospholipid syndrome (APS) exerts a stronger antifibrinolytic effect. The prolongation of the lysis time with the APS-IgG is up to 6-fold compared to the pure fibrin. These effects are caused by the variable Fab portion of the IgG molecules. The IgGs do not affect the amidolytic activity of plasmin, but the APS-IgGs obliterate the competition of the fibrin and the peptidyl-p-nitroanilide for the protease suggesting interference of the IgGs with the plasmin action on the fibrin.

The combined modulating effects of IgGs and phospholipids (PLs) were investigated in the elementary steps of the fibrinolytic process. In fibrin clots containing PLs the N-IgG enhanced its fibrinolytic resistance to the diffusion of tissue-type plasminogen activator (tPA) and to plasminogen activation, but did not modify the lysis by plasmin. The impact

of APSIgGs, unlike the pattern of N-IgG effects, can be either pro- or antifibrinolytic in the consecutive steps of the fibrinolysis. Myosin also renders thrombi more resistant to fibrinolytic agents. It forms reversible noncovalent complexes with fibrinogen (K_d=1.35–1.70 μM), and both the association and the dissociation phases of the interaction are slow (ka=180.6 $M^{-1}s^{-1}$; kd=3.07x$10^{-4}s^{-1}$).

In the thrombus compartment the "α_1-PI+NE ↔ α_1-PI–NE" reaction seems to be second order and not pseudo-first order as generally assumed. The elevated levels of α_1-PI are associated with NE-mediated suppressed plasma fibrinolytic potential. The inhibition of thrombin activity by higher α_1-PI-level results in a coarse fibrin structure more resistant to the fibrinolytic process.

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ANDRÁS ILLÉS (2006)

Investigation of cortactin function in different signalisation pathways

Supervisor: László Buday

Cortactin was discovered as a substrate of Src kinase. It has been well established that cortactin is an activator of the Arp2/3 complex, the initiator of the branching actin polymerization. Therefore, it plays crucial role in the regulation of several Arp2/3 complex dependent processes, such as cell motility, intracellular movement of pathogens, or endocytosis. Although cortactin was discovered 16 years ago, little is known about its regulation and the reasons of its translocation to cell periphery, moreover about the role of its tyrosine- or serine-phosphorylation. Therefore, we aimed to examine the role of cortactin phosphorylation in its translocation or activation in several signalisation pathways, as well as to verify observations that the Rac effector PAK kinase can phosphorylate cortactin, in this manner making a direct connection between Rac and cortactin.

We wanted to investigate whether cortactin is involved in the integrin pathway and to test the influence of dominant negative cortactin constructs, as well as the cortactin silencing with siRNA in the integrin α5β1-dependent cell spreading.

Phorbol ester treatment of HepG2 cells also leads to cortactin translocation. Therefore it makes another possibility for the examination of cortactin activation. In our experiments, we found that phosphorylation of cortactin on its serine or tyrosine residues is not necessary for cortactin translocation or cortical actin polymerisation in short-term EGF stimulation. We also found that cortactin is not a substrate for the PAK1 serine/threonine kinase. Using dominant negative constructs of cortactin, or siRNA technique, we established a role for cortactin in integrin signalling. We demonstrate that cortactin is necessary for the integrin-mediated cell adhesion and spreading, and the inhibition of cortactin function, or expression eventuate robust dysfunction in the integrin signalling.

Using a cell migration model, in HepG2 cells, we found that, although PKC-activator phorbol ester treatment results in cortactin tyrosine phosphorylation in an Src-dependent

manner, this phosphorylation is not necessary for cortactin translocation, and what's more, this is a PI3-kinase-, or Rac-independent process. We suppose that interaction of a currently unknown, cell-specific substrate of PKC with cortactin may contribute to the translocation of cortactin to the cell periphery.

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ÉVA KERESZTURI (2007)

Functional analysis of the dopamine D4 receptor gene promoter region

Supervisor: Mária Sasvári

The human dopamine D4 receptor (DRD4) gene has been extensively studied as a candidate gene for attention deficit hyperactivity disorder (ADHD). Both the 5' regulatory region and the coding sequence contain a number of polymorphisms. The promoter variants have received particular attention in the past few years due to their possible role in the regulation of gene expression. In these theses I describe an association analysis of the 120 bp duplication and three SNPs (–616 C/G, –615 A/G, –521 C/T) in the 5' region of the DRD4 gene are presented among children with ADHD. No preferential allele transmission could be demonstrated when investigating the four polymorphisms separately using the Transmission Disequilibrium Test (TDT). In case-control approach, however, the 1-repeat form of the 120 bp duplication had a significantly higher frequency among ADHD children than controls, both in allele- ($p=0.047$), and genotype ($p=0.019$) distributions. When studying haplotype distribution among ADHD children, the preferential accumulation of the 1-C-A-T combination of promoter polymorphisms was observed ($p=0.002$).
To understand better the transcriptional regulation of the DRD4 gene, a series of 5' promoter deletion mutants were created. Transcriptional activity of the promoter constructs was determined by transient transfection of luciferase reporter vectors. The activity profile of these promoter fragments was similar in each of the cell lines tested. The highest luciferase reporter activity was obtained with a construct containing promoter sequences between nucleotides –668 to –389, while a putative silencer region was localised spanning from nucleotide –1571 to –800. The three SNPs had no significant effect on transcriptional activity. The different 120 bp duplication alleles (1-repeat, 2-repeat and the newly identified 4-repeat allele) were found to have an effect on transcriptional activity of the DRD4 gene in both neuroblastoma and retinoblastoma cell lines in a dose-dependent manner. The higher was the repeat number of the 120 bp sequence in the promoter, the stronger was the decrease in gene transcription (1-repeat>2-repeat>4-repeat; $p<0.01$). These results from association and functional analyses suggest that the 1-repeat form of the 120 bp duplication might be a risk factor of ADHD, especially in the homozygous form and/or in the context of certain haplotypes.

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ORSOLYA KIRÁLY (2007)

Pathobiochemistry of pancreatic secretory trypsin inhibitor (SPINK1)

Supervisor: Miklós Sahin-Tóth

Pancreatitis is caused by the premature activation of digestive zymogens in the pancreatic parenchyma and the resulting autodigestion of the pancreas. While most cases of the disease are associated with environmental factors, hereditary pancreatitis is caused by mutations in genes involved in pancreatic function. Pathogenic mutations in human cationic trypsinogen cause a gain of function by increasing autoactivation. Conversely, disease-associated mutations in the pancreatic secretory trypsin inhibitor (SPINK1) are believed to be loss-of-function mutations but their functional effects are largely unknown.

The aim of the present study was to undertake a systematic functional analysis of missense SPINK1 mutations associated with pancreatitis. We have identified a new mutation in the signal peptide of SPINK1, L14R, as well as a polymorphic variant, L12F. Mutation L14R and the previously reported L14P mutation cause autosomal dominant hereditary pancreatitis. We studied the functional effects of SPINK1 variants in transiently transfected HEK 293T cells. Mutations L14P and L14R abolish SPINK1 secretion, while the L12F variant has no effect. Missense mutations N34S, R65Q, R67C, P55S, D50E and Y54H in the mature protein have no major effect on trypsin inhibitory activity, but mutations R65Q, R67C, D50E and Y54H impair secretion of SPINK1 by causing intracellular retention and degradation of mutant proteins, probably due to mutation-induced misfolding. The most frequently studied mutation in SPINK1, N34S, and the polymorphic variant P55S had no effect on either trypsin inhibitory activity or SPINK1 secretion. Thus, the functional effect of the N34S mutation remains unknown and might be caused by the intronic mutations reported to be in linkage disequilibrium with N34S.

Our results show that disease-associated mutations in SPINK1 are indeed loss-of-function mutations impairing SPINK1 function in the pancreas. Missense mutations in the signal peptide and in the mature protein both result in decreased SPINK1 secretion and thus share a common pathomechanism in generating a protease-antiprotease imbalance that results in susceptibility to pancreatitis.

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IMRE KOVÁCS (2006)

Clinical significance of flow-mediated dilatation (FMD) of brachial artery in evaluation of endothel function and efficacy of therapeutic interventions

Supervisor: Albert Császár

Impairment of vascular function constitutes the first phase of the development of vascular diseases. Therefore, endothelial function, i.e. functional status of the vasculature is very important in the clinical practice. This can be studied by measuring the flow-mediated dilatation (FMD) in the large vessels. We have investigated FMD in different cardiovascular patients belonging to primary and secondary prevention risk groups. Furthermore, we studied the efficacy of non-pharmacological and pharmacological interventions in patient groups mentioned above in order to follow the changes in endothel function and correlate them with the severity of disease. We demonstrated in patients with combined dyslipidaemia that their FMD is abnormal, whereas the levels of cytokines, acute phase proteins and adhesion molecules are significantly elevated compared to healthy controls. Ciprofibrate has normalized FMD within a short period of time, supposedly due to its anti-inflammatory effect. We have found that elevated cytokine and acute phase protein (CRP) levels in postinfarction patients with reduced left ventricle function correlate with the severity of endothel dysfunction. Treatment with quinapril, a tissue ACE inhibitor, resulted in significant FMD improvement and elicited anti-inflammatory action compared to enalapril. Thus tissue ACE inhibition has a central role in protection of endothel function by ACE inhibitors. We demonstrated that severity of FMD impairment is closely related to the 5-year incidence of cardiovascular events, therefore, has an importance in early prognosis. Alos, BMI, CRP and FMD are closely interrelated. A relationship has been demonstrated between inflammation caused by visceral obesity and endothel dysfunction caused by inflammation and that regular dynamic training has an anti-inflammatory effect. Furthermore, we have found that in patients with erectile dysfunction, but without known vascular risk factors there is a positive correlation between severity of FMD and erectile dysfunction. Overall these findings provided evidence for a close correlation between endothel dysfunction (characterised by FMD), vascular risk factors and inflammatory markers. Therefore, FMD is suitable for determination of vascular diseases prognosis and evaluation of the efficacy of therapeutic interventions.

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ZOLTÁN KUKOR (2006)

Characterisation of trypsinogen and mutations of trypsinogen activation peptide and their role in the pathogenesis of hereditary chronic pancreatitis

Supervisor: Miklós Tóth

Introduction: Incidence of chronic pancreatitis is 3.5–35/100000 in advanced countries, 10–20% of the cases are hereditary pancreatitis. The initial step of pancreatitis is premature trypsin generation in the pancreas, that can activate pancreatic digestive enzymes and initiate inflammation processes. Three trypsinogen isoforms are expressed in the pancreas. Two forms—the cationic trypsinogen (CatTg) and the anionic trypsinogen (AnTg)—express in bulk, the third one, mesotrypsinogen is approximately 5% of the total trypsinogen quantity. The ratio of CatTg vs. AnTg is 2/1 under physiological conditions, that can change to 1/2 in pancreatitis. The first pancreatitis associated mutation, CatTg R122H was described in 1996, followed by about 20 others later. **Aims:** (1) Biochemical characterization of recombinant human pancreatic trypsinogens. (2) *In vitro* investigation of CatTg-AnTg mixtures of physiological (2/1) and pathological (1/2) composition. (3) Characterization of mutations of trypsinogen activation peptide (TAP) of CatTg. (4) Validation and characterization of the inhibitory property of CatTg cleaved at Arg122 (CatTg*) on trypsin activity. **Methods:** Determination of trypsin activity was based on the cleavage of a chromogenic substrate, activation and degradation of trypsinogen was studied by gel electrophoresis. For analysis of activation and autolysis pancreatic acinar (pH 8.0, absence of Ca^{2+}), pancreatic lysosomal (pH 5.0) and duodenic (pH 8.0, 1 mM Ca^{2+}) conditions were used. **Results:** In contrast to the CatTg, AnTg suffers rapid autodegradation in Ca^{2+} deficient, pH=8.0 medium whereas at pH=5.0 its autoactivation is extremely slow, therefore under these conditions only trace amounts of trypsin is formed. In acinar conditions the trypsin activity of pathological composition of trypsinogens is roughly half of that of the physiological composition. Significant change in the duodenal milieu was not found. The mutations of TAP (D19A, D22G, K23R) cause increased autoactivation under all of the conditions studied. Hydrolysis of the Arg122-Val123 peptide bond of CatTg by trypsin is followed by resynthesis of the bond. Accordingly, Arg122 is not an initiation site for autolysis, rather the CatTg* behaves as a unique canonical inhibitor of trypsin. The CatTg ↔ Tg1* conversion is Ca^{2+} dependent, absence of Ca^{2+} favors CatTg* formation. CatTg* is a weak inhibitor, Ki = 80 μM. **Conclusions:** (1) AnTg is rapidly degraded or inactivated under acinar conditions of pancreas whereas CatTg is less affected. (2) Change of the 2/1 ratio of CatTg vs. AnTg to 1/2 could be protective for the pancreas, because it decreases the pancreatic trypsin level. (3) The TAP mutations of CatTg lead to increased rate of autoactivation resulting in an augmented risk of pancreatitis. (4) The behavior of human CatTg* as a protease inhibitor is a genuine and unique observation. The CatTg R122H mutation causes loss of this inhibitor property and may therefore be a risk factor of chronic pancreatitis.

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ROZÁLIA LACZKÓ (2007)

The role of lysyl oxidase in astrocytic tumor progression, and the importance of lysyl oxidase and fibronectin interaction

Supervisor: Balázs Sarkadi

Lysyl oxidase (LOX) is a copper-containing amine oxidase known to catalyze the covalent cross-linking of fibrillary collagens and elastin. Recent implication of LOX in cancer, wound healing, cell motility, chemotaxis and differentiation reflects its remarkable functional diversity. Few recent reports have indicated the role of LOX in the central nervous system pathologies. Results of this study demonstrate up-regulation and increased activity of LOX in anaplastic astrocytoma cells that positively correlates with invasion of malignant astrocytes. Immunohistochemistry revealed increased LOX expression in grade I–IV astrocytic neoplasms compared to normal brain and coincidence of increased LOX with the loss of astrocyte marker, GFAP in higher-grade tumors. Increased activity and expression of LOX in invasive astrocytes were accompanied by phosphorylation of FAK and paxillin, furthermore, both FAK and paxillin tyrosine phosphorylations were diminished by inhibition of LOX activity and depletion of peroxide, a byproduct of LOX catalytic activity. Additionally, LOX is likely processed by bone morphogenic protein-1 in anaplastic astrocytes and LOX activity might be further stimulated by fibronectin (FN) expressed by these cells. FN, as a binding partner of LOX, was identified by our previously performed yeast two hybrid screen, therefore we further investigated this novel regulation mechanism. GST pull-downs and solid phase binding assays confirmed LOX-FN interaction. LOX bound to the cellular form of FN (cFN) with high affinity comparable to the affinity of LOX binding to its substrates, tropoelastin and type I collagen, while LOX demonstrated a much lower binding affinity to the plasma form of FN (pFN). *In vivo* co-localization of FN and LOX was detected in various normal human tissues by immunohistochemistry such as lung, esophagus, kidney, stomach, heart and vascular tissues. *In vitro* LOX activity assays showed that cFN is not a substrate of LOX, but in FN-null mouse embryonic fibroblasts, we observed dramatically decreased LOX processing and enzyme activity. Therefore, we hypothesize that FN acts as a scaffold for enzymatically active LOX. Overall, our studies on anaplastic astrocytes demonstrated a LOX mediated mechanism that promoted migratory/ invasive behavior of malignant astrocytes, and results of the LOX-FN interaction studies suggested that the FN matrix provides specific microenvironment to regulate LOX catalytic activity.

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BEÁTA LIZÁK (2008)

The role of translocon peptide channel in the transport of small molecules across the endoplasmic reticulum membrane

Supervisor: Gábor Bánhegyi

Several enzyme activities localized in the luminal compartment of the ER require the transmembrane fluxes of their substrates, products and/or cofactors. Accordingly, a variety of structurally unrelated compounds is (or should be) able to cross the ER membrane, but only a few transporters have been reported so far. A low affinity, low capacity transporter with low selectivity might have an important physiological function. Cotranslational protein translocation and integration into the rough ER membrane occur at sites termed translocon. Translocon is composed of the Sec61 heterotrimer protein complex which forms a large aqueous pore in the membrane. At the end of translation most of the ribosomes do not dissociate from the channel, therefore a free pore remains transitionally in the membrane of the ER. Previous works on cell experimental systems indicated that a small, neutral molecule and Ca^{2+} permeate the translocon. Contribution of translocon peptide channel in the aspecific transport of low molecular weight anions (UDP-glucuronic acid, hexose-phosphates) was investigated in rat liver microsomes. Puromycin, an antibiotic which purges the translocon from the nascent peptide and create additional empty pores, raised the microsomal uptake of radiolabelled UDP-glucuronic acid, while did not increase the uptake of glucose-6-phosphate or glutathione. The transport of small anions was also envisaged by measuring the activity of microsomal enzymes with intraluminal active sites. The activities of UDP-glucuronosyltransferase and mannose-6-phosphatase were elevated upon addition of puromycin, but glucose-6-phosphatase and β-glucuronidase activities were not changed. The increase in enzyme activities was due to a better access of the substrates to the luminal compartment rather than to activation of the enzymes. The effect of puromycin could be prevented by the addition of its antagonist anisomycin, an antibody against Sec61 translocon component and the release of ribosomes. UDP-glucuronosyltransferase and mannose-6-phosphatase activities of smooth microsomal vesicles showed higher basal latencies, which were not affected by puromycin. In conclusion, translationally inactive, ribosome-bound translocons allow small anions to cross the ER membrane. This pathway can contribute to the non-specific substrate supply of enzymes with intraluminal active centers.

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MARIANNE VARSÁNYI (LÓTOSNÉ) (2008)

The role of FAD transport in oxidative protein folding in rat liver microsomes

Supervisor: Gábor Bánhegyi

The oxidative folding of secretory and membrane proteins takes place in the luminal compartment of the endoplasmic reticulum in eukaryotic cells. The electrons generated in the process are transferred to molecular oxygen by an electron transfer chain. Although the protein components of the chain are well known, the role of low molecular weight redox-active compounds is less elucidated. The aim of our work was to investigate the role of FAD and ascorbate in the process. The transport of FAD and its effect on disulfide bond formation was investigated in rat liver microsomal vesicles. By measuring the intravesicular FAD accessible space we observed that FAD permeates across the microsomal membrane and accumulates in the lumen. Rapid filtration experiments also demonstrated the uptake and efflux of the compound, which could be inhibited by atractyloside and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid. FAD entering the lumen promoted the oxidation of protein thiols and increased the intraluminal oxidation of glucose 6-phosphate. The uptake of FAD was necessary for the intraluminal oxidation of thiols and glucose 6-phosphate; the inhibition of the transport prevented the oxidative effects of FAD. The main characteristics of microsomal FAD transport are different from those described in mitochondria. These findings support the notion that, similarly to yeast, free FAD may have an important role in the mechanism of oxidative protein folding in the endoplasmic reticulum lumen of mammalian cells.

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ÉVA MARGITTAI (2007)

Endoplasmic reticulum stress in scurvy

Supervisor: Miklós Csala

Disulfide bond formation, a major co-/posttranslational modification of secretory proteins, takes place in the lumen of the endoplasmic reticulum (ER) in mammalian cells. Oxidation of thiol groups to disulfide bonds in nascent proteins is heavily dependent on the oxidative environment in the lumen of the ER. This oxidative environment is also reflected by the thiol redox potential, which is significantly higher in the ER lumen than in the cytoplasm. However, the particulars of the generation and maintenance of this environment are still unknown. Our *in vitro* experiments provided convincing evidence for the participation of ascorbate (vitamin C) and tocopherol (vitamin E) in the oxidative protein folding. The aim of the present study was to investigate the role of these antioxidant molecules in the oxidative protein folding *in vivo*. We wanted to verify the hypothesis by investigating the appearance of ER stress and apoptosis in ascorbate- or tocopherol-deficient animals.

Vitamin E deficiency and scurvy was induced with a long-term vitamin E- and ascorbate-free diet in rats and guinea pigs. The elements of the ER stress, the induction of chaperones and foldases, were demonstrated in the liver of scorbutic guinea pigs. The ER stress anticipated the appearance of increased lipid peroxidation caused by the missing antioxidant action of ascorbate. Besides the ER stress, an increased apoptosis was also observed in the liver. Both ER stress and apoptosis provoked by ascorbate deficiency could be reversed by ascorbate readdition. Neither the ER stress, nor the increased apoptosis was observed in tocopherol deficient rats. The present results confirm our previous *in vitro* observations and show that ascorbate is important in the oxidative protein folding also *in vivo*. It is also concluded that tocopherol can be substituted by alternative electron carriers in the ER membrane; therefore this antioxidant is dispensable with regards to the oxidative protein folding.

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ATTILA MOLVAREC (2007)

Serum levels and genetic polymorphisms of the 70 kDa heat shock protein in preeclampsia

Supervisor: István Karádi

Heat shock proteins (Hsps) are primarily known to be intracellular proteins with molecular chaperone and cytoprotective functions. However, Hsp60 and Hsp70 are present in the peripheral circulation of healthy non-pregnant individuals. Nevertheless, little is known about circulating Hsp70 in physiological and pathological pregnancies. Therefore, we determined serum Hsp70 concentrations of healthy pregnant and non-pregnant women, as well as of preeclamptic patients and patients with HELLP syndrome using a sandwich enzyme-linked immunosorbent assay. In healthy pregnant women, preeclamptic patients and patients with HELLP syndrome, we measured also serum concentrations of three acute phase proteins: C-reactive protein (with particle-enhanced immunoturbidimetric assay), α_2-macroglobulin and α_2-HS glycoprotein (with radial immunodiffusion). In addition, we determined genotypes of HspA1B (Hsp70-2) 1267A>G and HspA1L (Hsp70-Hom) 2437C>T single nucleotide polymorphisms in healthy pregnant women and preeclamptic patients using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

According to our results, Hsp70 is present in the peripheral circulation of healthy pregnant individuals. Serum Hsp70 concentrations were significantly lower in healthy pregnant women than in healthy non-pregnant women and showed a positive correlation with gestational age and a negative correlation with maternal age. Body mass index, smoking status, as well as systolic and diastolic blood pressure did not influence serum Hsp70 levels in pregnancy. The capacity of extracellular Hsp70 to elicit innate and adaptive proinflam-

matory (Th1) immune responses might be harmful in pregnancy and lead to immune rejection of the fetal semi-allograft. We hypothesize that extracellular Hsp70 is removed by the activated innate immune system in physiological pregnancy. Decreased circulating Hsp70 level, as a consequence, may promote the maintenance of immunological tolerance to the fetus.

Serum Hsp70 levels were significantly higher in preeclamptic patients compared to normotensive, healthy pregnant women. However, the severity, early or late onset of the disease, as well as the presence or absence of fetal growth restriction did not affect serum Hsp70 concentrations in preeclamptic patients. Serum Hsp70 levels of patients with HELLP syndrome were significantly higher than those of severely preeclamptic patients. Serum Hsp70 concentration correlated inversely with platelet count in the HELLP group, which also suggests a positive correlation of serum Hsp70 concentration with severity of the disease. Nevertheless, the source of soluble Hsp70 in physiological and pathological pregnancies is unknown. Elevated circulating Hsp70 level may not only be a marker of preeclampsia and HELLP syndrome, but might also play a role in their pathogenesis. Extracellular Hsp70 derived from stressed and damaged, necrotized cells elicits a proinflammatory (Th1) immune response, which might be involved in the development of maternal systemic inflammatory response and resultant endothelial damage in preeclampsia and HELLP syndrome.

Serum α_2-HS glycoprotein (AHSG) concentrations of preeclamptic patients were significantly lower than those of healthy pregnant women, which along with the significantly higher serum CRP levels supports the presence of maternal systemic inflammatory response in preeclampsia. We found significantly higher CRP and significantly lower AHSG serum concentrations in the HELLP group than in the severely preeclamptic group, which indicates a much more intensive systemic inflammation in HELLP syndrome than in severe preeclampsia without HELLP syndrome. However, it is still unclear whether these changes are causes or consequences of HELLP syndrome. Serum levels of CRP and AHSG did not correlate with serum Hsp70 concentrations in healthy pregnant women, preeclamptic patients, as well as in patients with HELLP syndrome, suggesting lack of a strong association between elevated serum Hsp70 levels and systemic inflammation in preeclampsia and HELLP syndrome. Serum α_2-macroglobulin concentrations did not differ significantly among the three groups.

There was no association between Hsp70-2 1267A>G and Hsp70-Hom 2437C>T single nucleotide polymorphisms and preeclampsia. Furthermore, the two polymorphisms were not associated with serum Hsp70 levels in either healthy pregnant women or preeclamptic patients.

However, it requires further investigation to explain the observed differences in serum Hsp70 levels between healthy pregnant and non-pregnant women, as well as to determine whether elevated serum Hsp70 level precedes the development of preeclampsia and HELLP syndrome and thus can help predict these serious conditions in pregnancy.

- *Molvarec A, Prohászka Z, Nagy B, Szalay J, Füst G, Karádi I, Rigó JJr (2006) Association of elevated serum heat shock protein 70 concentration with transient hypertension of pregnancy, preeclampsia and superimposed preeclampsia: a case-control study. J Hum Hypertens 20: 780–786.*
- *Molvarec A, Prohászka Z, Nagy B, Kalabay L, Szalay J, Füst G, Karádi I, Rigó JJr (2007) Association of increased serum heat shock protein 70 and C-reactive protein concentrations and decreased serum α_2-HS glycoprotein concentration with the syndrome of hemolysis, elevated liver enzymes, and low platelet count. J Reprod Immunol 73: 172–179.*

GÁBOR NAGY (2008)

Acetaminophen-induced redox stress in the endoplasmic reticulum

Supervisor: József Mandl

My study focused on the possible role of the hepatic endoplasmic reticulum in acetaminophen-induced liver damage. Sublethal dose of AAP caused an altered redox status of the ER. I observed that active thiols of the most prominent luminal oxidoreductases, protein disulfide isomerase and ERp72, became oxidized upon AAP insult. As a novelty, I measured microsomal glutathione levels instead of measuring whole cellular glutathione in AAP-toxicity, and observed a significant decrease in both total microsomal glutathione and reduced-to-total ratio. Several components of the ER stress response were activated. Proteolytical activation of ATF6, phosphorylation of eIF2α and JNK were detected. Transcriptional activation and elevated expression of GADD153/CHOP, an ER stress-responsive proapoptotic transcription factor, was observed upon AAP addition. Transient activation of the ER-resident caspase-12 was shown followed by an elevation in procaspase-12 level. Caspase-3 and caspase-8 activation could not be detected. AAP treatment resulted in an increased apoptosis of hepatocytes. Buthionine-sulfoximine treatment was unable to mimic the effects of AAP indicating that glutathione depletion alone is insufficient to provoke apoptosis. These results show that intraluminal redox imbalance of the ER and consequential activation of signaling processes and proapoptotic events are involved in hepatocellular damage caused by AAP overdose. Several components of ER stress response were observed on mouse hepatoma cells upon AAP, without decrease of cellular glutathione. My results give clear evidence for the involvement of the ER in AAP-induced hepatocellular damage. Luminal redox imbalance, activation of certain UPR components, proapoptotic signaling are the most prominent features of an active ER involvement.

- *Nagy G, Kardon T, Wunderlich L, Szarka A, Kiss A, Schaff Zs, Banhegyi G, Mandl J (2007) Acetaminophen induces ER dependent signaling in mouse liver. Arch Biochem Biophys 459: 273–279.*
- *Kardon T, Nagy G , Csala M, Kiss A, Schaff Z, Nagy PL, Wunderlich L, Banhegyi G, Mandl J (2006) Influence of BGP-15, a nicotinic amidoxime derivative, on the vascularization and growth of murine hepatoma xenografts. Anticancer Res 26: 1023–1028.*
- *Banhegyi G, Csala M, Nagy G, Sorrentino V, Fulceri R, Benedetti A (2003) Evidence for the transport of glutathione through ryanodine receptor channel type 1. Biochem J 376: 807–812.*

MELINDA OROSZLÁN (2007)

Interaction of C1q, mannose binding lectin and creative protein on endothelial cells, their role in endothelial dysfunction

Supervisor: László Romics

The endothelium is a physical barrier between the tissues and the blood. Disturbed balance between the anti- and proinflammatory phases alters the biological activities of endothelial cells and induces endothelial dysfunction, which is the initial step of atherosclerosis.

Endothelial cells are exposed to humoral proteins in blood such as complement proteins and acute phase proteins.

The goal of the study was to investigate the interaction of C1q, the recognition molecule of classical complement cascade, mannose binding lectin (MBL), the recognition molecule of lectin pathway, and one of the acute phase proteins, the complement activating C-reactive protein (CRP) to endothelial cells (ECs) and evaluate their role in endothelial dysfunction. All three molecules are supposed to contribute to the pathogenesis of atherosclerosis. However, either their role is controversially discussed or there is a paucity of data concerning to their exact role.

The proinflammatory role of C-reactive protein has been controversially discussed. We determined the role of carefully characterized and LPS-free native and modified CRP in the activation of ECs. None of the CRP variants induced increased expression of accepted proinflammatory markers such as ICAM-1, VCAM-1, E-selectin, IL-8 and MCP-1. C1q is known as a potent proinflammatory stimulus of ECs. Our *in vitro* experiments proved that abundant extracellular matrix proteins decorin and biglycan—which have been shown to regulate the classical complement pathway—strongly inhibited the binding of C1q to ECs. Furthermore, biglycan suppressed C1q-induced MCP-1 and IL-8 production by ECs. However, much less is known about the cellular interactions of the structurally similar molecule, MBL. We showed that MBL exhibited a dose-dependent binding to ECs under calcium-free conditions, which indicates an indirect way of involvement of its calcium-independent collagenous domains. We showed in cross-competition experiments that the binding can be inhibited by C1q and vice versa, suggesting that MBL and C1q recognize shared receptor(s) on the endothelial cell surface. ECs activated with LPS activation showed increased C1q and MBL binding and TNF-α led to elevated C1q binding. In contrast to C1q, stimulation of ECs with MBL did not result in a detectable increase in the expression of ICAM-1 and production of IL-6, IL-8 and MCP-1.

The complement system is a key component of the innate immune system involved in protection against invading pathogens. However, their uncontroled activity might contribute to the pathogenesis of several inflammatory diseases such as cardiovascular and autoimmune diseases.

In summary, our findings do not support the notion that different isoforms of CRP alone have significant effects on inflammation of the vessel wall via interacting with endothelial cells. We hypothesize that local expression of decorin and biglycan might regulate the proinflammatory role of C1q and thereby attenuate the inflammation and limit tissue damage. Furthermore, we speculate that binding of MBL to endothelial cells might be involved in the non-inflammatory clearance of atherogenic antigens via endothelial cells, which might indicate a protective role of MBL in the pathogenesis of atherosclerosis.

- *Oroszlan M, Herczenik E, Rugonfalvi-Kiss S, Roos A, Nauta AJ, Daha MR, Gombos I, Karadi I, Romics L, Prohaszka Z, Fust G, Cervenak L (2006) Proinflammatory changes in human umbilical cord vein endothelial cells can be induced neither by native nor by modified CRP. Int Immunol 18: 871–878.*
- *Oroszlan M, Daha MR, Cervenak L, Prohaszka Z, Fust G, Roos A (2007) MBL and C1q compete for interaction with human endothelial cells. Mol Immunol 44: 1150–1158.*

ESZTER PAPP (2006)

Changes of the redox state of the endoplasmic reticulum and their consequences during stress

Supervisor: Péter Csermely

Stress response is an important and highly conserved phenomenon of cellular defense. Stress proteins protect from oxidative injuries, increase defense against neurodegenerative diseases and cancer.

The endoplasmic reticulum contains many specific stress proteins. These proteins and their complexes assist in transport, folding, posttranslational modification and complex assembly of secreted proteins. One of the most important, specific luminal processes is the oxidative disulfide-bond formation, which is performed by the chaperone PDI in complex with its oxidizer protein, Ero1-L. The generation of the redox cycle and the contribution of small molecules in the process is not known yet. During my Ph. D. work I examined which small molecules can accept electrons from the redox folding machinery, maintaining its oxidase capacity in mammalian systems. I also investigated what role membrane-integrity plays in the process of luminal electron transfer. I proved that flavine-adenin dinucleotide (FAD) is potently promoting the oxidation of the endoplasmic reticulum proteins, acting exclusively via the Ero1-L/PDI pathway. My experiments demonstrated that the integrity of the microsomal membranes is needed for performing FAD driven protein oxidation. Since FAD and other flavines are supplied by the nutrition, their level can regulate the rate of protein folding depending on the metabolic state of the cells.

Little is known about the changes of chaperones in chronic stress. Alpha-1 antitrypsin deficiency is a chronic disease in which the mutant, unfolded alpha1-antitrypsin (PiZ) protein aggregates inside the lumen of the endoplasmic reticulum of liver cells. Symptoms of the disease are liver cirrhosis, sterile hepatitis and elevated risk of cancer. With the help of PiZ synthesizing transgenic mice, I examined what processes contribute to survival during long-term stress. My experiments demonstrated that ER resident chaperones are not induced during PiZ protein aggregation. In contrast, levels of cytoplasmic stress proteins are elevated. These changes are accompanied with a shift in the redox state of the endoplasmic reticulum, decrease in the redox isomerase activity, elevation in antioxidant enzyme levels and glutathion induction in both compartments. PDI remains bound to the PiZ protein, the protein folding complex is present in lesser extent in transgenic mice. These data suggest that chaperones promote degradation rather than folding of newly synthesized proteins during chronic stress.

- *Papp E, Nardai G, Mandl J, Banhegyi G, Csermely P (2005) FAD oxidizes the ERO1-PDI electron transfer chain: The role of membrane integrity. Biochem Biophys Res Commun 338: 938–945.*
- *Papp E, Száraz P, Korcsmáros T, Csermely P (2006) Changes of endoplasmic reticulum chaperone complexes, redox state and impaired protein disulfide oxidoreductase activity in misfolding α_1-antitrypsin transgenic mice. FASEB J 20: 1018–1020.*

ORSOLYA POLGÁR (2007)

Mutational studies aimed at understanding the dimerization of human ABCG2

Supervisor: András Váradi

ABCG2 is an ATP-binding casette half-transporter associated with multidrug resistance in cancer that is presumed to homodimerize for function. A well-conserved GXXXG motif is found in TM1 of ABCG2, a motif that is involved in the dimerization of several membrane proteins, most extensively studied in glycophorin A. We found that mutating these glycine residues to leucine (G406L/G410L) renders the protein inactive, while surface expression and susceptibility to chemical cross-linking is retained. Substituting these glycines with alanine, thus creating the AXXXA putative dimerization motif, has no effect on the protein. In glycophorin A, dimerization is mediated by an extended seven-residue sequence containing the GXXXG motif (LIXXGVXXGVXXT). Remarkably, ABCG2 also has a threonine separated by 3 amino acids from the GXXXG motif. Mutating this threonine to arginine together with the glycine to leucine mutations in the GXXXG motif (T402R/G406L/G410L) results in a protein with no transport capacity that is primarily localized to the ER in immunofluorescence studies. When only threonine 402 is mutated the protein traffics to the cell surface and is functional. Another conserved region potentially involved in ABCG2 dimerization is a 3-amino acid sequence in TM5 (residues 552-554). Mutations of the corresponding residues in the *Drosophila* white protein (an orthologue of ABCG2) presumably disrupt heterodimerization. We found that substituting glycine 553 with leucine (G553L) results in significant decrease in protein expression with mostly ER localization, and deficient N-linked glycosylation, but intact cross-linking. Interestingly, the same mutant, when expressed in Sf9 insect cells, traffics to the cell membrane but is inactive, as revealed by its inability to hydrolyze ATP. Coexpressing the G553L mutant together with the wildtype protein in HEK 293 cells does not change the mutant's altered localization, suggesting that no mutant/wild-type dimers are formed. Mutation of the same residue to glutamic acid (G553E) also leads to significantly decreased protein expression level, altered glycosylation and ER retention. These data suggest that the above-mentioned residues in TM1 and TM5 of ABCG2 are important in protein trafficking and are consistent with, but do not yet prove, involvement in dimerization. We also report the creation and limited experimental testing of a computer model of ABCG2 based on homology to bacterial ABC transporters with available crystal structures. The dimeric model is very informative and can serve as a template for future experiments aimed at understanding the ABCG2 molecule.

- *Polgar O, Robey RW, Morisaki K, Dean M, Michejda C, Sauna ZE, Ambudkar SV, Tarasova N, Bates SE (2004) Mutational analysis of ABCG2: role of the GXXXG motif. Biochem 43: 9448–9456.*
- *Polgar O, Ozvegy-Laczka C, Robey RW, Morisaki K, Okada M, Tamaki A, Koblos G, Elkind NB, Ward Y, Dean M, Sarkadi B, Bates, SE (2006) Mutational studies of G553 in TM5 of ABCG2: a residue potentially involved in dimerization. Biochem 45: 5251–5260.*

RICHÁRD SZMOLA (2008)

Regulatory digestive proteases in the pathomechanism of chronic pancreatitis

Supervisor: Miklós Sahin-Tóth

Chronic pancreatitis is an incurable, persistent inflammatory disease, characterized by progressive and ultimately irreversible damage to both exocrine and endocrine functions of the pancreas. Genetic and biochemical evidence defines a pathological pathway in which a sustained imbalance between intrapancreatic trypsinogen activation and trypsin inactivation results in the development of the disease. Inactivation of potentially harmful intrapancreatic trypsin by proteolytic degradation has been discussed as a possible protective mechanism against chronic pancreatitis for decades. In humans the inhibitor resistant mesotrypsin has been labeled a candidate for this function. Later, the presence of another unknown enzymatic activity referred to as enzyme Y was found effective in the degradation of pancreatic zymogens in human pancreatic juice.

The aim of this work was to study and characterize the protective trypsin-degrading activity of mesotrypsin and chymotrypsin C, and to investigate their possible impact on pancreatic disease. Human digestive proteases were purified with ion-exchange and inhibitor affinity chromatography. Enzymatic interactions were followed by activity assays, SDS-PAGE, and N-terminal sequencing.

Our results demonstrate that human trypsinogens are not degraded by mesotrypsin, and dispute a protective role for mesotrypsin-mediated trypsin degradation in pancreatic physiology. However, we identified a unique role for mesotrypsin in the degradation of trypsin inhibitors. Furthermore, we found that this distinctive enzymatic activity was endowed by the mesotrypsin-specific amino-acid Arg198. The observations not only indicate a physiological role for mesotrypsin in the degradation of dietary trypsin inhibitors, but also suggest that premature activation of mesotrypsinogen could contribute to the pathogenesis of human pancreatitis by reducing the protective levels of pancreatic secretory trypsin inhibitor.

Furthermore, we identified chymotrypsin C as enzyme Y, the so far obscure trypsinogen-degrading activity from human pancreatic juice. Chymotrypsin C can specifically promote inactivation of all human trypsin and trypsinogen isoforms by selective cleavage of the Leu81-Glu82 peptide bond within the Ca^{2+} binding loop. Our results support a defense mechanism against pancreatitis, in which chymotrypsin C mediated trypsin degradation mitigates unwanted intrapancreatic trypsin activity.

- *Szmola R, Kukor Z, Sahin-Toth M (2003) Human mesotrypsin is a unique digestive protease specialized for the degradation of trypsin inhibitors. J Biol Chem 278: 48580–48589.*
- *Szmola R, Sahin-Toth M (2007) Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: Identity with Rinderknecht's enzyme. Proc Natl Acad Sci USA 104: 11227–11232.*

BALÁZS VÁRADI (2008)

The molecular basis of thrombolytic resistance

Supervisor: Rajmund Machovich

The presented results provide evidence that arterial thrombi contain massive quantity of phospholipid and myosin, originating from platelets occluded in thrombi. In our experiments we searched for the answer how these materials affect the process of fibrinolysis. Protein-free platelet membrane and vesicles containing synthetic phospholipids inhibit the tPA-induced fibrinolysis, affecting the plasminogen activation and plasmin function. Synthetic phospholipid vesicles inhibit the plasminogen activation in correlation with the anionic phospholipid fraction. Applying tPA and plasmin on the surface of fibrin, phospholipid vesicles reduce the amount of tPA penetrated into the clot by 75% and the depth of the layer occupied by the tPA by 30%, whereas for plasmin both of these parameters decrease by approximately 50%. The phospholipids form not only diffusion barrier, they also bind the components of the fibrinolytic system. Isothermal titration calorimetry shows binding characterized with dissociation constants in the range of 0.35–7.64 μM. The binding between the phospholipids and the fibrinolytic proteins is accompanied by increase in the entropy.

Myosin also inhibits the tPA-induced fibrinolysis, maximally when its concentration is 1 μM. If the concentration of myosin is higher than this value, the rate of inhibition decreases because of the cofactor role of myosin in the plasminogen activation. In addition to the inhibition of fibrinolysis, myosin also affects the fibrin polymerization evidenced by the changes of fibrin dissolution under flow conditions (disintegration of fibrin starts at a lower degree of digestion despite the slower fibrinolysis in the presence of myosin). These results contribute to our better understanding of the mechanism of action of thrombolytics applied currently in human therapy and to the development of new agents designed to act more efficiently in the complex *in vivo* environment.

- *Váradi B, Kolev K, Tenekedjiev K, Mészáros G, Kovalszky I, Longstaff C, Machovich R (2004) Phospholipid-barrier to fibrinolysis: role for the anionic polar head charge and the gel-phase crystalline structure. J Biol Chem 279: 39863–39871.*
- *Kolev K, Tenekedjiev K, Gombás J, Váradi B, Ajtai K, Kovalszky I, Machovich R (2003) Myosin: a non-covalent stabilizer of fibrin in the process of clot dissolution. Blood 101: 4380–4386.*

PROGRAM 7/3.

EMBRYOLOGY, THEORETICAL, EXPERIMENTAL AND CLINICAL DEVELOPMENTAL BIOLOGY

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Program overview

The program deals with the progressive changes of the vertebrate organisms from the primordial germ cells through the fertilization up to the organogenesis. During this progressive development primary tissues (epithelial, supporting, muscle and nervous tissues) are formed from different stem cells or progenitors, what is timely and spatially strictly regulated at both genetic and molecular levels. The Program focuses on the early formation of lymphoid organs including the epithelio-mesenchymal interactions (thymus and bursa of Fabricii), formation of hemopoetic sites, cardiac anlage, accessory dendritic cells of the immune system and the effect of different environmental agents on the ontogeny. The ontogeny of the visual system and its relationship with the circadian rhytms (pineal body) is a significant and progressive topic of the Program. The endocytosis by the monocyte-macrophage system is also a rapidly expanding area of the Program. Finally, characterization of the accessory cells of the MALT in avian species and human completes the series of scientific topics. The methods used by the Program incorporate a wide range of different techniques like light- and electron microscopy, immunocytochemistry, immunofluorescence combined with confocal microscopy, monoclonal antibody production, tissue culture, embryo manipulation (ablation and transplantation of embryonic organ rudiments, chimaerism, parabiosis) and Western blotting.

Titles of research projects	***Supervisors***
Role of caveolae and caveolin isoforms in various function of the cells	Anna L. Kiss
Developmental biology of the hemopoietic organs	Nándor Nagy
Development of lympho-myeloid organs and supporting tissue	Imre Oláh
Photosensitive molecules and photoreceptors in the vertebrate retina and pineal gland	Ágoston Szél
Embryonal and postembryonal development of the pineal organ and epithalamus	Béla Vígh

Ph. D. students		***Supervisors***
Ágnes Ida Berta	ft	Ágoston Szél
Ildikó Bódi	ft	Imre Oláh
Gergely Halász	ft	Ágoston Szél

Erzsébet Lackó	ft	Imre Oláh
Dávid Molnár	ft	Nándor Nagy

Ph. D. candidates		***Supervisors***
Éva Bíró	ft	Imre Oláh
Erzsébet Botos	ft	Anna K. Kiss
Zsolt Fejér	i	Béla Vígh
Zoltán Hajdú	ft	Imre Oláh

Ph. D. graduates		***Supervisors***
Botond Zoltán Igyártó	ft	Attila Magyar
Vanda Olga Szlávik	ft	Gábor Varga
Miklós Tóth	ft	Imre Oláh

ft, full-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

BOTOND ZOLTÁN IGYÁRTÓ (2007)

Antigen presenting dendritic cells of birds

Supervisor: Attila Magyar

The first part of the thesis deals with the epidermal dendritic cells of the chicken. It is known from the 90's, that the epidermis of the chicken skin contains ATPase+ and MHCII+ dendritic cells. These cells were designated as Langerhans cells but neither their detailed phenotype nor their function was further investigated. In the present paper we demonstrate a complete overlapping of ATPase, CD45 and vimentin in all dendritic cells of the chicken epidermis. The $CD45^+$/$ATPase^+$/$vimentin^+$ dendritic cells could be divided into three subpopulations: (1) an $MHCII^+$/$CD3^-$/$KUL01^+$ and 68.1^+ (monocyte-macrophage markers) one, (2) an $MHCII^-$/$CD3^-$/$KUL01^-$ and 68.1^- one and (3) a $MHCII^-$/$CD3^+$/$KUL01^-$ and 68.1^- one. The first population could be designated as Langerhans cells. The last population represents $CD4^-$/$CD8^-$/αβ and γδTCR^- NK cells with cytoplasmic CD3 positivity. The epidermal dendritic cells have a low proliferation rate as assessed by BrdU incorporation. Both *in vivo* and *in vitro* experiments showed that dendritic cells could be mobilized from the epidermis. Hapten treatment of epidermis resulted in the decrease of the frequency of epidermal dendritic cells and hapten-loaded dendritic cells appeared in the dermis or in *in vitro* culture of isolated epidermis. Hapten-positive cells were also found in the so called dermal lymphoid nodules. We suggest that these dermal nodules maintain some regional immunological function similar to the mammalian lymph nodes.
The second part of the thesis discusses the origin and function of splenic FDCs. The paper deals with the β-galactosidase uptake, migration and differentiation of ellipsoid associated cells (EAC) to follicular dendritic cell (FDC). The EAC is a blood-borne, phagocytic cell, which is located in the antigen-rich zone of the spleen. A novel mAb (E5G12) recognizes

both EAC(s) and FDC(s) and monitors the migration of EAC(s). 45 minutes postinjection, β-gal positive EAC(s) are on the surface of the ellipsoid, while 24 hours later begin to accumulate in the PALS, several pre-existed GC and the B cells as well as β-gal loaded EAC(s) form "clusters". These B cells and β-gal loaded EAC clusters are the rudiments of the early GC. During migration, the differentiation of the EAC is indicated by ceasing of 68.2, an EAC marker and expression of the 74.3 dendritic cell marker. Double positive (E5G12 versus β-gal) cells on the surface of the ellipsoid and the GC indicate that the antigen transporting cell is the same as the FDC. The β-gal binding of EAC is independent from the FcR and CR receptors and sulfated polysaccharide inhibits the bacterial antigen uptake. The EAC-FDC axis works exclusively inside the spleen, therefore we propose, that this system may operate in the pneumoccocus infection.

- *Igyarto BZ, Magyar A, Olah I (2007) Origin of follicular dendritic cell in the chicken spleen. Cell Tissue Res 327: 83–92.*
- *Igyarto BZ, Lacko E, Olah I, Magyar A (2006) Characterization of chicken epidermal dendritic cells. Immunology 119: 278–288.*

VANDA OLGA SZLÁVIK (2008)

In vitro differentiation of primary human submandibular gland cells and immortalized cell line

Supervisor: Gábor Varga

There is no effective treatment for loss of functional salivary tissue after diseases or injury. One possible approach is regeneration of salivary glands from stem cells. It has been shown that the intercalar duct cell line HSG is capable of differentiation into gland-like structures, though little is known of how morphological features are formed or controlled. We investigated the changes in cell proliferation and apoptosis upon terminal differentiation of HSG cells in Matrigel®, an extracellular matrix derivative. Changes in the expression of survivin, a prominent antiapoptotic factor, and caspase-3, an apoptotic factor were also measured. In order to better understand the involvement of key signal transduction pathways in this system we pharmacologically blocked the activity of tyrosine kinases, NFκB, PKC, PI3K and MMPs. Results of these studies demonstrate that cytodifferentiation of HSG cells to acinar phenotype is accompanied first by a decrease of cell proliferation and then by a massive programmed cell death, affected by multiple signal transduction pathways. Thus, Matrigel® alone is insufficient for the full maturation and long term survival of the newly formed acini: the presence of other factors is necessary to complete the acinar differentiation of HSG cells.

We aimed to investigate whether small pieces of human submandiubular gland tissue contain elements to reconstruct salivary rudiments *in vitro* from single-cell culture via acinar and ductal differentiation. Primary suibmandibular gland cells (PTHSG) were isolated from human tissue and cultured *in vitro* by a new method, in which single cells form an expanding epithelial monolayer on plastic substrates. Differentiation, morphology, number and organization of these cells were then followed on plastic and basal membrane extract (BME) by RNA quantitation, immunohistochemistry, viability assay and microscopy. On the surface of BME PTHSG cells formed acinotubular structures within 24 hours but did not proliferate, and stained more intensively for amylase. In cultures

derived from half of the donors, the acinar markers amylase and claudin 3 were upregulated. The PTHSG culture model suggests that the human salivary gland may be capable of regeneration via reorganization and differentiation, and basal membrane components play a crucial role in morphological and functional differentiation.

- *Szlavik V, Vag J, Marko K, Demeter K, Madarasz E, Olah I, Zelles T, O'Connell BC, Varga G (2008) Matrigel-induced acinar differentiation is followed by apoptosis in HSG cells. J Cell Biochem 103: 284–295.*
- *Szlavik V, Szabó B, Vicsek T, Barabás J, Bogdán S, Gresz V, Varga G, O'Connell BC, Vág J (2008) Differentiation of primary human submandibular gland cells cultured on basement membrane extract. Tissue Eng Part A 14: 1915–1926.*

MIKLÓS TÓTH (2007)

Pre- and postnatal changes in the human tympanic cavity

Supervisor: Imre Oláh

The middle ear is the typical place of ear surgical interventions that requires exact anatomical knowledge of the territory from the surgeon. The rapid development of technical utensils has not been followed by the demanded, detailed anatomical description of the tympanic cavity. The thesis summarizes the analyses of over thousand fetal or adult temporal bones and sheds light upon developmental reasons that lead to the considerable variability of tympanic structures.

The results of the thesis show that the carotid canal develops from two bony plates and that the failure of their fusion leads to dehiscences of the canal observed during the first two years of childhood. The walls of protympanum are formed by the petrous part of temporal bone; the cavity of protympanum is principally narrowed by the carotid canal. In the lateral wall of the protympanum the chorda tympani passes in a separate bony canal, however, related developmental cues have been unrevealed. The present investigations show that this canal, too, forms from two plates and that incomplete fusion of these plates leaves the nerve unprotected against mechanical challenge or may account for the so-called Costen syndrome. The structure of the round window is already established by birth, further changes are caused by pathological events. Nonpathological anatomical variants of the round window niche are classified in nine groups in the thesis. The roof of the tympanic cavity is formed by the tympanic tegmen; its congenital bony dehiscences appear supposedly in two different ways. The present anatomical investigations suggest that disturbances in the ossification process around the 25th week lead to this bone defect. The anatomy of human cochlea is known for several centuries; however, its exact relation to the medial wall of tympanic cavity has not been clarified. Now cochlea is reconstructed using serial cuts in anatomical and surgical directions also indicating the projection of tympanic structures.

According to the anatomical investigations included in the thesis it can be concluded that development of the inner ear exerts a powerful impact upon the formation of middle ear since its walls rather exclusively arise from the petrous part of temporal bone. Tympanic and squamous parts contribute to approximately 10% of the upper-lateral wall of the tympanic cavity.

- *Tóth M, Alpár A, Bodon G, Moser G, Patonay L (2006) Surgical anatomy of the cochlea for cochlear implantation. Ann Anat 188: 363–370.*
- *Tóth M, Medvegy T, Moser G, Patonay L (2006) Development of the protympanum. Ann Anat 188: 267–273.*
- *Tóth M, Alpár A, Patonay L, Oláh I (2006) Development and surgical anatomy of the round window niche. Ann Anat 188: 93–101.*

PROGRAM 7/4.

BASIS OF HUMAN MOLECULAR GENETICS AND GENE DIAGNOSTICS

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Program overview

To provide an overview on various fields of human medical and molecular genetics, genomics, including theory and methodology.

Titles of research projects	*Supervisors*
Studies on the pathomechanism of rheumatological diseases	Edit Buzás
Regulation of histidine decarboxylase (HDC) gene expression in physiological and pathological processes	Zsuzsanna Darvas
Study on the inflammation agents and regulatory molecules of the acute phase response	András Falus
Diagnostic methods of gene analysis in clinical paediatrics	György Fekete
Examination of the influence of genetical and immunological factors in rheumatological conditions	Pál Géher
Molecular biological methods in examining patients with muscular dystrophies	Veronika Karcagi
Chemotaxis: Its biological and clinical significance	László Kőhidai
Role of histamine in antigen presentation and cell differentiation	Valéria László
The cell cycle in different phases: monitoring changes in the expression of Waf-1/p53/PCNA/cyclines/Cdks with Northern and Western techniques	Zoltán Marcsek
Genetical and immunological factors in rheumatological conditions	György Nagy, Pál Géher
The active immunologic relationship between foetus and mother means fetal antigen presentation, maternal antigen recognition and immunologic response	Erna Pap
Studies on the genomic background of haematological malignancies	Éva Pálinger

DNA examination in forensic medicine	Péter Sótonyi
Study on the pathomechanism of asthma with molecular genetic methods	Csaba Szalai
Pharmacogenomics of childhood acute lymphoblastic leukaemia	Csaba Szalai
Analysis of cardiac functions in histamine-free transgenic murine model	Sára Tóth
Single nucleotide polymorphisms in the development of paradontal disease and missing tooth-germs	Gábor Varga
Isolation and characterization of dental postnatal stem cells	Gábor Varga
The significance of genetic polymorphism studies in systemic mastocytosis	Judit Várkonyi

Ph. D. students		***Supervisors***
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Orsolya Hegyesi	a	Gábor Varga
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Ágnes Koncz	i	Edit Buzás
Zsuzsanna Ortutay	ft	Edit Buzás

Éva Pállinger	i	András Falus
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a, absolutorium; ft, full-time, pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

GUSZTÁV BÉLTEKI (2007)

Novel methods in transgenic mouse technology

Supervisor: András Falus

Understanding the human gene function can be facilitated by studying orthologous mouse genes. Genetic manipulation of mouse embryonic stem (ES) cells enables us to change gene dosage and study the consequences *in vivo*. Both inactivation (knock-out) and overexpression of a gene can lead to aberrant phenotypes and the nature of these can provide clues about the gene's function under normal circumstances. However, understanding gene function in great detail will require the generation of numerous alleles as well as spatially and temporally regulatable overexpression. In my work I searched for novel methods which can simplify *in vivo* gene studies.

We studied the utility of the C31 I integrase in mouse embryonic stem cells. This site-specific recombinase had not been used for manipulation of the mouse genome previously. I showed that the integrase is operational in mouse ES cells without any additional cofactor. Site-specific genomic integration of transgenes is possible when a suitable C31 I integrase selection strategy is applied. Finally, expression of the C31 I integrase not affect the viability and developmental potential of ES cells. This C31 I integrase could turn into a useful tool in transgenic mouse technology.

My other aim was to develop a strategy for tissue-specific and inducible overexpression in ES cells and transgenic mice. I combined the tissue-specificity of the Cre recombinase with the inducibility of the tetracycline-responsive systems. I generated a transgenic mouse strain that expresses the reverse tetracycline transactivator (rtTA) and the enhanced green fluorescent protein (eGFP) genes after Cre-mediated recombination. Gene expression is driven by the ubiquitous Rosa26 promoter. Presence of the mutant allele did not cause any phenotypic abnormality in mice. The utility of the strategy was tested in triple transgenic mice that overexpressed the vascular endothelial growth factor (VEGF-A) following gene induction. Without induction the triple transgenic animals were normal and the induction resulted in characteristic histologic abnormalities and lethality. In conclusion, tight control of gene expression is possible with our strategy.

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- *Belteki G, Haigh J, Kabacs N, Haigh K, Sison K, Costantini F, Whitsett J, Quaggin S, Nagy A (2005) Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. Nucleic Acids Res 33: 51.*

KATALIN BOÉR (2008)

Histamine metabolism and histamine receptor expression pattern in human colorectal cancer

Supervisor: Zsuzsa Darvas

It was repeatedly demonstrated that histamine synthesis in tumours is significantly increased compared to the surrounding normal tissues, such as in breast cancer, colon carcinoma, malignant melanoma and hematological malignancies. Previous research has revealed the local production of histamine in colon tumours, and the significance of histamine in the development and progression of cancer. The local effect of histamine is largely determined by the actual histamine receptor (H1R-H4R) expression pattern. It has been shown that in experimental carcinomas endogenous histamine may act as an autocrine growth factor by stimulating tumour cell proliferation via H2 receptors. In humans, several clinical trials have been performed with H2 receptor antagonists as additive treatment to surgical resection, with contradictory results so far. Colon adenomas represent an increased risk for developing colon cancer, it is well documented that adenomatous polyps may undergo malignant transformation.

We intended to analyse the histamine metabolism and receptor expression profile in human colorectal carcinoma. In our experiments we perfomed a comparative analysis of histamine metabolism and receptor expression in colorectal cancer, adenoma and normal colonic mucosa. Our results showed an elevated histamine concentration, increased HDC activity and decreased DAO content in adenocarcinoma in comparison to the normal mucosa and adenoma. Based on our original hypothesis we expected to find altered histamine metabolism in adenomas suggesting a transition to the metabolic pattern found in carcinomas. In contrast we could not demonstrate any altered metabolism in the benign lesions of the colon. The analysis of histamine receptor expression profile showed a marked difference in malignant tumours and normal mucosa. A well defined distribution and expression pattern of receptors was found in selected tumour samples, namely significantly downregulated H1R and H4R, with unchanged H2R expression. These findings are just opposite to the results found in normal colonic mucosa and inflammatory bowel disease published by others. In addition to these results there was no difference between the receptor expression profile according to different Dukes' stages. The role of histamine with respect to the intracellular mechanisms involved in the colon tumour development and progression has not been clarified yet. More details are needed to clarify the role of histamine and its receptors as prognostic factors in the treatment a colorectal cancer patients.

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- *Hegyesi H, Colombo L, Pállinger E, Tóth S, Boér K, Molnár V, Falus A (2007) Impact of systemic histamine deficiency on the crosstalk between mammary adenocarcinoma and T cells. J Pharmacol Sci 105: 66–73.*
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IRÉN HALTRICH (2006)

Multipoint interphase FISH analysis of chromosome 3 abnormalities in childhood leukemia

Supervisor: György Fekete

In the model system called "elimination test" and in different solid tumors it was proven that certain chromosome 3p regions are non-randomly deleted and 3q regions retained in a manner that transgresses tissue and species barriers. This finding suggests the possibility that deletion hot spots on 3p containing tumor suppressor genes and "oncogene rich" 3q regions may be involved in childhood hematological malignancies including ALL, AML and CML. The further aim of this investigation was to identify chromosome 3 abnormalities at high resolution using a novel multipoint I-FISH technique. In the analyzed 60 childhood acute leukemia cases the author identified two cases of interstitial 3p deletions and 9 cases of 3q gains. The two deletions were detected at 3p12-p13 regions in T-ALL. The overlapping interstitial deletions harbor a known tumor suppressor region (72.6-78.8 Mb), described in solid tumors and contains the "Deleted in–U-Twenty-Twenty "gene (DUTT1/ROBO1), associated with early phases of malignancy. Increased copy number of 3q including the trisomy of whole chromosome 3, 3q trisomy and 3q tetrasomy (isodisomy), in the 4 T-cell ALL was identified in smaller, in the 5 AML cases in larger subpopulations. The majority of chromosome 3 structural abnormalities (71%) were identified in myeloid leukemia, involving mostly 3q21 and/or 3q26 breakpoints. The cytogenetic syndrome involving bands 3q21 and 3q26 simultaneously, known as "3q21q26 syndrome", has been detected in two patients. The 3q26 breakpoint involved the EVI1 gene. The EVI1 protein is a zinc finger transcriptional factor which plays a key role in normal hematopoetic differentiation. The 3q21 breakpoint was identified close to the RPN1 gene, which exerts oncogenic effect through mechanism acting over some genomic distance. The author identified in a t-AML case an AML1/EVI1 fusion gene, which is extremely rare in childhood leukemia. Both partners of the chimeric protein may disturb the normal hematopoesis. The author identified also a t(3;8)(q21;q24), previously never reported in childhood AML and mentioned only a single adult t-AML case. In the investigated CML case an unbalanced jumping translocation of 17q11-qter was revealed, which has never been reported in hematological malignancies and a complex 3q rearrangement. The high specificity and sensitivity of the interphase multipoint FISH method, introduced first by the author for the examination of hematological malignancies, identified subtle aberrations (small subpopulations, interstitial gain and loss) that would remain undetected by current methods based on total tumor DNA analysis and conventional cytogenetics. Similar cytogenetic changes of chromosome 3 could be identified in general in children and in adults, but differences in incidence have been noted. The poor outcome in patients with 3q rearrangements appears to be quite uniform. The combination of conventional and molecular cytogenetic techniques was helpful in identifying high-risk groups like the "3q21q26 syndrome" that needs another treatment than conventional chemotherapy.

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JUDIT HORVÁTH (2006)

Identification of the DNAL1 gene and development of a novel genetic based diagnostic method in primary ciliary dyskinesia

Supervisor: György Fekete

Background: Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder characterized by chronic infections of the upper and lower airways, situs inversus (50%) and reduced fertility. The phenotype of PCD results from dysfunction of motile cilia, which manifests in immotility and dysmotility. Diagnosis of PCD usually relies on electron microscopy, which is technically demanding and sometimes difficult to interpret. The frequent ultrastructural defects involve the absence of the outer dynein arms (ODAs), which are normally composed of several light (LCs), intermediate and heavy (HCs) dynein chains. Recessive mutations of DNAH5, the human ortholog of the biflagellate Chlamydomonas ODA γ-HC, cause PCD. In *Chlamydomonas*, motor protein activity of the γ-ODA-HC is regulated by binding of the axonemal LC1.
Aims: (1) Identification of the human (DNAL1) and murine (Dnal1) orthologs of *Chlamydomonas* LC1 and characterization of the encoding proteins. (2) Establishment of a novel, genetic based diagnostic method, which helps a non-invasive, clear (obvious) diagnosis of PCD.
Results: (1) The novel human DNAL1 is 97% homologous to the murine protein Dnal1. We demonstrated that both of these proteins, similarly to DNAH5 have tissue specific expression in testis, in embryonic node, in respiratory, and in ependymal cells. The LC1/g-HC binding motif in human DNAL1 is completely conserved. We were the first to show the interaction of human DNAH5 and human DNAL1 with co-immunoprecipitation method. Based on these findings, we considered DNAL1 a candidate for PCD. We also sequenced all exons of DNAL1 in 86 patients, the results of mutational analysis were negative. (2) Immunofluorescence staining of the inner dynein arm component of DNALI1 and the outer dynein arm component of DNAH5 in respiratory cells of 54 PCD patients and healthy controls were performed. In respiratory epithelial cells from healthy probands, specific DNALI1 and DNAH5 staining were observed along the entire length of the axonemes, respectively. These proteins were either completely or only distally absent from the respiratory ciliary axoneme in patients with PCD. We observed eight distinct aberrant DNALI1/DNAH5 staining patterns: two isolated inner and outer dynein arm defects and four combined outer and inner dynein arm defects.
Conclusions: (1) The strong expression in testis suggests that DNAL1 might play a particular role in sperm flagella, and raises the possibility that DNAL1 mutations are involved in male sterility, however the negative mutational analysis excluded the role of DNAL1 in

the pathogenesis of PCD.(2) Immunofluorescence staining with anti-DNAH5 and anti-DNALI1 antibody might be a novel diagnostic tool in the future, because it is a non-invasive and highly specific method.

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- *Kispert A, Petry M, Olbrich H, Volz A, Ketelsen UP, Horvath J, Melkaoui R, Omran H, Zariwala M, Noone PG, Knowles M (2003) Genotype-phenotype correlations in PCD patients carrying DNAH5 mutations. Thorax 58: 552–554.*

IVETT JELINEK (2007)

The effect of the lack of histamine and histamine H4R on the functional properties of dendritic cells

Supervisor: Valéria László

Dendritic cells (DCs) are the main antigen presenting cells. Their main function is antigen processing and presentation, which can be affected by several environmental factors such as histamine. Using a genetically histamine-free (histidine decarboxylase knock out, HDC–/–) mouse model, we examined the effects of histamine on DC-mediated antigen presentation and cytokine production. We found that spleen DCs, derived from HDC–/– mice, display a higher efficiency in antigen presentation compared to wild type cells. After flow cytometric analysis of the main DC cell surface markers and costimulatory molecules we found that these two DC groups do not differ in their phenotype or maturation status. We also analyzed their cytokine expression profile by real-time PCR. We measured enhanced Th1 cytokine profile (IL-12p35 and IFN-γ) in HDC–/– DCs compared to the wild type ones.

Beside histamine H1 and H2 receptors, DCs are known to express the newly discovered histamine H4 receptor (H4R) but until recently, its function on DCs has not been completely elucidated. In our experiments, we studied the involvement of H4R in the mediation of the above mentioned histamine effects. In these experiments we studied DCs which either do not have H4R (H4R–/– DCs), or we blocked the H4R functions with the specific H4R antagonist JNJ7777120. We found again increased antigen presentation in H4R–/– DCs, or when we blocked the H4R with its antagonist. Moreover, the selective H4R antagonist was able to compensate the suppressive effect of IFN-γ but not that of histamine on the Th1 cytokine production. Finally we found that H4R is essential for DC migration as H4R–/– DCs have decreased migratory capacity both *in vitro* and *in vivo*, which is in accordance with our findings that H4R–/– DCs express less CCR7 than their wild type counterparts.

These results indicate that histamine plays an essential role in DC functions, as it affects antigen presentation, T cell polarization, cytokine production, and migration of DCs. Our

analysis disclosed that H4R is crucial in mediating these effects. These observations suggest new potential applications for the novel H4R ligands in vaccination therapy of several immun-related disorders, such as allergy or cancer.

- *Jelinek I, Laszlo V, Buzas E, Pallinger E, Hangya B, Horvath Z, Falus A (2007) Increased antigen presentation and T(h)1 polarization in genetically histamine-free mice. Int Immunol 19: 51–58.*
- *Buzas EI, Gyorgy B, Pasztoi M, Jelinek I, Falus A, Gabius HJ (2006) Carbohydrate recognition systems in autoimmunity. Autoimmunity 39: 691–704.*
- *Kis Z, Pallinger E, Endresz V, Burian K, Jelinek I, Gonczol E, Valyi-Nagy I (2004) The interactions between human dendritic cells and microbes; possible clinical applications of dendritic cells. Inflamm Res 53: 413–423.*

ÁGNES KONCZ (2008)

Role of nitric oxide in T cell activation

Supervisor: Edit Buzás

Activation, proliferation, or programmed cell death of T lymphocytes is regulated by the mitochondrial transmembrane potential through controlling ATP synthesis, production of reactive oxygen intermediates (ROI). Mitochondrial hyperpolarization is an early and reversible event associated with T cell activation. CD3/CD28 costimulation of human PBL elevated cytoplasmic and mitochondrial Ca^{2+} levels, ROI and NO production, and elicited mitochondrial hyperpolarization. Although T cell activation-induced $Ca2^{2+}$ release, ROI levels, and NO production were diminished by inositol 1,4,5-triphosphate receptor antagonist, superoxide dismutase mimic and NO chelator, mitochondrial hyperpolarization was selectively inhibited by NO chelators. Moreover, the NO precursor NOC-18 elicited NO and ROI production, Ca^{2+} release, transient ATP depletion, and robust mitochondrial hyperpolarization. Ca^{2+} influx by ionomycin or Ca^{2+} release from intracellular stores by thapsigargin alone failed to induce NO synthase expression, NO production, or mitochondrial membrane potential elevation. Western blot analysis revealed expression of Ca-dependent endothelial NO synthase and neuronal NO synthase isoforms and absence of Ca-independent inducible NO synthase in PBL. CD3/CD28 costimulation elicited severalfold elevations of endothelial NO synthase and neuronal NO synthase expression. The results suggest that T cell activation-induced mitochondrial hyperpolarization is mediated by ROI- and $Ca2^{2+}$ dependent NO production.

In order to study the role of histamine in T cell activation, we investigated cytokine production and T cell signal transduction in HDC-KO and wild type mice. In the absence of histamine, an elevated INF-γ mRNA and protein levels of splenocytes were associated with a markedly increased NO production, compared to wild type animals. Furthermore, histamine treatment decreased the NO production of splenocytes from both wild type and HDC-KO mice. The NO precursor NOC-18 elicited IFN-γ production, while the NO synthase inhibitors NG-monomethyl-L-arginine and nitronidazole both inhibited IFN-γ production, suggesting the role of NO in regulating IFN-γ synthesis. Our present data indicate that in addition to its direct effects on T lymphocyte function, histamine regulates cytokine production and T cell signal transduction through regulating NO production.

- *Koncz A, Pasztoi M, Mazan M, Fazakas F, Buzas E, Falus A, Nagy G (2007) Nitric oxide mediates T cell cytokine production and signal transduction in histidine dexarboxylase knockout mice. J Immunol 179: 6613–6619.*
- *Nagy G, Koncz A, Fernandez D, Perl A (2007) Nitric oxide, mitochondrial hyperpolarization, and T cell activation. Free Radic Biol Med 42: 1625–1631.*
- *Nagy G, Koncz A, Perl A (2005) Mitochondrial T cell signal transduction abnormalities in systemic lupus erythematosus. Curr Immunol Rev 1: 62–68.*

ZSUZSANNA ORTUTAY (2007)

Studies on the autoimmune pathomechanism of rheumatology diseases

Supervisor: Edit Buzás

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint destruction. Several studies investigate the role of matrix metalloproteinases (MMPs) in cartilage degradation. We examined the role of glycosidase enzymes in the degradation of cartilage proteoglycans, since these enzymes are recently neglected by the literature. Determining the relative importance of the above groups of enzymes might be important for the understanding of the pathomechanism of arthritic diseases. In the case of RA the small, leucine-rich proteoglycans that are important components of the extracellular matrix might be trivial targets of the autoimmune processes. We examined these molecules. The activity of MMPs and glycosidases in the synovial fluid (SF) of patients with RA, osteoarthritis (OA), psoriatic arthritis (AP), seronegative spondylarthritides (SNSA) and acute sports injury have been demonstrated. We have shown that the activities of β-D-glucuronidase and β-D-N-acetylglucosaminidase in the SF are significant predictors of RA. We have detected anti β-D-glucuronidase antibodies in the serum and SF samples of patients with RA. Also, an elevated activity of β-D-glucuronidase has been detected in the blood cells of patients with RA compared to the healthy controls. Glycosidases alone or in combination with MMPs proved to be effective in depleting glycosaminoglycans (GAGs) from cartilage. We tested biglycan, decorin, aggrecan, collagen type II and fibronectin-specific IgG and IgM antibody levels in serum and SF of patients with RA, OA, AP and SNSA. We have detected antibodies reactive to these cartilage components in the SF samples. While the elevated biglycan-specific IgM response was characteristic for RA, the decorin-specific IgG response proved to be particularly elevated in SNSA.

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- *Polgar A, Falus A, Koo E, Ujfalussy I, Sesztak M, Szuts I, Konrad K, Hodinka L, Bene E, Meszaros G, Ortutay Z, Farkas E, Paksy A, Buzas EI (2003) Elevated levels of synovial fluid antibodies reactive with the small proteoglycans biglycan and decorin in patients with rheumatoid arthritis or other joint diseases. Rheumatology 42: 5227.*

ÉVA PÁLLINGER (2006)

Investigations of histamine dependent pathomechanisms of the hematopoiesis

Supervisor: András Falus

In *in vivo* and *in vitro* experiments we justified that histamine production depends on the functional state of the cells. On the one hand, in our *in vivo* system we could demonstrate that intracellular HDC and histamine content increases in those periods of bone marrow regeneration when the rate of cell proliferation is the highest. On the other hand, we found different levels of histamine in hematopoietic precursor cells which have different hematopoietic activity. In our *in vitro* system we could justify that anti-CD3 treatment increases the HDC expression and histamine production of Jurkat cells. It demonstrated that the endogenously synthesized histamine has a great role in the regulation of T cells. By the investigations of the effects of different chemicals on histamine production we could justify that both perinatal and adult treatments of animals could change the histamine producing ability of immune cells. The functional activity of the investigated cells can be changed in the long run with a single treatment. We found that the effects of different treatments strongly depend on the hormonal environment and on the animals' age.
By the investigation of histamine free (HDC-KO) mice we showed that histamine synthesis of the hematopoietic precursor cells have a great influence on hematopoiesis. An altered hematopoietic differentiation can be detected in the absence of endogenously synthesized histamine. The effects of histamine through H4R have a regulatory role in hematopoietic differentiation and T cell development as shown by the experiments on H4R-KO mice.
We could detect significantly lower amounts of HDC for the first time in the circulating monocytes of neonates suffering from fatal sepsis. Our results suggest that the measurement of HDC content of white blood cells could be a useful factor in screening the prognosis of sepsis.

- *Pállinger É, Tóthfalusi, Csaba G (2004) Prolonged effect of endorphin treatment during pregnancy of rat on the histamine content of immune cells of F1 and F2 offspring generations. Cell Biochem Funct 24: 287–290.*
- *Csaba G, Kovács P, Pállinger É (2004) Prolonged effect of an H1-receptor blocker antihistamine on the histamine content of white blood cells and mast cells. Cell Biochem Funct 22: 201–204.*

EMŐKE RÁCZ (2006)

Mutation analysis and gene expression studies in Darier's disease and Hailey-Hailey disease

Supervisor: Sarolta Kárpáti

Darier's disease (dyskeratosis follicularis, MIM 124200) is an autosomal dominantly inherited skin disorder with a prevalence of 1 in 30000–50000. It is characterized by skin-coloured or yellow-brown keratotic papules on the seborrheic areas of the skin; the papules often form large, crusted, confluent plaques. Mutations in the ATP2A2 gene

encoding the sarco/endoplasmic reticulum calcium ATPase 2 (SERCA2) were identified as the cause of the disease.

Hailey-Hailey disease (benign chronic familial pemphigus, MIM 169600) is characterized by recurrent vesicles and erosions, particularly involving flexural areas. Mutations in the ATP2C1 gene encoding the human secretory pathway calcium ATPase 1 (hSPCA1) were identified in the background of Hailey-Hailey disease, another autosomal dominantly inherited skin disease. A common histopathologic feature of the two diseases is the loss of cell-to-cell adhesion (acantholysis) and abnormal keratinization.

SERCA2 pumps are found in the membrane of the endoplasmic reticulum (ER). They catalyze the hydrolysis of ATP coupled with the translocation of Ca^{2+} ions from the cytosol to the lumen of the ER. The hSPCA1 protein is a transmembrane protein of the Golgi membrane. It has been shown to transport both Ca^{2+} and Mn^{2+} from the cytoplasm into the Golgi lumen. Both pumps play a pivotal role in intracellular Ca^{2+} homeostasis and signalling. SERCA2 and hSPCA1 are expressed in all tissues examined, however, the symptoms of Darier's disease and Hailey-Hailey disease are confined to the skin.

The aim of our study was to find Hungarian patients with Darier's disease and Hailey-Hailey disease, characterize their symptoms and to identify their disease causing mutations. By coupling the results of the mutation analysis to the clinical data of the patients we attempted to reveal genotype-phenotype correlations. Joining the dermatological research unit of the University of California, San Francisco, my task was to compare the process of calcium-induced differentiation of cultured keratinocytes from healthy controls and patients with Hailey-Hailey disease. Studying the ATP2A2 gene in Hungarian patients with Darier's disease, we found 11 distinct, heterozygous mutations, 9 of which were novel. Besides two deletions, one insertion and a new splice-site generating intronic nucleotid change we detected six missense and one non-sense mutations. In the ATP2C1 gene of Hungarian patients with Hailey-Hailey disease we identified three novel heterozygous mutations: one nucleotid change leading to a STOP codon, one insertion and one gross deletion affecting the 5' noncoding region of the gene. No clear correlation between genotype and phenotype could be observed in this cohort of patients. In our in vitro experiments we found that the levels of involucrin, a marker of the early keratinocyte differentiation was decreased in HHD keratinocytes, while the activation of the involucrin promoter upon calcium stimulation was normal or increased. The degradation of the involucrin mRNA transcripts was increased in HHD keratinocytes. The thesis summarizes the first mutation analysis data in patients with Darier and Hailey-Hailey diseases from Hungary.

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- *Rácz E, Csikós M, Benkó R, Kornsée Z, Kárpáti S (2005) Three novel mutations in the ATP2A2 gene in Hungarian families with Darier's disease, including a novel splice site generating intronic nucleotide change. J Dermatol Sci 38:231-234.*
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PROGRAM 7/5.

IMMUNOLOGY

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Program overview

The recognition of immunology as an independent scientific discipline is recent, therefore, in many universities immunological research is carried out in various (e.g. biochemical etc) departments. Qualified immunologists are greatly demanded in many areas, including clinical science. This project, completing the curriculum of undergraduate training, yields a perspective to qualify in several areas of immunology. The purpose of this Ph.D. Program is to train independent, reliable and competent research scientists. The Program emphasizes the importance of studying basic immunology and laboratory methods, both being prerequisites of any work in experimental and clinical immunology.

Titles of research projects	***Supervisors***
Expression of protooncogenes and tumor suppressor genes in gestational trophoblastic tumors and normal pregnancy	Vilmos Fülöp
Study of the associations with the major histocompatibility complex (MHC) with diseases	György Füst
Regulation of immune reactions and role of cytokines in the pathomechanism of autoimmune diseases	Péter Gergely
Links between naive and cellular immunreactions	Attila Horváth
Acute phase proteins in clinical diagnostics	László Kalabay
Effects of bacterial products on the progression of HIV infection	Károly Nagy
Genetical, molecular and clinical interactions in multifactorial diseases	Zoltán Prohászka
Infection and autoimmunity in inflammatory diseases	Gyula Poór

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Judit Czúcz	ft	György Füst
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Gyula Schäffer	ft	Zoltán Prohászka
Krisztián Vörös	pt	László Kalabay

Ph. D. candidates		*Supervisors*
Tamás Constantin	i	Péter Gergely
Tímea Gombos	ft	Zoltán Prohászka
Ferenc Harmos	ft	Károly Nagy
Gábor Hutás	i	Péter Gergely
Elyas Talha	it	Károly Nagy

Ph. D. graduates		*Supervisors*
Anikó Bányai	i	Katalin Pálóczi
József Bátorfi	i	Vilmos Fülöp
Adrienn Jókuti	pt	Sára Tóth
Lajos Kalmár	ft	Attila Tordai
Péter Kelemen	pt	Péter Gergely
Petra Kiszel	ft	György Füst
Judit Laki	ft	György Füst
Zoltán Nagymányoki	pt	Vilmos Fülöp
Éva Palkonyai	i	Péter Gergely
Szabolcs Rugonfalvi Kiss	i	György Füst
Krisztina Katalin Sallai	ft	Péter Gergely
Gábor Széplaki	pt	György Füst

ft, full-time; pt, part-time; it, international; i, individual

Abstracts of Ph. D. theses successfully defended in 2005, 2006, 2007 and 2008

ANIKÓ BÁNYAI (2005)

Study of complement functions in patients with malignant lymphomas based on experiences from SLE

Supervisor: Katalin Pálóczi

The complement system has essentially important effector functions in the immune defense mechanism. The complement can be activated by surface molecules of microorganisms and antigen-antibody complexes. The complement system also has an important role in the formation of immune complexes (IC) via determination of their structure, size, transport, elimination as well as a role in the pathomechanism of IC provoked inflammations. Connection between IC disease, defective complement functions and SLE has been explored extensively. However, little is known about connection between the complement system and malignant lymphomas despite clinical observations including high susceptibility to infections and paraneoplastic syndromes with IC pathomechanism. The aim of our work was to develop methods for characterization of the function and components of the complement system and circulating IC in SLE in order to study connections between activity of disease and changes of parameters. Using previous experience and adopting the methods obtained in SLE, the connection between the clinical course and comple-

ment alterations was studied in malignant lymphomas, too. On the basis of ELISA microtechnique, a new modified method was developed for studying IC composition in SLE. Quantification of results was carried out by using calibration curves for immunoglobulins and C3. Decreased C3 content of IC was observed in active stage of SLE and in cases with renal involvement suggesting incomplete IC solubilization resulting in further complement activation and inflammation. In malignant lymphomas defective complement functions and decreased levels of complement components were observed. Complement abnormalities were more frequent in high-grade malignancies. However, hypocomplementemia was observed in low-grade cases, too. Decrease of complement mediated immune complex solubilization and immune precipitation inhibition was also determined. Study and follow-up of complement functions, serum levels of complement components C3, C4, and circulating IC during autologous blood stem cell transplantation in malignant lymphoma patients were published by our group as novel findings. Results of complication-free cases were regarded as standard and alterations from these values were suggested as signs of different complications.

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- *Banyai A, Paloczi K, Csipo I, Csongor J, Szegedi G (1990) Complement-mediated immune complex solubilization and precipitation inhibition in sera of patients with non-Hodgkin's lymphoma. Haematologia 23: 87–95.*
- *Varga L, Czink E, Miszlai Z, Paloczi K, Banyai A, Szegedi G, Fust G (1995) Low activity of the classical complement pathway predicts short survival of patients with chronic lymphocytic leukaemia. Clin Exp Immunol 99: 112–116.*

JÓZSEF BÁTORFI (2006)

Recent progress in the diagnostics and follow-up of pathological pregnancies

Supervisor: Vilmos Fülöp

The placenta is the organ of normal pregnancy which is responsible for fetomaternal transport mechanisms, for supplying the fetus with oxygen and nutrition. But its biological role in pregnancy is more complex because the development of molecular medicine and immunology has led to realize other important placental functions such as regulation of tissue proliferation or immunological coordination of pregnancy through the barrier function, which avoids the rejection of immunologically partly alien fetus. That is why placental dysfunction can influence pregnancy outcome in several ways. In this dissertation I discussed two obstetrical diseases caused by abnormal placental function. First I analyzed the placental dysfunction in molar pregnancy through its anomalous protein expression features. I tried to collect new scientific information about the molecular background, which is partly unknown nowadays. Most intra- and extracellular mechanisms are closely related to each other. I hope my results could become a part or base of further research work in order to contribute to the permanently expanding general knowledge. Moreover, I made statistical analysis of data in the follow-up of molar pregnancies, sketching a new, cost-effective and safe protocol of post-disease monitoring, which additionally may be more acceptable for patients, and might potentionally be introduced to clinical practice.

Another area of abnormal placental function is the unexplained recurrent spontaneous abortion. I analyzed immunological aspects of this disease, introducing the first five years of the Multicentric Clinical Study of Recurrent Spontaneous Abortion, in which I have taken part since its beginning as a researcher and hanging committee member. My aim was to draw attention to the new and important aspects of our protocol in RSA by summarizing the results of immunopathological examination and applied immunotherapy.

- *Batorfi J, Bin Ye, Mok SC, Cseh I, Berkowitz RS, Fulop V (2003) Protein profiling of complete mole and normal placenta using ProteinChip analysis on laser capture microdissected cells. Gynecol Oncol 88: 424–428.*
- *Batorfi J, Fulop V, Jae-Hoon Kim, Genest DR, Doszpod J, Mok SC, Berkowitz RS (2003) Osteopontin is down-regulated in hydatidiform mole. Gynecol Oncol 89: 134–139.*
- *Feltmate CM, Batorfi J, Fulop V, Goldsetin DP, Doszpod J, Berkowitz RS (2003) Human chorionic gonadotropin follow-up in patients with molar pregnancy: a time for reevaluation. Obstet Gynecol 101: 732–736.*

ADRIENN JÓKUTI (2008)

Studying histamine, IL5, IgE and TGF-beta1 in the pathogenesis of nasal polyposis

Supervisor: Sára Tóth

Nasal polyps are benign mucosal protrusions into the nasal cavity of multifactorial origin, characterised by chronic eosinophil inflammation. The suggested pathomechanisms comprise several important molecules and cytokines. Our aim was to study IgE, IL-5, TGF-beta1, and histamine in the pathomechanism of nasal polyposis, as well as to find out which histamine receptors play a role in the effect of histamine in nasal polyp tissue. In our findings we found significantly higher tissue IgE in polyps compared to controls. Tissue IL-5 was significantly higher in polyp tissue compared to controls with no difference between allergic- and non-allergic polyps. TGF-beta1 proved to be significantly higher in controls than in polyps. Immunohistochemical analysis revealed numerous IL-5 positive eosinophil cells and TGF-beta1 positivity in the lamina propria of polyp samples, but none in controls. Both HDC gene expression and HDC protein were found to be higher in nasal polyps. HNMT activity was elevated also in nasal polyp tissue. There was no significant difference in the histamine content of NP and control mucosa. The expression of H1 and H4 receptors was elevated in polyp tissue, while the level of H2 and H3 receptors was not increased significantly. The concentration of eosinophil cationic protein (ECP) was significantly higher in polyp tissue and this elevation suggests an association with the increased H4 receptor expression. IL-5 plays a key role in the eosinophil recruitment and activation. Immediate hypersensitivity with systemic allergic reaction does not seem to be involved in the pathogenesis of this disease, but tissue IgE production might be due to local allergic mechanisms. High tissue TGF-beta1 quantity in healthy nasal mucosa without its active form on the cell surface and its low quantity in polyps may reflect its essential role in the regulatory mechanisms and pathogenesis of nasal polyposis. The main sources of IL-5 and TGF-beta1 are the eosinophils and macrophages. Histamine metabolism seems to be altered in polyp tissue, that can influence IL-5 release, and this may be an important factor in the pathomechanism of polyp formation. The histamine related mechanisms are preferentially mediated through H1 and H4 histamine receptors in the polyp tissue. Due

to this point one may speculate that the H4 receptor mediated histamine effects have a role in eosinophil accumulation and activation in inflammatory diseases of the nasal and paranasal sinus mucosa, like nasal polyposis.

- *Jókúti A, Hellinger E, Hellinger A, Darvas Z, Falus A, Thurmond RL, Hirschberg A (2007) Histamine H4 receptor expression is elevated in human nasal polyp tissue. Cell Biol Int 31: 1367–1370.*
- *Jókúti A, Schwelberger H, Darvas Z, Páli Z, Bösze S, Falus A, Hirschberg A (2004) Histamin metabolism is altered in nasal polyposis. Inflamm Res 53: 93–94.*
- *Hirschberg A, Jókúti A, Darvas Z, Almay K, Répássy G, Falus A (2003) The pathogenesis of nasal polyposis by immunoglobulin E and interleukin-5 is completed by transforming growth factor-beta1. Laryngoscope 113: 120–124.*

LAJOS KALMÁR (2007)

Identification of disease causing mutations in hereditary angioedema and the establishement of the C1-INH gene mutation database

Supervisor: Attila Tordai

Hereditary angioneurotic edema (HAE) is an autosomal dominant disorder characterized by episodic local subcutaneous and submucosal edema caused by the deficiency of activated C1 esterase inhibitor protein (C1-INH, type I: reduced serum antigen level, type II: reduced activity and normal serum antigen level). The aim of the present study was to determine the disease-causing mutations among Hungarian HAE-patients. The estimated number of affected HAE-families in Hungary is 40–50, out of which 26 families (type I: 23, type II: 3) treated in a single center were enrolled in the current study. To detect large deletions/insertions, we used Southern blotting analysis followed by real time PCR based gene dosage analysis. In the absence of large structural changes, we employed direct sequencing covering the whole coding region and splicing sites of the C1-INH gene. Large deletions were detected in 4/23 (17.4%) type I families. We found the g.16788C>T (p.Arg444Cys) mutation in all 3, type II HAE-families. In the remaining type I families, 13 previously unreported mutations (g.638G>A, g.2238C>T, g.2534_2535delCT, g.2579_2620del42, g.2533G>A, g.2695G>A, g.2696_2697insT, g.4467C>T, g.14224A>T, g.14107delA, g.16749_16775dup, g.16810T>A, g.16885C>G) were detected in 16 families affecting primarily exon 3 (6/13) of the C1-INH gene. In the 3 remaining families, known mutations were identified affecting primarily exon 8 (2/3).

Published C1-INH mutations are represented in large universal databases (e.g., OMIM, HGMD), but these databases update their data rather infrequently, they are not interactive, and they do not allow searches according to different criteria. The HAEdb, a C1-INH gene mutation database (http://hae.biomembrane.hu) was created to contribute to the following expectations: (1) help the comprehensive collection of information on genetic alterations of the C1-INH gene; (2) create a database in which data can be searched and compared according to several flexible criteria; and (3) provide additional help in new mutation identification. The website uses MySQL, an open-source, multithreaded, relational database management system. The user-friendly graphical interface was written in the PHP web programming language. Several attributes (e.g., affected exon, molecular consequence, family history) are collected for each mutation in a standardized form. This data-

base may facilitate future comprehensive analyses of C1-INH mutations and also provide regular help for molecular diagnostic testing of HAE patients in different centers.

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- *Kalmar L, Bors A, Farkas H, Vas S, Fandl B, Varga L, Fust G, Tordai A (2003) Mutation screening of the C1 inhibitor gene among Hungarian patients with hereditary angioedema. Human Mutation 22: 498.*
- *Kalmar L, Hegedus T, Farkas H, Nagy M, Tordai A (2005) HAEdb: a novel interactive, locus-specific mutation database for the C1 inhibitor gene. Human Mutation 25: 1–5.*

PÉTER KELEMEN (2005)

Tolerogenic dendritic cells: immunomodulation of monocyte-derived dendritic cells with n-Butyrate, the NF B inhibitor PDTC and the JAK3 inhibitor WHI-P-154

Supervisor: Péter Gergely

Dendritic cells (DC) can present antigens in immunogenic or tolerogenic fashion. Various attempts have been made to convert DC into tolerogenic antigen presenting cells (APC). These treatment modalities have in common the ability to interfere with the proper maturation of DC. Our aim was to generate tolerogenic monocyte-derived human dendritic cells *in vitro* by activating immature DC in the presence of different inhibitory substances.

In this study three independent attempts were made to convert DC into tolerogenic APC such as treatment of DC with n-butyrate, the NFκB inhibitor pyrrolidine dithiocarbamate (PDTC) and the Janus Kinase 3 inhibitor WHI-P-154. We aimed to characterize these *in vitro* modulated DC and to analyze the hyporesponsiveness induced by these APC. Furthermore, we aimed to study the influence of calcineurin inhibition with Cyclosporine A (CsA) in functional T-cell responses elicited by DC pretreatment with PDTC in primary and secondary mixed lymphocyte culture (MLC) and to establish T-cell hyporesponsiveness with PDTC-modulated dendritic cells in T cells from patients with renal allografts under CsA-based immunosuppression. These modulated DC exhibited phenotypical features and cytokine production of immature DC, as well as defective stimulatory capacity for allogeneic T-cell responses. Up-regulation of the costimulatory molecules CD80, CD86, CD40 and the MHC antigens as well as of the specific DC maturation marker CD83 was found to be clearly suppressed. Endocytosis and macropinocytosis were inhibited. We observed a clear inhibition of the production of the immunostimulatory cytokine IL-12. We found reduced T-cell stimulatory capacity of DC. These modulated DCs induced a state of hyporeactivity in alloreactive T cells in secondary cultures. Addition of CsA to primary MLC led to an inhibition of the allogeneic T-cell proliferation. Secondary MLC revealed that the presence of CsA during alloantigenic priming did not influence the induction of the hyporesponsive state by PDTC-modulated DC.

Findings, concerning the n-butyrate pretreatment of DC not only provide a novel interpretation of its potent anti-inflammatory properties but also suggest a possible *in vivo* immunomodulatory role via interference with the function of DC in the gastrointestinal tract. WHI-P-154 profoundly influenced the maturation of CD40-triggered DCs. Therefore,

this property of JAK3 inhibitors represents a further level of interference, which together with the well-established suppression of common-gamma chain signaling could be responsible for their clinical efficacy. We generated and described a novel form of tolerogenic DCs by activating immature DCs in the presence of PDTC. The successful establishment of allogeneic hyporesponsiveness is not prevented by concomitant calcineurin inhibition *in vitro* as well as in T cells from patients under Cyclosporine A-based immunosuppression *ex vivo*. This indicates that the potential benefit of DC-based protocols as a therapeutic strategy will not be lost if this treatment modality is incorporated into conventional clinical immunosuppressive treatment. In conclusion, given the therapeutical potential of dendritic cells to induce tolerance in general, these findings suggest that immunization strategies using *in vitro* modulated dendritic cells that are characterized by a maturation blockade might be potentially useful in the control of allograft rejection.

- *Säemann MD*, Kelemen P*, Böhmig GA, Hörl WH, Zlabinger GJ (2004) Hyporesponsiveness in alloreactive T-cells by NF-κB inhibitor-treated dendritic cells: resistance to calcineurin inhibition. Am J Transplant 4: 1448–1458 (*Co-first authors: Errata. Am J Transplant 2004[4]: 1928–1929).*
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- *Säemann MD, Diakos C, Kelemen P, Kriehuber E, Zeyda M, Böhmig GA, Hörl WH, Baumruker T, Zlabinger GJ (2003) Prevention of CD40-triggered dendritic cell maturation and induction of T-cell hyporeactivity by targeting of Janus Kinase 3. Am J Transplant 3: 1341–1349.*

PETRA KISZEL (2008)

Genetic polymorphism- and haplotype-based methods in different human model systems

Supervisor: György Füst

Single nucleotid polymorphism (SNP)- and haplotype-based methods offer a powerful approach to catch candidate genes for multifactorial diseases. Furthermore, *in vitro* model systems are used to examine the effect of maximally informative SNPs in haplotype blocks. In our studies, we showed the practical applications and difficulties of these haplotype-based methods in three different human model systems. The human MHC region is located in the short arm of chromosome 6, where the linkage disequilibrium is very high among the locuses. In this region the 8.1 ancestral haplotype (8.1 AH) was the most frequent in Caucasian populations. We examined the frequency of 8.1 AH and its recombinated variants in three Caucasian populations. Out of the examined 8.1 AH markers, the HSP70-2 +1267 G allele occurred most frequently in a recombinated 8.1 AH variant, which carried only one 8.1 AH marker. Therefore, we can conclude that hot-spot regions may be located around the HSP70-2 gene.

In the next studies the associations were examined between IL-6 -174 G/C SNP and anti-HSP60 autoantibody levels in the healthy Hungarian population. The IL-6 -174 G allele carriers have significantly higher autoantibody levels than subjects with CC genotype. The haplotype blocks were determined in the promoter and coding regions of IL-6 gene

with genotyping of 7 SNPs. Two 2000 bp long haplotype blocks were found. The most frequent haplotype (CGCCG), which carried the C allele at position -174, had low autoantibody levels. These results can be explained in several ways. High IL-6 levels can cause high autoantibody levels or other genes, which are in linkage disequilibrium with IL-6 gene on the chromosome 7, may have indirect effect on the production of autoantibodies. In our third study we examined the functional effect of IL-6 -174 G/C polymorphism in an *in vitro* model system. No significant differences were observed between IL-6 expression of HUVEC cells with different IL-6 -174 G/C genotypes. IL-6 production of HUVECs did not depend on different genotypes at IL-6 -174 position. However, we can not exclude that the IL-6 production can be influenced by the IL-6 -174 SNP in endothelial cells of different origin.

- *Kiszel P, Fust G, Pessi T, Hurme M, Prohaszka Z (2006) Associations between Interleukin-6 genetic polymorphisms and levels of autoantibodies to 60-kDa heat-shock proteins. Human Heredity 62: 77–83.*
- *Kiszel P, Mako V, Prohaszka Z, Cervenak L (2007) Interleukin-6 -174 promoter polymorphism does not influence IL-6 production after LPS and IL-1beta stimulation in human umbilical cord vein endothelial cells. Cytokine 40: 17–22.*
- *Kiszel P, Kovacs M, Szalai C, Yang Y, Pozsonyi E, Blasko B, Laki J, Prohaszka Z, Fazakas A, Panczel P, Hosszufalusi N, Rajczy K, Wu YL, Chung EK, Zhou B, Blanchong CA, Vatay A, Yu CY, Fust G (2007) Frequency of carriers of 8.1 ancestral haplotype and its fragments in two Caucasian populations. Immunol Invest 36: 307–319.*

JUDIT LAKI (2008)

The 8.1 ancestral MHC haplotype: a double-edged sword

Supervisor: György Füst

The term “conserved extended haplotype” or as also known “ancestral haplotype” (AH) defines highly conserved haplotype—blocks of highly conserved genomic sequences—derived from a common remote ancestor. The major histocompatibility complex (MHC) or human leukocyte antigen (HLA) regions harbour genes—such as HLA-A, B, C, DQ, DR, heat shock proteins, complement components and cytokines like TNF-α and LT-α—that play important roles in the innate or acquired immune response. Variations, alleles of these genes make up conserved extended haplotypes in the MHC regions, extending through class I, class II and class III regions over a few megabases. The so-called 8.1 AH consists of, among others, HLA-A1, HLA-B8, HLA-Cw7, TNFα -308A, C2*C, Bf*S, C4A*Q0, C4B*1, HLA-DRB1*0301, HLA-DQA1*0501 and HLA-DQB1*0201 alleles. Individuals carrying the 8.1 AH have altered cytokine profile, increased TNF-α production, high titers of autoantibodies and circulating immune complexes. These features are thought to be beneficial in response to infections and are thought to have had positive selection during evolution, thereby resulting in accumulation in the Caucasian population. However, the 8.1AH may lead to the development of autoimmune diseases as a long-term side effect. (1) We have shown the RAGE -429C and in another study the LTA +252G and HSP70-2 +1267G alleles to be member alleles of the 8.1AH. (2) The detection of TNF-α -308A, RAGE -429C, LTA +252G and HSP70-2 +1267G alleles may simplify the detection of the 8.1AH. (3) According to our studies carrier state of this haplotype may modify the clinical

course of cystic fibrosis by delaying the onset of colonization, possibly by exerting a more efficient immune response against bacterial infections. (4) In another study we found carriers of the 8.1 AH to have a significantly higher risk for colorectal cancer than non-carriers. So the genetically encoded alteration of the immune response of 8.1AH carriers which is more efficient against infections may not be advantageous in terms of tumours. Disease association studies focusing on single alleles lose the context and detection of the effect of the complex haplotype they belong to, therefore the importance of haplotype analysis rather than determination of single alleles as modifier factors in disease association studies is to be emphasized.

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ZOLTÁN NAGYMÁNYOKI (2008)

Cellular immune response and other functional proteins in gestational trophoblastic diseases

Supervisor: Vilmos Fülöp

Gestational trophoblastic diseases are mostly the results of a multispermic conception. The molar pregnancies containing extremely heterogenic mainly paternal chromosomes are characterized by excessive trophoblast proliferation. The complete mole which contains only paternal chromosomes has significant invasive potential and in 20% of cases it persists in the uterus after evacuation. The persistent mole can compromise the life of the patient without effective chemotherapy. I investigated the maternal-molar interface, the implantation site, to better understand the mechanisms of the trophoblast invasion, and to find a better marker than hCG follow-up to predict molar persistence. According to our understanding 3 mechanisms can control the invasion and the persistence of the molar pregnancy in the uterus: the maternal immune system, the expression profile of the decidual cells and the blood supply of the endometrium. On histologic slides I investigated the number and function of the maternal immune cells, the expression profile of the decidual cells and the level of angiogenesis at the implantation site and the associated angiogenic factors produced in different gestational tissues. Besides, from fresh frozen normal placental and molar tissues I characterized the T cell receptor variable beta chain usage to determine the specificity of the T cells at the implantation site. The results demonstrate that in molar tissues the vigorous immune response is specific to one or few conservative placental antigens. In molar pregnancy, as a result of the T cell activation, the regulatory T cells appear at the implantation site. However, at the normal implantation site regulatory T cells can not be found therefore they do not play a role in the development of the maternal immunosuppression. Besides, higher expression of the laminin receptor 1 was noted on

decidual cells at the molar implantation sites and the higher level of hCG as an angiogenic factor might contribute to a higher microvessel density (MVD) at the placental implantation site. Evaluation of MVD at the implantation site might also be a useful predictor of persistence in complete moles.

- *Nagymanyoki Z, Callahan MJ, Parast MM, Berkowitz RS, Mok SC, Fulop V (2008) Overexpression of laminin receptor 1 on decidual cells in partial and complete mole. Gynecol Oncol 108: 121–125.*
- *Nagymanyoki Z, Callahan MJ, Parast MM, Fulop V, Mok SC, Berkowitz RS (2007) Immune cell profiling in normal pregnancy, partial and complete molar pregnancy. Gynecol Oncol 107: 292–297.*
- *Fülöp V, Végh G, Bátorfi J, Nagymányoki Z, Berkowitz RS, Dancsó J (2006) A terhességi trofoblaszt daganatok molekuláris biológiája. Magy Nőorv L 69: 297–306.*

ÉVA PALKONYAI (2006)

Prognostic factors in rheumatoid arthritis

Supervisor: Péter Gergely

In a multicenter transcultural prospective study a cohort of 172 Austrian, Hungarian and Slovenian patients suffering from early rheumatoid arthritis (RA) were followed over 3 years at 6 monthly intervals. The relevant clinical, laboratory, serological, genetic, radiological and psychosocial features of early RA were correlated to unfavourable outcome of the disease in order to find prognostic markers for a poor prognosis. Poor outcome was defined by the following variables: significant deterioration of Health Assessment Questionnaire (HAQ) score; need for treatment with biologicals, cytotoxic drugs or corticosteroid pulse therapy; surgery of joints or tendons; death of the patient.

According to the data of our multicenter investigations in the Central European region, 24.4% of early RA patients had a poor outcome. Higher initial values for IgG and IgM rheumatoid factor, COMP, HAQ score, Larsen score of feet, DAS score, ESR, swollen and tender joint counts predicted worse outcome but not the IgA rheumatoid factor, a-CCP antibody level, Larsen score of hands or educational level. Furthermore, a comparison of clinical presentation, laboratory values and outcome of patients with late-onset RA versus adult-onset RA was carried out, but no significant differences were found. The significantly higher HAQ score and significantly higher serum COMP-levels found in late-onset RA patients could be explained by concomitant osteoarthritis.

For the first time in the literature we introduced a new modified short Larsen score based on the evaluation of 12 key areas in the hands and wrists. In a cross-sectional as well as a longitudinal analysis we proved that the short Larsen score works as well as the original one, which is based on the evaluation of 40 areas of the hands and feet.

In a transcultural study in early RA, also first in the literature, we compared the prevalence of depression. There was a higher prevalence of depression among Hungarian patients as compared with Austrian ones, in spite of their geographical and cultural proximity.

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- *Palkonyai É, Larsen A, Temesvári IP, Géher P, Kaposi NP, Szilágyi M, Ratkó I, Kolarz G (2006) A rheumatoid arthritis okozta radiológiai elváltozások számszerű meghatározása, különös tekintettel a módosított rövid Larsen-skálára. Magy Reumatol 47: 70–76.*
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SZABOLCS RUGONFALVI KISS (2006)

Influence of congenital and infective factors on the progression of atherosclerotic disease

Supervisor: György Füst

Based on literature data and our previous studies it seemed reasionable that a pattern recognition molecule (mannan binding lectin)—or rather its genetically defective mutant form—which plays a role in the innate immunity may contribute to the progression of severe atherosclerosis. We also wondered if this molecule, wich is able to activate the complement system, has any role at another vascular territory of the arterial system (carotid artery). Our work was to investigate the relation of *Chlamydia* and the mannan binding lectin for the first time in vascular disease. The mutant variant has lower plasma concentration of the functionally active mannan binding lectin protein and seemed to put the patients at 2–3 times higher risk for having coronary artery disease and for developing severe complication in anti-*Chlamydia* positive individuals. This correlation existed even after adjustment for sex, age, smoking habits and total cholesterol level. There was no correlation among patients carrying homozygote wild genotype (A/A).

Patients carrying the wild A allele developed restenosis after eversion type carotid endarterectomy more frequently as our retrospective analysis revealed. This effect seemed to be gene-dose dependent: patients with the homozygote variant allele (O/O) are more protected compared to heterozygotes. None of the 6 patients carrying homozygote genotype has developed restenosis. According to the data of the prospectie study a higher degree of restenosis was present in women carrying the homozygote wild-type allele (A/A) compared to the A/O and O/O genotype at 7 month postoperatively and they kept on having this tendency all the way.

Significant difference could only be measured at the end of the follow-up in men. Deficient mannan binding lectin has a lower affinity to microorganism and carries a higher chance for the inflammation to persist chronically and may contribute to the development of vascular wall inflammation. On the contrary, high levels of mannan binding lectin may cause tissue destruction through complement activation.

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carotid eversion endarterectomy in homozygous carriers of the normal mannose-binding lectin genotype. Stroke 36: 944–948.
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KRISZTINA KATALIN SALLAI (2007)

The role of pathogenic autoantibodies and their detection in systemic lupus erythematosus

Supervisor: Péter Gergely

Systemic lupus erythematosus is an autoimmune disorder characterized by a systemic inflammatory state and the presence of a wide range of pathogenic autoantibodies. My research focused on the detection and characterization of these autoantibodies, since many details about their development and their pathogenic effects are still unclear. My work also included the evaluation of laboratory tests which might help the diagnosis, disease activity or prediction about the outcome of the SLE. The aims of my work were: (1) to measure serum DNase activity in the SLE population with the aim of evaluating the significance of DNase activity in the development of SLE, especially in lupus nephritis; (2) to study the association between the DNase activity and the production of antinucleosome antibodies; (3) to examine the presence of antiphospholipid antibodies in SLE, and their role in the development of thromboembolic episodes; (4) to examine the role of other, SLE independent thromboembolic risk factors, and evaluate the clinical value of thrombophilia screen in SLE; (5) to analyze the inhibitory effect of synthetic peptides from the Fc region of the antibodies on the Fcγ receptor mediated effector functions of human monocytes, and evaluate the possible use of these in the treatment of SLE. My results confirm that patients with SLE have reduced serum DNase activity, but the enzyme activity does not show association with disease activity or organ involvements such as lupus glomerulonephritis, but it negatively correlates with serum antinucleosome antibody concentration. According to my results, the incidence of thromboembolism is about 10-fold higher in SLE than that in the normal population, and the antiphospholipid antibodies play the primary role in the development of thrombosis in SLE. Other, inherited risk factors seem to be of lesser importance. The IgG Fc peptides, although in conjugates they could trigger macrophage effector functions via the FcRs, were unable to inhibit either the binding of immuncomplex-bound antibodies to the receptors or the IL-6 cytokine production induced by the antibodies.

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- *Sallai K, Nagy E, Gergely P (2004) SS-A(Ro) és SS-B(La) autoantitestek előfordulása szisztémás lupus erythematosusban. Magy Immunol 3: 16–21.*

GÁBOR SZÉPLAKI (2008)

Role of the complement system in the pathogenesis of atherosclerotic vascular diseases

Supervisor: György Füst

Atherosclerosis is a chronic inflammatory disease with multiplex etiology: genetic predisposition and environmental factors both play essential roles in the pathogenesis. The complement system—which is part of the innate immune system—has essential roles in the defense against microbial infection, clearance of immune complexes, modulation of the inflammatory response and bridging innate and adaptive immunity. The complement system plays an important role in the pathogenesis of atherosclerosis and in the mediation of ischemia/reperfusion (IR/) injury as well.

The aim of the present study was to investigate the prognostic values of certain complement proteins (C3) and regulators (C1-inhibitor) in atherosclerotic vascular diseases. We studied the associations of complement proteins and (1) the occurrence of new vascular events in patients with severe coronary artery disease (CAD) who underwent coronary artery bypass graft (CABG) surgery and (2) the development of early restenosis in patients who underwent carotid endarterectomy (CEA) in two prospective studies, respectively. Additionally, we aimed to study the presence and mechanism of early complement activation following CEA and its association with the degree of I/R injury.

We found that women with severe CAD have nearly 4-times higher risk to develop new vascular events following CABG surgery. High C3 levels were associated with the onset of early restenosis following CEA, partly dependent of the mannose-binding lectin (MBL2) genotypes. We found that low C1-inhibitor levels predicted the development of an early restenosis following CEA, in homozygous carriers of the wild-type alleles of MBL2 and were associated with the degree of restenosis. We demonstrated that early complement activation followed CEA and were associated with the time of I/R injury.

Our results suggest that C3 might be a novel specific inflammatory marker of the progression of severe CAD and contributes to the development of an early restenosis following CEA. The complement system plays an essential role in the pathophysiology of restenosis; dysregulation of complement might enhance the process. According to our data, monitoring of C1-inhibitor levels and genotyping of MBL2 might be useful during the follow-up of patients who underwent CEA.

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SCHOOL OF PH. D. STUDIES

8. PATHOLOGICAL SCIENCES

Chairman:
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General overview

The Doctoral School of Pathology includes five postgraduate teaching Programs as follows: Oncology, Experimental and Diagnostic Pathomorphology, Microbiology, Transplantation studies, Health Sciences. Consequently, the training covers a rather broad area of medical sciences involving both the etiopathogenesis, diagnostic and therapeutic activities of the most common human diseases and health education. The training concentrates on individual research work guided by the tutors who makes proposal for the topic of the research, provides the facilites, warrant the technical/intellectual up-to-dateness and the progress of study. For the introduction to basic and applied pathology it is compulsory to attend the regular courses with final examinations. At present 27 Ph. D. students with diploma in medicine, pharmacy, or biology are holders of fellowship, in addition 19 medical doctors as corresponding Ph. D. students are preparing their dissertation. The Ph. D. degree has been awarded to 102 students trained in the frame of Doctorate School of Pathology in the last years.

PROGRAM 8/1.

ONCOLOGY

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Program overview

The Program invites those who intended to learn and study tumor biology and basic science in experimental and clinical oncology. The Ph. D. students are also trained in updated techniques in cell biology, pathology, biochemistry and recombinant gene technology. The following courses are organized by the program: Experimental Oncology, Clinical Oncology, Molecular Oncology.

Titles of research projects	*Supervisors*
Epigenetic gene inactivation in breast carcinoma and in head and neck tumors	Orsolya Csuka
Pediatric oncology	Miklós Garami
System biological modelling of chemoresistance	Balázs Győrffy
Experimental modelling, characterization and chemotherapeutic response in osteosarcoma	András Jeney
Regulation of cell proliferation and cell death	László Kopper
Role of extracellular matrix elements in the regulation of liver behavior	Ilona Kovalszky
Role of proteoglycans in carcinogenesis	Ilona Kovalszky
Gene defects related to malignancy	Ilona Kovalszky
Clinical and experimental aspects of oncotherapeutic markers in colorectal cancer	Judit Kralovánszky
Epidermal growth factor receptor (EGFR) in giant cell bone tumors in the progression of giant cell bone tumors	Tibor Krenács
Upregulation of collagen XVII in malignant transformation and tumor progression	Tibor Krenács
Defects of direct cell-cell communication in malignant melanoma due to failures in connexin junctions	Tibor Krenács
Tumor immunology—tumor infiltrating immunocells in human tumors and immunological parameters in sentinel lymph nodes	Andrea Ladányi
Molecular genetics in genesis and progression of lymphomas	András Matolcsy
Signalling pathways directed by receptors and metabolism in cell death and their pharmacological characterization	Rudolf Mihalik
Role of liver stem cells in hepatic disorders and tumors	Péter Nagy
Tumor induced angiogenesis	Sándor Paku
Morphological study on the biliary tract and its vascular network during regeneration and carcinogenesis	Sándor Paku
Death receptor signalling as target for tumor therapy	István Peták
Host and tumor factors in metastatization (mainly in melanomas)	Erzsébet Rásó
The examination of individual beam sensitivity in radiation therapy patients, the identification of genes is responsible for the beam sensitivity	Géza Sáfrány
The increase of the sensitivity of tumours for radiotherapy with gene therapy procedures	Géza Sáfrány
The examination of the late genetic effects of the ionising radiation	Géza Sáfrány
Chromosomal instability in giant cell bone tumors	Zoltán Sápi
Repair of the function of proapoptotic regulators to increase the effect of chemotherapy	Anna Sebestyén
Clinical progression and ECM components in oral precancerosis and squamous cell cc	Zsuzsanna Suba
Induction of apoptosis	Béla Szende
Role of microRNA in the pathogenezis of non-Hodgkin lymphomas	Ágota Szepesi
Gene expression maps to predict individual behavior and response of tumors	József Tímár
Metastatization and angiogenesis	József Tímár

Modelling and regulation of the movement of human tumor cells	József Tóvári
The effect of ionizing radiation on the immune system and its role in the modulation of antitumor immune response	Katalin Lumniczky

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Zsuzsanna Dunai	ft	Rudolf Mihalik
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Tamás Márton Garay	ft	József Tímár
András Lőrincz	ft	István Peták
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Melinda Hajdu	ft	Anna Sebestyén
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Enikő Kis	pt	Géza Sáfrány
Lajos Viktor Komlósi	ft	Judit Kralovánszky
Katalin Kristó	i	László Kopper
Viktória László	ft	Sándor Paku
Judit Müller	i	Judit Kralovánszky
Judit Pápay	i	József Tímár
Andrea Réti	ft	Judit Kralovánszky
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Tibor Füle	ft	Ilona Kovalszky
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Csaba Galambos	i	Sándor Paku
Beáta Hargitai	i	Béla Szende
Revekka Harisi	it	András Jeney
Péter Hauser	i	László Kopper
Erika Hitre	i	Judit Kralovánszky
Gergely Imre	ft	Rudolf Mihalik
Pál Kaposi Novák	ft	András Kiss
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Júlia Lukits	it	József Tímár
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Lilla Reiniger	ft	Ágota Szepesi
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Botond Tímár	ft	András Matolcsy
Gyula Végső	pt	László Kopper
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ft, full-time; pt, part-time; it: international; i, individual; crc: Cooperation Research Centre

Abstracts of Ph. D. theses successfully defended in 2006, 2007 and 2008

GÁBOR BARNA (2006)

The regulation of apoptosis by physiological and synthetic drugs

Supervisor: László Kopper

The cell death program (apoptosis) is one of the most important regulatory function to maintain the homeostasis in a living organism. In some diseases this regulation is damaged, the rate of apoptosis pathologically increased (e.g. neurodegenerative diseases) or decreased (e.g. cancer). In cancer the restoration of normal rate of cell death could be a valuable tool to manage tumor growth. Our goal was to study apoptosis induced by natural or synthetic drugs, focusing on the mechanism of actions, including the two main apoptotic pathways.

The apoptotic effect of exogenous TGFβ1 was studied in a TGFβ1 producing B-lymphoma cell line (HT58) which cells were insensitive to their endogenous TGFβ1. It has been shown that exogenous TGFβ1 induces apoptosis in HT58 cells increasing mitochondrium permeability by reactive oxigen species and activating caspases. In this case the apoptotic machinery of TGFβ1 in HT58 cells was intact because exogenous TGFβ1 was able to induce apoptosis, deregulating survival signals. In another set of experiments we compared the apoptotic effect of two retinoids, a natural and its synthetic analogue in B-lymphoma cells. It is concluded that both retinoids induced apoptosis via mitochondrial pathway, in caspase-dependent or caspase-independent way, respectively. Finally we studied the conformational changes in Bak molecule during cisplatin treatment in melanoma cell lines. It turned out that Bak is activated differently in various melanoma cell lines. These results call the attention to the optimal use of apoptosis induction in tumor cells, using either physiological regulators or artificial compounds.

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- *Dobos J, Timar J, Bocsi J, Burian Z, Nagy K, Barna G, Petak I, Ladanyi A (2004) In vitro and in vivo antitumor effect of 2-methoxyestradiol on human melanoma. Int J Cancer 112: 771–776.*

ÁGNES BOGNÁR (2007)

Clonal selection in the bone marrow involvement of follicular lymphoma

Supervisor: Ágota Szepesi

To characterize the pathways of bone marrow involvement of follicular lymphoma, we performed morphological and immunophenotypical analysis of tumor cells from lymph nodes and corresponding bone marrows in 21 patients with FL. In three cases, genealogical trees were constructed based on the immunoglobulin heavy chain gene variable region (IgVH) sequences of tumor clones from lymph nodes and bone marrows. Results showed that follicular lymphomas within the bone marrows display identical or lower cytological grades than in the lymph nodes. In the majority of cases, different proportion of tumor cells expressed bcl-2, CD10 and Ki67 in lymph nodes and bone marrows. Tumor cells in the bone marrow showed ongoing somatic hypermutation of the IgVH genes; the distribution of these mutations was highly consistent with antigen selection. The topology of the genealogical trees revealed that different subclones populate the lymph nodes and bone marrow, and bone marrow infiltration may occur at different points of the clonal evolution of follicular lymphoma. Early descendants of the original tumor clone and derivatives of diversified tumor clones may invade the bone marrow. These results suggest that the bone marrow involvement of follicular lymphoma is associated with intensive clonal selection of tumor cells, and the bone marrow provides a microenvironment similar to the germinal centers of lymph nodes, where tumor cells retain their biological nature.

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- *Timár B, Fülöp Z, Csernus B, Angster C, Bognár Á, Szepesi Á, Kopper L, Matolcsy A (2004) Relationship between the mutational status of VH genes and pathogenesis of diffuse large B-cell lymphoma in Richer's syndrome. Leukemia 18: 326–330.*

CSABA BÖDÖR (2008)

The role of the aberrant somatic hypermutation and activation-induced cytidine deaminase in the pathogenesis of mediastinal large B-cell lymphoma

Supervisor: András Matolcsy

Mediastinal large B-cell lymphoma (MBL) is a highly aggressive B-cell non-Hodgkin lymphoma (B-NHL), categorized as a subtype of diffuse large B-cell lymphomas (DLBL), with distinct clinicopathological characteristics. The specific genetic alterations associated with the pathogenesis of the disease are not fully understood and the cellular origin of MBL is still a matter of debate. A recently described form of genetic instability, termed aberrant somatic hypermutation (ASHM), plays a role in the pathogenesis of more than 50% of DLBLs. ASHM is regarded as a malfunction of the physiological somatic hypermutational mechanism (SHM) of immunoglobulin (Ig) and BCL-6 genes in B-cells. ASHM targets multiple loci outside Ig genes, including proto-oncogenes c-MYC, PAX-5, RhoH and PIM-1. Activation-induced cytidine deaminase (AID) is essential for SHM. The expression of AID is normally restricted to CD19+/CD38+ germinal centre (GC) B-cells. Constitutive expression of AID also plays a part in the pathogenesis of different types of lymphomas by generating illegitimate DNA recombinations and somatic mutations in different genes. To determine the possible role of ASHM and AID expression in the pathogenesis of MBL, we have determined the expression level of AID mRNA by real-time polymerase chain reaction, and we have analyzed the mutational status of genes affected by ASHM, including c-MYC, PAX-5 and RhoH, in tumor specimens from six patients with MBL and in different cell lines. Mutations in one or more genes and expression of AID mRNA were detected in all the six cases of MBL and in the MBL derived cell line MedB-1. Our results were proven also on protein level using immunohistochemistry. These observations suggest that ASHM and AID expression may have a role in the pathogenesis of MBL.

- *Bödör Cs, Bognár Á, Reiniger L, Szepesi Á, Tóth E, Kopper L, Matolcsy A (2005) Aberrant somatic hypermutation and expression of activation induced cytidine deaminase mRNA in mediastinal large B-cell lymphoma. Br J Haematol 129: 373–376.*
- *Reiniger L, Bödör Cs, Bognár Á, Balogh Zs, Csomor J, Szepesi Á, Matolcsy A (2006) Richter's and prolymphocytic transformation of chronic lymphocytic leukemia are associated with high mRNA expression of activation-induced cytidine deaminase and aberrant somatic hypermutation. Leukemia 20: 1089–1095.*

JUDIT DOBOS (2008)

Endocrine factors influencing melanoma progression

Supervisor: Andrea Ladány

According to recent findings that beside cancers traditionally considered as hormone-dependent, several other tumor types show different behavior in the two sexes, indicating the possible role of endocrine factors in the course of these diseases. The possibility that endocrine factors may influence the clinical course of human malignant melanoma is suggested by the higher survival rate in premenopausal vs. postmenopausal women or men of any ages. However, investigations on the sex hormone receptor status of human cutaneous melanomas and experiments attempting to support the epidemiological results yielded conflicting results. In our human melanoma cell lines we failed to detect steroid receptors at protein level, while quantitative PCR demonstrated that their mRNA expression level was orders of magnitude lower compared to the positive control cell lines. Sex hormones did not influence the *in vitro* features of the human melanoma cells considerably. On the other hand, glucocorticoid receptor was present both at mRNA and protein level, although, dexamethasone was effective *in vitro* only at high doses. Our previous experiments showed that intrasplenic injection of human melanoma cells resulted in a significantly higher number of liver colonies in male than in female SCID mice. We now show that this difference evolves during the first day. After injection into the tail vein we did not observe gender-dependent difference in the efficiency of pulmonary colonization. Examining the pattern of metastasis formation after intracardiac injection, we have found differences between the two sexes in the incidence or number of colonies only in the case of the liver but no other organs. We concluded that the observed phenomenon is specific to the liver; therefore we investigated the effects of 2-methoxyestradiol, an endogenous metabolite of estradiol produced mainly in the liver, known from its estrogen receptor-independent antitumor activity. 2ME2 effectively inhibited melanoma cell proliferation by inducing apoptosis and an arrest in the G2/M phase. The mechanism of action involved microtubules, mitochondrial damage and caspase activation as well. In SCID mice, 2ME2 was effective in reducing primary tumor weight and the number of liver metastases after intrasplenic injection of human melanoma cells, and causing significantly higher rate of apoptotic cells in the metastases.

- *Dobos J, Tímár J, Bocsi J, Burián Z, Nagy K, Barna G, Peták I, Ladányi A (2004) In vitro and in vivo antitumor effect of 2-methoxyestradiol on human melanoma. Int J Cancer 112: 771–776.*
- *Döme B, Rásó E, Dobos J, Mészáros L, Varga N, Puskás LG, Fehér LZ, Lőrincz T, Ladányi A, Trikha M, Honn KV, Tímár J (2005) Parallel expression of alphaIIbbeta3 and alphavbeta3 integrins in human melanoma cells upregulates bFGF expression and promotes their angiogenic phenotype. Int J Cancer 116: 27–35.*

CSABA FORSTER HORVÁTH (2006)

Cell adhesion molecules (CD44v3, VAP-1) on human tumor tissue T- and endothelial cells

Supervisor: József Tímár

Cell adhesion molecules play an important role in the interactions between immune cells and the endothelium but also in homo- and heterotypic interactions of cancer cells. In this work we have studied the tumoral and stromal expression of v3 variant of CD44 and VAP-1 adhesion molecules in head and neck cancer and malignant melanoma. According to our observations the normal epithelium of the larynx is characterised by the expression of CD44v6/CD44v3 and this phenotype change during carcinogenesis: both molecules are downregulated. In parallel to this, expression of NM23H1 and MMP-2 are upregulated in cancer cells. We also observed that in the tumor stroma mononuclear cells and endothelial ones were also positive for CD44v3. We have shown that resting T cells constitutively express the CD44v3 gene, but the protein is not present at the cell surface. Stimulation of T cells by anti-CD3 antibody and IL-2 or by the phorbol ester (PMA) *in vitro* resulted in the up-regulation of CD44s, and CD44v3 in parallel with CD44v6 and CD25 at the surface, therefore can be considered as an activation marker. Tumoral microvessels are different from normal counterparts, which could be reflected in their adhesion molecule pattern. We have confirmed the CD44v3 expression of peritumoral microvessels in skin melanoma and head and neck cancers. *In vitro* studies identified the CD44 exon v3 transcription in HUVEC and KS Imm cells and demonstrated the protein expression *in situ* on the surface of cultured endothelial cells (occasionally colocalized with MMP-2). Functional analysis identified its role in the migration of endothelial cells. VAP-1 expression was analysed in skin melanoma samples. We found a significantly decreased VAP-1 protein expression in intratumoral microvessels compared to peritumoral ones, the loss of expression involved both endothelial and smooth muscle cell components. The decrease of VAP-1 expression was independent of the tumour thickness (a strong prognostic factor). However, the 5-year survival of melanoma patients with low VAP-1 protein expression in intratumoral blood vessels was lower compared to those patients with high expression, suggesting a role in melanoma progression. These studies demonstrated the importance of cell adhesion molecules in various cancers but provided examples also for their different role in cancer cells compared to stromal counterparts.

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- *Forster-Horváth C, Döme B, Paku S, Ladányi A, Somlai B, Jalkanen S, Tímár J (2004) Loss of vascular adhesion protein-1 expression in intratumoral microvessels of human skin melanoma. Melanoma Res 14: 135–140.*
- *Forster-Horváth C, Döme B, Rásó E, Morini M, Albini A, Tímár J (2004) Expression of CD44v3 protein in human endothelial cells in vitro and in tumoral microvessels in vivo. Microvasc Res 68: 110–118.*

TIBOR FÜLE (2006)

Human papillomavirus in cervical cancer and in peritumoral tissues

Supervisor: Ilona Kovalszky

It is generally accepted that high-risk HPV types act as effective carcinogens in the development of the carcinoma of the uterine cervix. In our experiments, we aimed at investigating how frequently the three most common high risk HPV types (16, 18 and 33) occur in the tumor specimens of patients who had undergone surgery for in situ and/or invasive cancer of the cervix at the 1st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary. Furthermore, we investigated the occurrence of these HPV types in the lymph node metastases of these tumors, and in lymph nodes without metastasis. Finally, we analyzed our data to calculate whether the presence of HPVs, also regarding the specific types detected, affects tumor progression. An existing correlation would indicate that the presence of HPVs can be regarded as a prognostic factor in the cancers of the uterine cervix. In our study, the HPV types examined were found less frequently in CIN III specimens than in invasive cancers. This finding was already reported by other groups who compared HPV infection of *in situ* and invasive cervical carcinomas. In our patient group consisting of Hungarian women, the percentage of HPV-positive samples was 56% in 50 surgically removed *in situ* cervical cancers, whereas 92% of invasive cancers was related to infection with HPV 16, 18 and 33. According to our results, lymph nodes with metastasis are significantly more likely to contain HR-HPV sequences than those without metastasis; however, the presence of HPVs is not necessarily an indicator of micrometastases. In our follow-up studies, no correlation was demonstrated between the HPV status of lymph nodes and patient survival. Supposedly, viral sequences and viral oncoproteins that are protagonists in the early steps of tumorigenesis play only a minor or irrelevant role in the later phases of tumor progression, e.g. in the transition to the invasive phenotype. Hence, in contrary with our initial expectations, the presence of HPVs has no significant influence on the survival of patients.

According to our investigations, the DNA and certain proteins of HPVs are present in non-epithelial cells. In a subset of samples, HPV 16 E6 and E7 immunostaining was observed in peripheral nerves and in vascular endothelia in the vicinity of the tumor. The presence of the entire viral genome was also verified. These data suggest that HPV may contribute to the development of non-epithelial tumors, and it might be an etiological factor in non-malignant pathological processes of which the origin is presently unclear. HPV affectedness of non-epithelial tissues may also be related to a novel spreading pathway of these viruses; the exploration of such processes is a major research challenge for our future work.

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- *Lotz G, Simon S, Patonai A, Sotonyi P, Nemes B, Sergi C, Glasz T, Fule T, Nashan B, Schaff Z (2004) Detection of Chlamydia pneumoniae in liver transplant patients with chronic allograft rejection. Transplantation 77: 1522–1528.*

ZSOLT FERENC FÜLÖP (2006)

Analysis of genetic instability in Richter's transformation of B-cell chronic lymphotic leukemia

Supervisor: András Matolcsy

B-cell chronic lymphocytic leukemia (B-CLL) is an indolent non-Hodgkin lymphoma that may transform into diffuse large B-cell lymphoma (DLBL). DLBL arising from the transformation of B-CLL is referred to as Richter's syndrome or Richter's transformation. To analyze whether microsatellite instability (MSI) and DNA mismatch repair defects are associated with Richter's transformation, we have performed microsatellite analysis, mutational analysis of the hMLH1 and hMSH2 mismatch repair genes and methylation status analysis of the CpG island of the hMLH1 promoter on serial biopsy specimens from 19 patients with B-CLL. Ten cases of B-CLL showed no histologic alteration in the second biopsy, and nine cases of B-CLL underwent morphologic transformation to DLBL in the second biopsy. Investigating eight microsatellite loci, a high level of MSI was associated with Richter's transformation in four cases of B-CLL, but none of the B-CLL displayed this level of MSI without transformation. Mutations of the hMLH1 and hMSH2 genes were not detected in any of the lymphoma samples. In five cases of Richter's transformation the hMLH1 promoter was hypermethylated both in the B-CLL and in the DLBL samples. Hypermethylation of the hMLH1 promoter associated with high levels of MSI in four cases, and low levels of MSI in one case. These findings suggest that in some cases of Richter's transformation the genetic instability initiated by the defect of the DNA mismatch repair system plays a role in tumor progression.

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CSABA GALAMBOS (2008)

The role of vascular endothelial growth factor isoforms in lung development and neonatal lung disorders

Supervisor: Sándor Paku

Introduction: Lung vascular development is a carefully orchestrated multistep process consisting of complex interactions among growth factors, matrix proteins, and cytokines. Understanding the functional reach of several members of growth factor families is integral to an appreciation of the etiology and pathogenesis of developmental lung disorders affecting newborns. One example, alveolar capillary dysplasia (ACD), a rare, fatal disease,

is characterized by failure of formation of air-blood barriers, the presence of dysplastic, thin-walled vessels in the central part of the alveolar septa, and often by misalignment of pulmonary veins. One of the growth factors involved in pulmonary angiogenesis, vascular endothelial growth factor (VEGF), is so critical for embryonic development that in the mouse, elimination of just a single allele is lethal. In the early stages of lung development, the mouse VEGF gene expresses three isoforms (120, 164, and 188) in a distinct temporospatial pattern, suggesting a specific function for each. We engineered mice that express only VEGF120, to study the role of VEGF isoforms in lung development, and to assess their possible involvement in the pathogenesis of ACD. **Methods:** Lung vessel development in these mice was studied by scanning electron microscopy of Mercox casts of lung vasculature. Airway and air-blood barrier development was analyzed by light microscopy, transmission electron microscopy, immunohistochemistry, and morphometry. D2-40 antibody was utilized to identify lymphatic versus vascular endothelium in lung tissues obtained from patients with ACD. **Results:** In all VEGF120/120 fetuses and pups, lung vascular casts were smaller and less dense compared with 120/+ and wild-type littermates. Although the generation count of pre-acinar vessels was similar in all three genotypes, the most peripheral vessels were more expanded and sparse in 120/120 fetuses of all ages, compared with 120/+ and wild-type littermates. In addition, 120/120 animals had fewer air-blood barriers and a decreased airspace-parenchyma ratio compared with 120/+ and wild-type littermates. D2-40 antibody reliably distinguished lymphatic channels from misaligned veins in lungs of patients with ACD. **Conclusions:** The absence of VEGF 164 and 188 isoforms impairs lung microvascular development and delays airspace maturation, pointing to an essential role for heparin-binding VEGF isoforms in normal lung development. D2-40 antibody proved to be a very useful tool in the histologic diagnosis of ACD. The pulmonary phenotype found in VEGF120/120 mice strongly resembles those of newborns suffering from alveolar capillary dysplasia. Therefore, we propose that altered VEGF signaling is one of the causative factors in the pathogenesis of ACD and we speculate that the VEGF120 mouse could be further utilized to study the pathomechanisms of ACD.

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BEÁTA HARGITAI (2006)

Etiology and pathogenesis of chronic lung disease and hypoxic-ischaemicic brain damage in term and preterm neonates

Supervisor: Béla Szende

Recent advances in obstetric and perinatal intensive care made a great impact on frequency of intra and post partum hypoxic injuries and resulted in a longer survival time of preterm babies. However, the frequency of severe neurological damage and cerebral palsy has not diminished and long term complications of prematurity cause major problems in

well developed countries. In the course of our prospective study, we investigated the morphological evidence and potential role of apoptosis in various organs of preterm babies, suffering from Infantile Respiratory Distress Syndrome, Chronic Lung Disease (CLD) and hypoxic ischaemic encephalopathy. We examined the etiology of cerebral palsy in a case control cohort study, using placentas from children suffering from cerebral palsy (21), and placentas with ultrasonographic evidence of a vanishing twin (49). Our target was to test the vanishing twin hypothesis, to investigate the role of early embryonic loss in the development of cerebral palsy. In the course of detailed morphological examination we aimed to describe placental lesions associated with the vanishing twin phenomenon and neurological impairment.

As a result, we observed programmed cell death in chronic lung disease, hypoxic ischaemic encephalopathy, and renal failure of ventilated preterm infants and described morphologic features of apoptosis using TUNEL reaction. This was the first report in the literature on the role of apoptosis in complications associated with prematurity.

We concluded that apoptosis of alveolar and bronchiolar epithelial cells plays important role in the development of chronic lung disease of preterm neonates during the subacute and chronic stage of the disease. In hypoxic brain damage of preterm babies, complicated with intraventricular haemorrhage or periventricular leukomalacia, the programmed cell death activity in the neuro-gial cells of the germinal layer is usually mild or at most moderate.

Higher apoptotic activity in the lung and brain tissue was associated with severe bacterial or fungal infection in several cases of our study. Based on our observations during the vanishing twin study, we concluded that morphological study of term placentas is not a sensitive method to identify remnants of early pregnancy loss and that fibrin plaques are not specific indicators of a vanished twin. Large, sacculated subchorionic haemorrhages and subchorionic cysts may cause a diagnostic problem during ultrasound screening and may be misinterpreted as a twin pregnancy. Placentas, with ultrasonographic evidence of a vanished twin, showed significant association with marginal cord insertion and multiple histological lesions. Our results did not support the vanishing twin hypotheses and thus the vanishing twin phenomenon is not likely to be a risk factor for cerebral palsy or might be responsible for only a small proportion of cases wich our study was not able to detect. We described significant association between fetal stem vessel thrombosis, presence of avascular villi and villitis of unknown etiology in placentas.

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REVEKKA HARISI (2006)

Role of the extracellular matrix in the invasive growth and response on cytotoxic treatment in human osteosarcoma

Supervisor: András Jeney

Osteosarcoma is the most common primary malignancy of the bone in childhood and adolescence. In case of chemotherapy resistant (Huvos grade I–II) patients even the more aggressive chemotherapy was not successful, which indicated the search for new therapeutical targets, outside of the cells, in the extracellular matrix. In this work it was investigated: how the three-dimensional extracellular matrix (ECM) may influence the biological features and response to cytostatic treatments of a self-established osteosarcoma cell line (OSCORT). The ECM induced cell proliferation in OSCORT cells, among the ECM-components: this effect was mainly due to the heparan sulphate proteoglycan (HSPG) and fibronectin. The ECM, and mainly HSPG increased the synthesis of: β1 integrin, p36 (cyclin D1)-p34(cdk4) complex, PCNA and Ki-67, and the activity of topoisomerase II. In the presence of the ECM components the OSCORT cells produced matrix metalloproteinase 9 and its active form, the main induction was observed for HSPG. The proliferation and viability of doxorubicin-treated and in the ECM cultured cells were significantly higher than that of the on plastic monolayer cultured cells. The apoptosis rate in the to ECM-attached OSCORT cells was lower by all doses of doxorubicin than in cells cultured on plastic monolayer, moreover, a repair induced by ECM was assumed. The significantly lower apoptosis rate could be explained by the decreased p53 synthesis and cell nuclear localization. The repair might be confirmed by the fact that a nuclease was found in OSCORT cells induced by ECM. The results shown in my dissertation might give new aspects to the doxorubicin therapy of osteosarcoma: a high dose–short-term therapy against tumor growth, and a low dose–long-term therapy against tumor invasion. A novel HSPG, agrin, was detected in the OSCORT-ECM, and was described at the first time. It was found that the doxorubicin increased the proteoglycan synthesis, and its extracellular localization, while the clodronat—mainly in the extracellular fraction—decreased the glycosamin incorporation into the proteoglycan molecules. Further investigation proved that clodronat could abrogate the resistance of osteosarcoma cells against doxorubicin, by its effects on proteoglycan synthesis. The detected novel especially for the osteosarcoma tumorous extracellular matrix characteristic proteoglycans might serve as potent therapeutic targets of osteosarcoma in the future.

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PÉTER HAUSER (2007)

Examination of epidemiology, possible prognostic factors and treatment of pediatric brain tumors

Supervisor: László Kopper

Pediatric central nervous system tumors (CNS) are crucial important among the pediatric malignant tumors due to their high relative frequency. However, as a consequence of their moderate chemosensitivity and their low survival rate, CNS tumors called for special attention only in the last decade. Our aims were to determine the exact incidence of the pediatric CNS tumors in Hungary and compare it to that of other countries; to study the prognostic significance of the presence of anti-apoptotic heat shock proteins (HSP27, HSP 70, HSP 90) in medulloblastoma, and to examine treatment results of patients with medulloblastoma/primitive neuroectodermal tumor (PNET) using the Hungarian Brain Tumor Protocol. All over the world pediatric patients with CNS metastasis of non-CNS tumors are overshadowed, while this phenomenon principally influences the prognosis of these patients. Another important aim of my study was to estimate the incidence, treatment and outcome of patients with CNS metastasis of non-CNS tumors in Hungary.

In Hungary the incidence of pediatric primary CNS tumors is 3.4/100.000, which is surprisingly high comparing to other countries. However, the exact cause of this phenomenon is not known yet. We demonstrated that the presence of anti-apoptotic HSPs in medulloblastoma is common. However, their pretreatment constitutional level does not influence patients' survival. We set out, that the Hungarian Brain Tumor Protocol introduced in 1998, resulted in a similar survival (44%) for patients with medulloblastoma/PNET to other countries. However, there is a remarkable difference between low (74%) and high risk (17%) patients. That is why I suggested the introduction of the high dose chemotherapy with stem cell rescue as a standard treatment for high risk patients in Hungary.

Furthermore, CNS metastasis of non-CNS tumors is a rare phenomenon in Hungary (4%) with extremely bad prognosis. Treatment of CNS metastasis of non-CNS tumors similarly to other countries is unsolved in Hungary.

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ERIKA HITRE (2006)

Importance of pharmacogenetic markers in the fluoropyrimidine-based therapy of colorectal tumours

Supervisor: Judit Kralovánszky

Regarding the colorectal cancer (CRC) mortality among European countries Hungary is on the first place in case of women and the second in case of man. The combinations of low penetrancy genes, the "western lifestyle" and the diet together may play role in the pathogenesis of sporadic CRCs. The known polymorphisms of the thymidylate synthase (TS) gene depending on the folate intake modify the personal susceptibilty to CRC. Based on our studies it seems that the two most characteristic polymorphisms of the TS (5'-TSER and 3'-TSUTR) influence the CRC susceptibilty and in the Hungarian population the TS heterozygtes are less susceptible for CRC development. The professional therapy of CRC needs collaboration of many medical specialities. The aim of the adjuvant chemotherapy after the primary surgical resection is to eradicate the residual tumour cells. In the adjuvant therapy of CRC the 5-fluorouracil/leucovorin (5-FU/LV) combination is the "gold standard", which is applied as bolus treatment or continuous infusion. In case of bolus treatment the relapse risk has been proved to be significantly higher compared to the continuous infusional therapy. In our study it has been demonstrated for the first time that those germline TS genotypes, which had been shown to induce high TS expression predict significantly longer disease-free and overall survival compared to those combinations involving low TS expression. It has been confirmed that the combination of TS polimorphisms is an independent prognostic factor of survival of CRC patients receiving 5-FU-based adjuvant chemotherapy. In case of metastatic CRC patients it has been shown by us that the methylenetetrahydrofolate reductase gene polymorphism (MTHFR C677T) has prognostic value for 5-FU-based treatment. The importance of pharmacokinetic investigations has been demonstrated in connection with the therapy of metastatic CRC patients with continuous infusional 5-FU, and in addition the dihydropyrimidine dehydrogenase activity and the serum creatinine levels of patients control the efficacy of 5-FU therapy, as well. The genetic background of patients influences the efficacy of therapy and the chance for survival. Accordingly, there is a need to develop pharmacogenetic methods, which allow a more accurate prediction of therapy outcome and the planning of individually tailored treatments.

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GERGELY IMRE (2008)

Role of proteases in the regulation of cell daeth in caspase-inhibited leukemia cells induced by staurosporine

Supervisor: Rudolf Mihalik

A type of cystein proteases, called caspases are the main regulators of apoptosis. However, more and more data support the idea that the inhibition of caspase activity is not by all means sufficient to inhibit cell death. These caspase activity-independent cell death forms may be diverse according to morphological and even biochemical studies. Increasing amount of evidence suggests that the alternative forms of cell death are actively regulated by cells and in some cases their role in physiological and developmental situations has also been demonstrated. However, the regulator components and mechanisms of caspase-compromised cell death are less understood. Knowledge of these alternative cell death pathways may help us to overcome chemotherapy resistance of tumors or to decrease the intensity of necrosis resulting in secondary tissue damage. By examining alternative cell death pathways we found that staurosporine (STS), a model compound of apoptotic death, resulted in both apoptotic and necrotic cell death even in the presence of z-VAD.fmk, a caspase inhibitor. Light microscopic and flow cytometry analysis confirmed that the two distinct forms of cell death occurred in parallel in separate cells. Inhibition of cystein cathepsins by z-FA.fmk prevented STS induced apoptosis in caspase-inhibited cells, however, cells did not survive but died with necrosis. Inhibition of Hsp90 chaperon activity with geldanamycin (GA) blocked necrotic cell death in caspase compromised cells. In this case cells died with apoptosis. Cotreatment with z-FA.fmk and GA inhibited both forms of caspase compromised cell death by preserving mitochondrial membrane potential and lysosomes. Cotreatment with these two inhibitors was not effective in the inhibiton of STS only induced (caspase dependent) apoptosis and H_2O_2 stimulated necrosis, indicating the specificity of inhibitors. In conclusion, STS induced cell death diverges in STS treated, caspase-inhibited U937 cells. Decision between the two types of death might depend on the activity of cystein cathepsins and the Hsp90 chaperon protein.

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PÁL KAPOSI NOVÁK (2007)

Comparative genomic classification of human hepatocellular carcinoma

Supervisor: András Kiss

Global transcriptome analysis has been successfully applied to characterize various human tumors, including hepatocellular carcinomas. This novel technology can facilitate early discovery as well as prognostic and therapeutic diversification of cancer patients. To enhance access to the genomic information buried in archived pathology samples, we assessed RT-PCR amplification rates in paraffin embedded tissues preserved in three different fixatives. Reliable amplification could be achieved from all paraffin embedded specimens, when the amplicon size did not exceed 225 bp. A longer amplicon size resulted in rapid decrease of yield and reproducibility. In addition, formalin provided superior morphology and better reactivity with claudin-4 and -7 immunohistochemistry. Amplification of the initial sample is often required before transcriptome analysis of clinical specimens could be performed. We introduced a random nonamer primed T3 polymerase reaction into the conventional linear RNA amplification protocol. The modified T3T7 method generated a sense strand product ideal for synthesizing indirectly labeled cDNA templates. Microarray analysis of amplified frozen and laser micro-dissected Myc and mouse liver tumors confirmed good reproducibility (r=0.9) of the Myc/TGF reaction and conservation of original transcriptional patterns (r=0.79). Finally, we tested the utility of expression profiling for the classification of human HCC samples. By comparing expression data from HGF treated c-Met conditional knock-out and control primary mouse hepatocytes, we identified 690 HGF/c-Met target genes. Functional analysis of the significant gene set implicated c-Met as key regulator of hepatocyte motility and oxidative homeostasis. Cross comparison of the c-Met induced transcription signature with human HCC expression profiles revealed a group of tumors (27%) with potentially activated c-Met signaling (MET+). These tumors were characterized by higher vascular invasion rate, increased microvessel density, and shortened survival. A prediction model based on 111 cross-species conserved c-Met signature genes was able to diversify HCC patients into good and bad prognostic groups with 83–95% accuracy. Our results therefore demonstrate that careful experimental design and state-of-the-art laboratory methods could open the way for global expression profiling of archived and limited availability pathologic samples. Comparative functional genomics based analysis of the cancer transcriptome could lead to novel molecular classification systems which are essential for the introduction of individualized cancer therapeutics.

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JUDIT KISS (2008)

Examination of different factors influencing the vascularization of human cutaneous melanoma

Supervisor: József Tímár

We analyzed the relationship among microvessel density (MVD) and tumor infiltrating cells in cutaneous malignant melanoma. We also studied the effect of hyperforin on tumor and endothelial cell growth in vitro and *in vivo*.

The density of lymphocyte subpopulations, macrophages, dendritic cells and CD34+ microvessels was determined by immunohistochemistry in primary tumor samples from fifty-two patients with melanoma thicker than 1 mm. The antiproliferative effect of hyperforin was studied on 16 human and 7 rat cell lines and on human dermal microvascular endothelial cells (HDMEC). Intratumoral MVD did not show significant association with infiltration for any of these cell types. In the case of peritumoral reactive cell densities analyzed in the whole patient population, significant correlation was found with CD3+ T-cell density. This association was stronger in melanomas >4.0 mm and in visceral metastatic tumors. In these subgroups similar phenomenon was observed for CD8+ cells. We found significant correlation of MVD with CD68+ macrophage density only in the highest thickness category, and weak associations with B-cell and dendritic cell infiltration in visceral metastatic cases. MVD did not vary significantly in tumors categorized according to thickness, localization, ulceration or histological type. However, both intratumoral MVD and macrophage infiltration were significantly higher in male patients compared to females. Hyperforin inhibited tumor cell proliferation and induced apoptosis. *In vitro*, hyperforin blocked microvessel formation of HDMEC on a complex extracellular matrix. Furthermore, hyperforin reduced proliferation of HDMEC, without displaying toxic effects or inducing apoptosis. In Wistar rats hyperforin inhibited tumor growth and reduced tumor vascularization. Since the net outcome of the enrichment in tumor-infiltrating host cells and in tumor vascularization cannot be easily predicted, further clinicopathological studies are needed on human skin melanoma patients. Hyperforin holds the promise of being an interesting antineoplastic and antiangiogenic agent with low toxicity.

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JÚLIA LUKITS (2008)

The effect of the microenvironment of head and neck cancers on tumor progression

Supervisor: József Tímár

In the last 20 years the incidence and mortality of head and neck squamous cell carcinomas showed an increasing rate in Hungary. In our work we examined the microenvironment of head and neck cancers localized in different anatomical regions. Clinical evidence shows that the prognosis of hypopharyngeal tumors is poorer than that of head and neck cancers in other anatomical localizations. We investigated if tumor size or vascularity has correlation with the biological behavior of these tumors. The results showed that the tumor size of laryngeal cancers in T2 stage were significantly larger than that of hypopharyngeal cancers in T4 stage. Regarding the vascularity or expression of VEGF we did not find any difference between these two types of tumor, suggesting that the more aggressive behavior of hypopharyngeal cancers is probably due to the invasive phenotype of this tumor, an assumption supported by genomic examination. In a prospective study we examined the correlation between the microvascular density and treatment outcome in irradiated head and neck cancer patients. We demonstrated that the decreased vascularity induced by radiotherapy is a predictive marker of treatment success. We have investigated the role of the endocrine environment in tumor progression, determining the hormone receptor status of head and neck cancers using immunohistochemical and molecular methods. Results showed that ER and PGR are expressed in almost half of the examined tumors, and the presence of functional ER was also frequent in these cases (40.3%), while the solitaire hormone receptor expression was a rare phenomenon. Expression of hormone receptors in all the examined cases did not show any correlation with patients survival but in the laryngeal/hypopharyngeal group ER positivity was associated with a shortened survival ($p=0.0636$). In a multicenter phase I/II clinical trial we examined the tumoral and stromal effects of a natural leukocyte interleukin (LI) in oral squamous cell cancers. LI was administered locally in four different doses. The proportion of tumor cell nests and tumor stroma decreased significantly after LI treatment (induction of fibrosis), which was associated with the induction of necrosis. Morphometric determination of Ki-67+ cells showed a tendency of cycling stromal cells to decrease in response to treatment by the different doses of LI, while lower doses of LI produced a temporary increase in cycling tumor cells. Density of intraepithelial neutrophils was higher after LI treatment, and the stromal density of neutrophils was higher in the responder subgroup. In the tumor stroma macrophage density was similar in the treated and control cases, while a significant decrease of these cells was observed intraepithelially. Finally, we were not able to detect CD34+ immunosuppressive mononuclear cells in these tumors. Our examinations supported the theory that the tumor stroma and its components play an important role in tumor progression, and therapeutic modulation of these components can influence the progression.

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MIKLÓS MÁTHÉ (2007)

Alterations of syndecan-1 expression and HPV infection: independent and interacting factors in the prognosis and progression of squamous cell carcinomas

Supervisor: Ilona Kovalszky

Syndecan-1, a transmembrane proteoglycan may exert anti-proliferative effects, but may also promote cell growth by binding various growth factors. Malignant epithelial cells often down-regulate their own syndecan-1 production, whereas they are capable of inducing an aberrant syndecan-1 expression in stromal fibroid cells. Immunohistochemical analysis performed on 40 oral leukoplakias, 51 invasive oral squamous cell cancers and 35 *in situ* and 56 invasive carcinomas of uterine cervix revealed one or both of the above alterations concerning syndecan-1 expression. A decrease in syndecan-1 expression compared to normal epithelium could occasionally be detected as early as in leukoplakias, representing premalignant oral lesions. Syndecan-1 expression of tumor cells was decreased or even completely lost in 45/51 oral carcinomas and in all cervical carcinomas. Furthermore, tumor-induced stromal syndecan-1 immunoreaction appeared in 19/51 oral tumors. In the case of oral cancers the probability of postoperative progression showed some dependence on the degree of decrease in tumour cell syndecan-1 levels.Based on recurrence and overall survival data, stromal syndecan-1 expression in primary oral cancers appears to be a more reliable factor of adverse prognosis ($p=0.023$). We also detected the tumor-induced stromal syndecan-1 expression in the case of cancers of uterine cervix; however, the question whether the presence and extent of stromal syndecan-1 expression can be considered real risk factors of postoperative progression in these malignancies requires further clinical investigation.

Numerous lines of evidence indicate that head and neck squamous cell carcinomas associated with human papillomaviruses form a special molecular subgroup. However, HPVs' role in the pathogenesis of oral cavity cancers is still a matter of debate. Our aim was to analyze the presence of HR-HPVs DNA and E6 protein in oral cancers. Fifty-one oral carcinomas were assayed for HPV by PCR, and parallelly by E6 protein immunohistochemistry. Our results are consistent with the idea that a distinct subgroup of oral cancers are HR-HPV-associated, although not all PCR positivity means a real causative relationship between virus and malignancy. The syndecan-1 expression of the HPV 16 PCR/IHC positive tumours was higher than cancers lacking the virus, which support the idea, HPV-associated head and neck cancers form a distinct entity.

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KATALIN NAGY (2007)

The role of proteasome-ubiquitin system in the regulation of apoptosis induced by chemotherapeutic drugs and TRAIL in promyelocytic leukemia and colon cancer

Supervisor: László Kopper

Drugs which interfere with microtubules, such as taxol, are among the most successful chemotherapeutic agents of recent years, and they are used in the treatment of many solid tumors including breast and ovarian cancer. TRAIL, a tumor specific cytokine in phase I. clinical trial, represents the new type of chemotherapeutic drugs, simirarly to proteasome inhibitors which interact with the proteasome-ubiquitin pathway. PS-341/Bortezomib was the first proteasome inhibitor approved by the Food and Drug Administration for clinical use but only against myeloma. The effects of the combination treatment of proteasome inhibitors with other drugs against various tumors and the molecular and biochemical mechanisms of the synergism are barely explored, while it can offer a new effective opportunity to treat resistant tumors.

In our study human promyelocytic leukemia cell line, HL-60 was exposed to nocodazole or etoposide in combination with proteasome or caspase inhibitors. Nocodazole, a microtubule inhibitor, induced caspase-dependent apoptosis in the HL-60 cell line. At subcytotoxic concentrations, proteasome inhibitors—including MG-132 or clasto-β-lactone—decreased nocodazole-induced apoptotic DNA fragmentation without affecting the induction of caspase-3 activity. In contrast, MG-132 decreased both DNA fragmentation and caspase activation induced by etoposide, a topoisomerase-II inhibitor. In parallel, MG-132 upregulated Hsp70 protein expression both in the presence or absence of nocodazole. We showed that proteasome inhibitors decreased antimicrotubule drug-induced apoptotic DNA fragmentation downstream of caspase-3 activation, possibly due to increased Hsp70 expression. Results indicate that combination treatment with proteasome inhibitors and the more conventional drugs in leukemia requires careful evaluation of their interferences at the level of apoptosis signalling.

Next we focused on the sensitization effect of proteasome inhibitors to TRAIL in TRAIL-resistant colon carcinoma cells. Combination of various proteasome inhibitors (epoxomycin, bortezomib/PS-341 and MG-132) and TRAIL induced rapid apoptosis in TRAIL-resistant colon carcinoma cell lines. DNA fragmentation, mitochondrial membrane depolarization and increased caspase-3-like enzyme activity were induced only by the combined treatment (epoxomycin and TRAIL). DNA fragmentation was inhibited completely by the general caspase inhibitor Z-VAD-FMK and partially by the caspase-8 inhibitor IETD-FMK. Further, treatment with TRAIL alone induced partial activation of caspase-3

(p20 fragment), while the combination treatment led to the full proteolytic activation of caspase-3 (p17 fragment). At the same time point (5 hr), the combination treatment induced marked membrane depolarization and the release of cytochrome-c, HtrA2/OMI and SMAC/Diablo from the mitochondria. Epoxomycin alone induced the release of cytochrome-c and HtrA2/OMI, in the absence of significant mitochondrial membrane depolarization or release of SMAC/Diablo, while TRAIL treatment alone did not cause significant release of any of these mitochondrial proapoptotic proteins. AIF was not released by any of these treatments. Our results provide a model where enhanced release of Smac/Diablo has an important role in full caspase activation and subsequent DNA fragmentation induced by the combination of TRAIL and epoxomicin. We used siRNA technique to confirm this action and we found that apoptotic DNA fragmentation induced by the combination treatment was significantly inhibited in Smac/Diablo knock out cells. These results further underline that TRAIL and a proteasome inhibitor is a promising combination against colon carcinomas.

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GÁBOR PÉLEY (2006)

Radioisotope-guided surgical techniques for the treatment of early breast cancer

Supervisor: László Kopper

Radioisotope guided surgery has been increasingly used for the treatment of breast cancer since 1990. After introducing the technique of sentinel lymph node biopsy the radioguided occult breast lesion localization has been developed for treating non-palpable breast cancer. The feasibility and clinical significance of both techniques has been examined in this study. It has been found that the dual agent guided sentinel lymph node biopsy with preoperative lymphoscintigraphy and with adequetely prepared and practised technique is at least as an accurate method for staging the axilla as formal axillary lymph node dissection. The staging sensitivity can be significantly improved by the intensive histological work-up of the sentinel lymph nodes but the clinical significance of this improved sensitivity has not yet been known. We do not consider molecular biological techniques appropriate for examining the sentinel lymph nodes. Sentinel lymph node biopsy for staging ductal carcinoma *in situ* is clinically significant only in patients undergoing mastectomy. Sentinel lymph node biopsy is feasible after neoadjuvant chemotherapy but we had not detected any clinical benefit of using this method. Negative axillary lymphoscintigraphy after subareolar radiocolloid administration most likely predicts histologically positive axillary nodes and for this we perform formal axillary lymph node dissection in these patients. This statement may not be valid for other sites of radiocolloid injection for example for the intratumoral

radiocolloid administration technique. The radioguided occult breast lesion localization technique can be used successfully for excising non-palpable breast cancer and simultaneous sentinel lymph node biopsy can also be performed with a high identification rate. We also used this technique successfully for multifocal non-palpable breast cancer. The results of our prospective observational study has shown that completion axillary lymph node dissection can safely be omitted in patients with negative sentinel lymph node. We are now conducting a prospective randomized clinical trial for determining the optimal treatment of the axilla (completion axillary lymph node dissection versus axillary irradiation) in patients with positive sentinel lymph node.

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LILLA REINIGER (2007)

Richter's and prolymphocytic transformation of chronic lymphocytic leukemia are associated with high mRNA expression of activation-induced cytidine deaminase and aberrant somatic hypermutation

Supervisor: Ágota Szepesi

Chronic lymphocytic leukemia (CLL) is an indolent B-cell non-Hodgkin lymphoma (NHL) that may transform into higher-grade lymphoma. The transformation involves an increased number of prolymphocytic cells, termed prolymphocytic transformation (PLT) or the development of diffuse large B-cell lymphoma (DLBL), also referred to as Richter's transformation (RT). To analyze whether activation-induced cytidine deaminase (AID) which is essential for somatic hypermutation (SHM) of normal B-cells, and malfunction of SHM termed aberrant somatic hypermutation (ASHM) are associated with higher-grade transformation of CLL, AID mRNA expression and the mutation pattern of c-MYC, Pax-5 and RhoH genes were analyzed in 8 cases of CLL without transformation and in 21 cases that showed RT or PLT. CLL cases which showed no transformation or eventually transformed into higher-grade lymphoma showed low levels of AID mRNA expression and low frequency of mutations of c-MYC, Pax-5 and RhoH genes. In both RT and PLT, high-levels of AID mRNA expression and high-frequency mutations of c-MYC, Pax-5 and RhoH genes were detected. These results indicate that AID expression and ASHM are associated with higher-grade transformation of CLL and provide further evidences that AID expression and ASHM may be activated during the clonal history of B-cell lymphomas.

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PÉTER TÁTRAI (2008)

Selective deposition of agrin in the microvasculature of hepatocellular carcinoma: aspects in pathogenesis and differential diagnosis

Supervisor: Ilona Kovalszky

Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers and is the fifth most common malignancy worldwide. HCC typically develops in the cirrhotic liver. Our preliminary results indicated that agrin, a heparan sulfate proteoglycan (HSPG) previously not detected in the liver, accumulates in the basement membranes (BMs) of the cirrhotic liver and HCC. This novel finding prompted us to investigate the role of agrin in the pathogenesis and differential diagnosis of HCC. As a first step, the formerly unspecified monoclonal antibody we used was verified as anti-agrin using mass spectrometry. Our subsequent experiments were carried out on specimens from 131 liver disease patients and 18 individuals with healthy liver, from 4 rats subjected to cirrhosis/HCC induction and 1 untreated control, as well as from cultured cells. In both species, significantly increased agrin expression in cirrhosis and HCC compared to the normal liver was demonstrated by IHC, Western blot and quantitative RT-PCR. By IHC on serial sections and double immunofluorescence studies, agrin was localized to the muscular layer of blood vessel walls, the BM of bile ducts and ductular reaction, the microvessel walls in HCC, and occasionally the BM of hepatocellular tumor cells. (Co)localization, gene expression and mRNA *in situ* hybridization experiments suggested that the cellular sources of agrin include vascular smooth muscle cells, cholangiocytes and ductular epithelium, activated mesenchymal cells in the stroma of hepatocellular tumors and, in selected cases, tumor hepatocytes. Agrin in the BM of bile ducts and vascular endothelia is thought to play an important role in the survival of these structures; thus, it may contribute to the formation of ductular reaction and HCC angiogenesis. In sharp contrast with consistently agrin-positive HCC neovessels, normal and cirrhotic sinusoids were always devoid of agrin, which prompted us to investigate the applicability of agrin IHC in the detection of hepatocellular malignancy. Agrin (and, when needed, CD34) IHC was performed on 68 benign lesions (8 large regenerative nodules, 23 low-grade and 7 high-grade dysplastic nodules, 30 liver adenomas) and 29 malignant lesions (8 small HCC, 21 HCC), and evaluated semi-quantitatively. A decision algorithm was devised that, based on IHC results, differentiated benign and malignant parenchymal lesions with a sensitivity of 93.1% and a specificity of 92.6%. Hence we propose the inclusion of agrin in the IHC diagnostic panel used by liver pathologists.

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BOTOND TÍMÁR (2006)

Mutational profile of immunoglobulin heavy chain genes of chronic lymphocytic leukemia and diffuse large B-cell lymphoma in Richter's syndrome

Supervisor: András Matolcsy

Patients with chronic lymphocytic leukemia (CLL) may develop diffuse large B-cell lymphoma (DLBCL), also known as Richter's syndrome. Mutational status of immunoglobulin heavy chain variable region (IgVH) genes have prognostic impact in CLL. Patients with mutated IgVH genes have stable disease, whereas patients with unmutated IgVH gene have more aggressive disease. The mutational status of CLLs that transform to DLBCL is unknown. To reveal whether Richter's syndrome occurs in CLLs with mutated or unmutated IgVH genes, we have performed mutational analysis on serial specimens from eight patients. CLL and DLBCL tumorclones were identical in five cases and they were different in three cases. Six CLL expressed unmutated and two cases expressed mutated IgVH genes. In five of the six unmutated CLLs, the DLBCL clones evolved from CLL tumorclones and the IgVH genes expressed by DLBCLs were also unmutated. In one unmutated and two mutated CLLs, the DLBCL expressed mutated IgVH genes, but in these three cases the DLBCL tumorclones developed as independent secondary neoplasm. These result suggest that Richter's syndrome may develop in both mutated or unmutated CLLs, but clonal transformation of CLL to DLBCL occur only in the unmutated subgroup of CLL.

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GYULA VÉGSŐ (2008)

Post-transplantation malignant tumors and the challenges of immunosuppressive therapy in transplanted patients developing lymphoma. Mycophenolic ACID-A possibility

Supervisor: László Kopper

The increasing frequency of malignant tumors developing during chronic immunosuppression is an important determinant of the long-term survival of organ transplanted patients. This problem can be solved only if we are aware of the special characteristics concerning our patients. The incidence and frequency of tumors occurring in kidney transplant recipients differ from those of the Hungarian population. The increased tumor risk resulting from chronic renal failure and the increasing age of prospective kidney recipients, and—in addition—the increasing frequency of tumors diagnosed in the early post-transplantation period emphasize the importance of regular oncological screening of patients on the waiting list. Early diagnosis and treatment of tumors and precancerous conditions are equally important in transplanted patients as well, and the tumor risk could be decreased by applying low dose immunosuppression and the preferential usage of immunosuppressive drugs with an oncologically favorable effect. The prognosis of post-transplantation tumors is poor, as they respond poorly to therapy. Lymphomas are of great importance because of their frequency. Different immunosuppressive regimens represent varying degrees of risk in lymphoma development. This risk is lower in the case of mycophenolic acid. A major factor in treatment is the composition of immunosuppression. An oncologically ideal compound would prevent organ rejection, and at the same time, would not counteract oncological therapy. We have shown that mycophenolic acid inhibits the proliferation of human Bcell non-Hodgkin lymphomas and induces apoptosis by activating the intrinsic pathway, both *in vitro* and *in vivo*. The favorable properties of mycophenolic acid suggest that it can provide the necessary immunoprotection for the transplanted organ, and, given its anti-lymphoma effects, it may also prove useful in the therapy of lymphoma patients. It may also be helpful in the treatment of "traditional" lymphomas of the non-transplanted population, where the major cause of therapeutical failure is the development of apoptosis resistance. Mycophenolic acid, combined with other chemotherapeutical drugs, may enhance apoptosis in lymphoma cells. Our promising experimental results provide a basis for further, clinical studies.

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ÁRPÁD VIOLA (2007)

Intraoperative image fusion and linear-quadratic modelm for Iodine-125 interstitial irradiation of brain tumors

Supervisor: Jenő Julow

State-of-the-art treatment involves the use of multimodal imaging (CT, MRI, PET) for tumor localization and use of modern 3D radiation treatment planning programs to determine optimal access points and a dose distribution conforming to the tumour. While implanting seeds during a brachytherapy operation, a few millimeter variances from the planning of the operation may cause considerable changes in the radioactive outcome of the tumor volume and the normal tissue. As far as we know, there is no study available investigeting both, the accuracy of the stereotactic brachytherapy implantation in brain tumours and the dosimetric consequences of deviations from the planned implantation site. A number of comparisons between difrent stereotactic radiosurgery treatment techniques have been published, but none of them compared their radiobiological advantages, disadvantages and late radiobiological effect with I-125 interstitial brachytherapy treatment. After the renormalization of LINAC and brachytherapy referential doses, using the dose volume histograms and the linear-quadratic model the brachytherapy doses were compared to the brachytherapy equivalent LINAC radiosurgery doses with respect to late effect of irradiations on normal brain tissue.

It was found, that the application of the intraoperativ image fusion and linear quadratic model allows us to increase the quality of stereotactic interstitial irradiation, to choose the optimal stereotactic treatment method and to decrease the radiological exposure of normal tissue surrounding the target volume.

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PROGRAM 8/2.

ALTERATIONS OF CELLS, FIBRES AND EXTRACELLULAR MATRIX. DIAGNOSTIC PATHOMORPHOLOGICAL STUDIES IN THE COURSE OF HEART AND VASCULAR DISEASES AND IN CERTAIN TUMOURS. EXPERIMENTAL AND DIAGNOSTIC PATHOMORPHOLOGICAL STUDIES

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Program overview

Leading causes of morbidity and mortality in Hungary are the cardiovascular, gastrointestinal, hepatic, pancreatic diseases and malignant tumors. The Program offers a multi faceted analysis of the above diseases, completed with the development of liver diseases including liver, breast and pancreatic tumors. The research studies apply various pathohistological approaches with the extension of clinical retrospective and prospective studies. The project is dealing mostly with human materials, though experimental models are also induced. Several modern molecular pathological methods have been introduced in the well equipped laboratories. Recent studies focuses on the significance of microRNAs in chronic inflammations and human tumors and alterations of cell adhesion molecules during carcinogenesis.

Titles of research projects	***Supervisors***
Prognostic factors and locoregional staging	Gábor Cserni
Molecular genetic analysis of adhesion proteins	András Falus
Pathology of vascular network	Anna Kádár
Exogenous restrictive pulmonary diseases	Tibor Kerényi
Adhesion proteins and cell-connecting structures in viral hepatitis	András Kiss
Claudins in breast cancer	Janina Kulka
Prognostic factors in breast cancer	Janina Kulka
Effect of hepatotrop viruses and other hepatotoxins on the metabolism of liver cells and related disorders	Gábor Lotz
Factors in the development of liver diseases	Zsuzsa Schaff
Role of extracellular matrix in chronic liver diseases	Zsuzsa Schaff

Ph.D. students		***Supervisors***
Mónika Nóra Erős	pt	Janina Kulka
Anna Korompay	ft	Zsuzsa Schaff
Attila Patonay	ft	Zsuzsa Schaff
Áron Somorácz	ft	András Kiss
Attila Marcell Szász	ft	Janina Kulka
Péter Törzsök	ft	András Kiss

Ph. D. candidates		*Supervisors*
Batmunkh Enkhjargal	it	András Kiss
Katalin Borka	i	Zsuzsa Schaff
Hajnalka Győrffy	i	András Kiss
Judit Halász	i	Zsuzsa Schaff
Anikó Keller Pintér	ft	Janina Kulka
Csaba Lódi	ft	András Kiss
Zsuzsanna Németh	ft	András Kiss

Ph. D. graduates		*Supervisors*
Tibor Glasz	i	Anna Kádár
Attila Szijártó	pt	Zsuzsa Schaff

ft, full-time; pt, part-time; it, international; i, individual

Abstracts of Ph. D. theses successfully defended in 2006 and 2007

TIBOR GLASZ (2006)

Cardiovascular alterations following coronary artery bypass grafting procedures

Supervisor: Anna Kádár

Objectives: Coronary artery bypass grafting (CABG) belongs to the state-of-the-art of the surgery of atherosclerosis, one of the most important diseases in the western world. In all our investigations, we primarily sought what kind of gross and micromorphologic cardiovascular alterations develop following CABG procedures, and whether these have any pathophysiologic and clinical relevances? For the control of our observations, we performed a set of parallel studies to see if our original results were also valid in related states. Two main fields were observed. (1) On the one hand, we examined entities of the early postoperative period (0–30 days after surgery) following CABG to give clinico-pathologic evaluation on causes of death during this period. In view of the inconsistent use of terms in previous related reports, the unelaborated criteria applied and thus, the lack of their comparability, in order to meet these shortages we decided to produce a clear-cut system of criteria for the evaluation of death causes, providing basis for the comparability of similar studies in the future. As a further trial of our system of criteria, we attempted its use with two related open heart surgery cohorts (valvular operations and combined interventions/valvular + CABG operations) to see whether a better evaluation can be achieved, and whether the results so obtained correspond to the general pathophysiologic setting of the surgical intervention under scrutiny. (2) In the second part of our activities our attention was focused on chronic alterations following CABG, mainly on possible relations to the widely investigated infective agent of the vasculature, *Chlamydia pneumoniae*. Our objective was to study if accelerated long-term occlusion of coronary artery bypass grafts is related to infection by *C. pneumoniae*, and if conclusions on the pathophysiology of the infective process can be drawn from morphologic findings. For the control of our observations and conclusions, similar investigations were performed on different venous struc-

tures from various patient groups and coronary artery samples from young adults. Special attention was paid whether any of these results mutually confirmed each other, so implying a more general importance of the alterations observed.

Material and methods: (1) The evaluation study of early postoperative death was carried out in a collaboration between the Institute of Pathology, Bethesda Hospital and the Duisburg Heart Centre, Duisburg, Germany. The number of patients enrolled in this study was 32 following CABG, 9 following valvular operations and 7 following combined interventions. The hearts of deceased patients were conserved after routine post-mortem investigations and were further submitted to a highly detailed gross and micromorphologic investigation to obtain a thorough view on all parts including various segments of coronary arteries and grafts, myocardial areas, native and prosthetic valves. The obtained morphologic picture was evaluated against the records of the clinical history with the help of a cardiosurgeon, and a consensus was met on what mechanism of death is most probable on the basis of correspondence both to the pathomorphologic and clinical findings. (2) In our investigations on chronic alterations, 21 patients candidate for redo CABG due to long-term graft failure were selected in collaboration with three cardiosurgical centres in Budapest, Hungary (Semmelweis University, Faculty of Medicine, Department of Cardiovascular Surgery; National Medical Center, Department of Cardiovascular Surgery; Gyorgy Gottsegen National Institute of Cardiology). Coronary artery bypass graft segments with long-term occlusions and consequently no hemodinamical relevance to the patient, as well as venous and arterial samples from their native positions prepared for redo grafting (so called "new grafts": segments of the saphenous vein, radial artery and internal mammary artery) were collected. The total number of collected vessel samples was 62 (34 occluded and 28 new grafts) that, besides conventional histotechniques, were also processed with immunohistochemical and polymerase chain reaction (PCR) methods for the specific detection of *C. pneumoniae*. Blood samples were also collected for serologic examinations. Special emphasis was laid on accurate immunohistochemical evaluation. To this end, mural layers of the vessel samples were divided into two compartments (intima-media vs. adventitia) and a four-scale system (grade 0–3) was applied to separately define the severity of infection in each compartment. Specificity of our immunohistochemical reactions was controlled in various ways: positive and negative controls were applied and additionally, on one vessel sample showing grade 3 infection immunohistochemically, electronmicroscopic examination was performed for the direct visualization of the bacterium. In order to enhance reliability of PCR-detection, vessel samples were processed parallel in two independent laboratories according to two different methods. In our control series a similar detection methodology was applied on venous structures (69 samples from 51 subjects) from (a) patients undergoing pacemaker implantation, (b) patients undergoing varicectomy for lower limb varicosity and (c) subjects dying of sudden deaths for various (natural and violent) reasons on the one hand, as well as on coronary artery samples from young adults (222 samples from 74 subjects) on the other.

Results: (1) Early postoperative causes of death were devided into 4 groups, and the new system of criteria gave the following death cause prevalences according to surgical intervention types (CABG, valvular operations and combined interventions, respectively): (a) surgical complications: 14 (43%); 3 (33%); 4 (57%), (b) severe coronary artery disease: 13 (41%); 1 (11%); 1 (14%), (c) chronic heart failure 4 (13%); 3 (33%); 1 (14%), (d) non cardiac causes 1 (3%); 2 (22%); 1 (14%). (2) *Chlamydia pneumoniae* was detected immunhistochemically in all the 21 patients with chronic graft failure. With respect to graft samples and mural compartments, the following distribution was seen: occluded grafts (n=34)

were at least mildly infected in the intima-media in 28 vessels (82%), in the adventitia in 33 vessels (97%), whereas new grafts (n=28) showed infection in the intima-media in 13 vessels (46%), in the adventitia in 27 vessels (96%). Presence of *C. pneumoniae* in the total of 62 vessels sampled was 66% in the intima-media (n=41) and 97% in the adventitia (n=60) ($p<0,05$). Our statistical calculations demonstrated that chlamydial presence was significantly more frequent in the intima-media of venous grafts with long-term failure than in that of new venous grafts, while no statistically significant difference was demonstrable in the adventitia of these two vessel groups. Furthermore, severity of infection in the intima-media both of occluded and new grafts showed widely varying results (grade 0–3), while the adventitia—irrespective of the presence or absence and of the grade of infection in the intima-media—harboured a predominantly mild-to-moderate (grade 1–2) infection. Our observations on the adventitial infection were further confirmed by our findings in other venous structures and in coronary artery samples from young adults. **Conclusions:** (1) Clinico-pathologic evaluations of early postoperative mortality following open-heart surgery are surprisingly rare in the literature, terms used by the few available studies widely vary and hardly correspond to each other, and no generally accepted system for the applied criteria is available, which practically excludes any comparison between these studies and the results of the reported heart centres. The system of terms and criteria elaborated by us has substantially contributed to our evaluative work on the cohorts of patients who died early after CABG, valvular operations and combined interventions. Mortality rates obtained as results corresponded well to the general pathophysiologic characteristics of the surgical intervention types and of the basic cardiac alterations leading to surgery. The fact that these open-heart operations caused serious burden to the patients was shown by surgical complications ranking first among death causes in all three intervention groups. The second most important causes of death indicated well the basic cardiac alterations leading to surgery: it was severe coronary artery disease in the CABG-group and chronic heart failure in the valvular operation group, the latter corresponding to an already preoperatively restricted pump capacity secondary to long-term valve dysfunction. It is interesting to note that the number of cases examined in the combined intervention group was too low to allow for statistical evaluations, yet there was a tendency of an exceptionally high rate of deaths due to surgical complications corresponding to the enhanced operative burden, followed by an equal mortality representation both of severe coronary artery disease and chronic heart failure corresponding to the doubled heart pathology (i.e. coexistence of both coronary artery and valvular disease). The evaluation system elaborated by us may be a first step towards the standardization of related future studies. (2) The characteristics of *C. pneumoniae* infection detected as well in chronic coronary bypass graft occlusions, as in new grafts, different venous structures from various anatomic sites and coronary samples from young adults—with special respect to the high adventitial frequency of the bacterium and to the monotonous mild-to-moderate grade of infection in the adventitia—imply the possibility of an "adventitial baseline infection" from which infection of the inner wall layers (intima-media) may develop according to actual microenvironmental conditions. This would mean that—contrary to former views—plaque-forming mural layers are not so much infected from the main lumen, but rather from the periphery through adventitial lymphatic and small vessels. The fact that these characteristics were detectable not only in coronary bypass grafts, but also in other vessel types and in various conditions suggests that our observations may have more general angiopathologic implications.

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ATTILA SZIJÁRTÓ (2007)

Increasing ischemic tolerance in liver surgery

Supervisor: Zsuzsa Schaff

Introduction: The author reviews the international literature regarding ischemiareperfusion pathophysiology, and evaluates an experimental model of hepatic resection. Ischemiareperfusion (IR) injury plays an important role during liver resection or liver transplantation. Oxygenderived free radicals activate poly(ADPribose) polymerase (PARP) and decrease the available antioxidant pools. The aim of the study was to investigate ischemic tolerance during liver resection. In this model, we also investigated the protective effects of ischemic preconditioning (IP), PARP enzyme inhibitor (PJ34), and glutamine (Gln), an antioxidant. Inbred male Wistar rats were used for the experiment (n=130). Complete segmental ischemia of the liver was achieved by clamping of the portal triad with, or without, IP, PJ34 or Gln pretreatment before the ischemia. The microcirculation was monitored using laser Doppler flowmeter (LDF) throughout the ischemiareperfusion period. Required standardizations and mathematical analyses were performed in order to validate the statistical data. Histological alterations, immunohistochemistry (TUNEL, caspase), liver enzymes, bilirubin, and TNFα levels were all measured simultaneously. **Results, conclusion:** 30 minutes ischemia is well tolerated by the liver and IP does not cause further improvement. 45 to 60 minutes ischemia results in serious microcirculatory changes during reperfusion. 90 minutes ischemia is unequivocally intolerable. The liver injury and microcirculatory changes caused by 45 or 60 minutes of ischemia could be reduced with IP. Improvement was observed both, in the histological samples and in the survival rates. 45 and 60 minutes, IP+IR caused a significant decline in the TNFα level as well as in the laboratory blood samples. IP, PJ34 PARPinhibitor and glutamine pretreatment prior to 60 minutes of ischemia, all resulted in significant improvement of the microcirculation. We conclude that the widely used TUNEL assay is inadequate in the present IR model to detect apoptosis. Immunohistochemistry against activated caspase3 showed significantly higher apoptosis rates in the PJ34 PARPinhibitor and glutaminepretreated groups, in contrast to the sham and IR groups. Antioxidant levels in the serum and in the liver homogenates showed significant improvement in the IP and glutaminepretreated groups, in contrast to the PJ34 PARPinhibitor pretreatment, where only slight improvement was seen.

- *Szijártó A, Hahn O, Lotz G, Schaff Z, Madarász E, Kupcsulik P (2006) Effect of ischemic preconditioning on rat liver microcirculation monitored with laser Doppler flowmetry. J Surg Res 131: 150–157.*

• *Szijártó A, Batmunkh E, Hahn O, Mihály Z, Kreiss Á, Kiss A, Lotz G, Schaff Zs, Váli L, Blázovics A, Gerő D, Szabó Cs, Kupcsulik P (2007) Effect of PJ34 PARPinhibitor on rat liver microcirculation and antioxidant status. J Surg Res 142: 72–80.*

PROGRAM 8/3.

STUDY OF THE IMMUNOBIOLOGICAL EFFECTS OF MICROORGANISMS AND OF THEIR COMPONENTS AT MOLECULAR AND CELLULAR LEVEL AND IN MICROORGANISMS

Coordinators:
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Program overview

Infections caused by bacteria, viruses, parasites and fungi have shown significant leading roles in morbidity, mortality and health economy all over the world including Hungary. This Program has offered a variety of studying the causative agents, pathomechanisms, pathogenesis, transmission, epidemiology, control, rapid diagnosis and prevention of most frequent infections in Hungary from molecular to host level. Outstanding parts of the Program have been the in vitro and in vivo pathomechanism and pathogenesis of natural and nosocomial infections including the pheno- and genotypical features of infectious agents with special regard to the presence and expression of genes responsible for toxin production, cell-surface properties, and resistance to different antimicrobial groups. It has been extended to investigate the structure and function of adenovirus epitopes, the interaction between virus and host cell the origin and spread of hepatitis viruses, the regulation of viral oncogen expression. Most recently the effects of infections on the quality of life as well as on health care economy have been involved in the Program. Molecular mechanisms of the virus-cell interaction of human retroviruses, such as HTLV and the AIDS virus HIV, as well as the analysis of drug resistance of and the effect of antiretrovirals on HIV were added to the program as new fields

Titles of research projects	*Supervisors*
Differential diagnostics of viral respiratory	György Berencsi
Pathogenicity and antibioticum resistancy of streptococcus and enterococcus	Orsolya Dobay
Molecular biology of the resistancy against Gram-negative bacteria	Miklós Füzi
Role of chlamydias in the pathomechanism of chronic diseases (atherosclerosis, infertility)	Éva Gönczöl
Control in infections and clinical epidemiology	László Gulácsi
Health economy of infections and their effect on quality of life	László Gulácsi

Antibiotic policy based on microbiological and clinical studies	László Gulácsi
Health economy of vaccination	László Gulácsi
Health economy of diseases with great importance in public health and their modern (biological) therapy	László Gulácsi
Host dependent methylation pattern of latent Epstein-Barr viral genomes with automatic fluorescent genomic hybridization	János Minárovits
Regulation of expression of latent oncogenes in cells carrying latent Epstein-Barr viral genomes	János Minárovits
Molecular study on human retroviruses and their role in immuno-pathological diseases	Károly Nagy
Antibacterial peptides and their target proteins	László Ötvös
MRSA strains in streptococcus genome	Ferenc Rozgonyi
Antibiotic resistancy in Gram-negative bacteria	Dóra Szabó
Mechanism of resistance against Gram-negative non fermenting bacteria	Dóra Szabó
Resistant microorganisms in nosocomial infections	Dóra Szabó
Molecular study on hepatitis virus carrying individuals in order to identify origin and spreading of agents	Mária Takács

Ph. D. students		***Supervisors***
Emese Réka Juhász	a	Károly Nagy
Máté Sándor Szász	ft	Dóra Szabó

Ph. D. candidates		***Supervisors***
Valentin Brodszky	pt	László Gulácsi
Tamás András Koncz	pt	László Gulácsi
Katalin Kristóf	i	Dóra Szabó
Tünde Mag	ft	Ferenc Rozgonyi
Fruzsina Petrovay	ft	Éva Gönczöl
Ákos Tóth	pt	Miklós Füzi

Ph. D. graduates		***Supervisors***
Ágoston Ghidán	i	Ferenc Rozgonyi
Csaba Jeney	i	Ferenc Rozgonyi
András Máthé	pt	Ferenc Rozgonyi
Márta Péntek	pt	László Gulácsi

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2007 and 2008

ÁGOSTON GHIDÁN (2007)

Epidemiology of vanA gene carrier enterococci: molecular characterisation, antibiotic sensitivity and phylogenetic relationship of Hungarian isolates

Supervisor: Ferenc Rozgonyi

The thesis is discussing the examination of the vancomycin-resistant *Enterococcus* (VRE) strains. The VRE isolates of animal origin derived from the Central Veterinary Institute, collected continuously at the abattoirs in the frame of the Antibiotic Monitoring System between 2001–2004, from the intestines of healthy poultry (broiler chickens). Avoparcin as a growth promoter was used for many years in animal feeding, creating a selective pressure on the normal intestinal flora of the animals. Among the obtained samples, most VRE strains were isolated from poultry, especially from broiler chickens. We have examined these strains in the study, with both conventional as molecular methods. Despite the ban on the use of avoparcin in Hungary since 1998, the animals are still carrying VRE strains, with the possibility of entering also to the human population. These strains can serve as a source of glycopeptide resistance which can be transmitted to even much more pathogenic bacteria. This phenomenon is not unknown and was published several times. We have identified the isolates at genus and species level with molecular methods, adapting the protocols described in the international literature to the local situation. The glycopeptide MICs of the positively identified strains were determined by the agar dilution method, using a multipoint inoculator. Most of the strains were resistant to both vancomycin and teicoplanin. Out of the examined resistance determinants, we found only the presence of the vanA gene by PCR, which is located on the Tn1546 transposon.

It is worth to ponder that although these animals are not exposed to glycopeptides, why are the *Enterococcus* strains found in their intestines resistant? All isolates carrying the vanA gene, from both animal and human origin, were compared at molecular level. As human VRE are fortunately still extremely rare in Hungary, we could involve only two human isolates in the study. We detected the presence of the vanA gene in the animal isolates in 2001 and 2002, but in 2003 and 2004 we found only intermediately vancomycin-resistant strains, that did not carry any of the common van genes (vanA, vanB, vanC). During the species determination, we could identify not only the most frequent species, but on one occasion we managed to identify an *Enterococcus mundtii*, which is a very rare vanA carrier, even according to the literature.

The results of the phylogenetic examinations support the predominantly polyclonal origin of animal isolates, as well as the lack of their relatedness with human strains. It would be necessary to perform a thorough surveillance to screen for the source of resistance at those farms, where even five years after the ban of avoparcin VRE strains could be isolated, and if necessary, even the poultry-breeding technology could be altered in co-operation with the farmers.

• *Ghidan A, Jeney Cs, Marodi CL, Csiszár K, Rozgonyi F (2000) PCR detection of the vanA gene in a vancomycin-resistant Enterococcus faecalis clinical isolate from Hungary. J Antimicrob Chemother 46: 325–326.*

- *Kaszanyitzky EJ, Tenk M, Ghidan A, Fehervari GY, Papp M (2007) Antimicrobial susceptibility of enterococci strains isolated from slaughter animals on the data of Hungarian resistance monitoring system from 2001 to 2004. Int J Food Microbiol 115: 119–123.*
- *Ghidan A, Kaszanyitzky EJ, Dobay O, Nagy K, Amyes SGB, Rozgonyi F (2008) Distribution and genetic relatedness of vancomycin-resistant enterococci (VRE) isolated from healthy slaughtered chicken in Hungary from 2001 to 2004. Acta Vet Hung 56: 13–25.*

CSABA JENEY (2008)

New molecular diagnostic approach of human papillomavirus detection: the role of experimental design in the development, analytical and clinical evaluation

Supervisor: Ferenc Rozgonyi

By introducing the Taguchi method to optimize ELISA procedures we demonstrate the advantages of this approach over the more traditional labor-intensive methods. We have described a new experimental design method, which better handles the basic parameters of the underlying system. The modified strategy was further exemplified by the demonstration of the existence of a general primer optimum in a highly multiplex PCR reaction. On the basis of these work we developed a new detection method (L1F/L1R) that is suitable for sensitive and balanced amplification and specific genotyping of HPV DNA from clinical samples. It amplifies 46 HPV genotypes and the key elements of the system: the special selection of the amplified region, a novel and optimized amplification primer set, circumspectly designed genus and genotype specific oligonucleotide probes. The detection is based on solid phase hybridization in microtiter plate format using geno- and type specific probes at medium stringency, which makes the detection robust in case of small sequence variants. The assay is highly reproducible and suitable for automatization. The method was compared to Hybrid Capture II test. This study revealed excellent performance of the L1F/L1R for the examined parameters. L1F/L1R's estimated sensitivity was 91.1% and estimated specificity was 100%. As a part of the clinical evaluation the pelvic inflammatory disease as a risk factor were studied. The presence of HPV and other STD agents in cervical smears was detected with L1F/L1R reaction and other PCR methods. HPV prevalence was 33.74% in patients with PID and 26.40% in the group of women without PID ($p<0.001$). This suggests that patients suffering from PID apparently have a higher risk for aquiration of HPV infections.

- *Jeney C, Takacs T, Sebe A, Schaff Z (2006) Detection and typing of 46 genital human papillomaviruses by the L1F/L1R primer system based multiplex PCR and hybridization. J Virol Methods 149: 153–162.*
- *Skapinyecz J, Smid I, Horvath A, Jeney C, Kardos L, Kovacs P (2003) Pelvic inflammatory disease is a risk factor for cervical cancer. Eur J Gynaecol Oncol 24: 401–404.*
- *Jeney C, Dobay O, Lengyel A, Adam E, Nasz I (1999) Taguchi optimisation of ELISA procedures. J Immunol Methods 223: 137–146.*

ANDRÁS MÁTHÉ (2007)

Antimicrobial treatment of experimental infection due to an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strain

Supervisor: Ferenc Rozgonyi

The resistance to the third generation cephalosporins, mediated by extended-spectrum β-lactamases (ESBLs) is an increasing problem. The antimicrobial agents of choice for infections due to these strains have been the carbapenems, however the emergence of carbapenem-resistant ESBL-producing *K. pneumoniae* strains warrant the use of alternative agents.

Relevance of animal models for the determination of antimicrobial agents active in an infection surpass that of *in vitro* studies. However, major limitation of animal studies is the pharmacokinetic dissimilarities between animals and humans. The simulation of human pharmacokinetic parameters of renally excreted antibiotics can be reached most simply by the administration of a nephrotoxic drug. Different doses of cisplatin (from 0 to 26 mg/kg) were administered to CD-1 mice 3 days before cefepime administration. Pharmacokimetic parameters of cefepime and survival of mice for 8 days were determined at each cisplatin dose. At 18 and 22 mg/kg of cisplatin elimination half-life of cefepime significantly increased while lethality did not differ significantly from the control. Using 18 mg/kg of cisplatin the serum concentrations of cefepime were comparable to that of humans. Cisplatin pretreatment is a simple method to simulate human-like pharmacokinetics of renally excreted drugs.

Activities of amikacin, cefepime, amikacin plus cefepime, imipenem, and amikacin plus imipenem were studied against an SHV-5 ESBL-producing *K. pneumoniae* strain using high inoculum, *in vitro* and *in vivo* in septic mice. Cefepime showed inoculum effect. Susceptibility to amikacin and imipenem was independent from the inoculum size. In killing curve studies the bacterial count increased in the presence of cefepime and in the absence of antibiotic, while it was reduced by the other antibiotics and combinations. *In vitro* synergy between amikacin and imipenem was not detected using killing curve study and chequerboard technique. The blood bacterial count was reduced significantly by amikacin, amikacin plus cefepime, imipenem, and amikacin plus imipenem. Amikacin, amikacin plus cefepime and imipenem prolonged significantly the survival of mice compared to cefepime and to the infected untreated group (survival analysis in the amikacin plus imipenem treated group was not was not performed). Increased resistance due to high inoculum could be responsible for the ineffectiveness of cefepime. The combination of amikacin plus imipenem did not have any advantage over monotherapy.

- *Szabó D, Máthé A, Filetóth Zs, Anderlik P, Rókusz L, Rozgonyi F (2001) In vitro and in vivo activities of amikacin, cefepime, amikacin plus cefepime and imipenem against an SHV-5 extended-spectrum β-lactamase-producing Klebsiella pneumoniae strain. Antimicrob Agents Chemother 45: 1287–1291.*
- *Szabó D, Máthé A, Lengyel J, Filetóth Zs, Anderlik P, Rókusz L, Rozgonyi F (2002) In vitro and in vivo activities of ciprofloxacin and levofloxacin against an SHV-5 extended-spectrum β-lactamase-producing Klebsiella pneumoniae strain. Curr Med Chem 9: 437–442.*
- *Máthé A, Komka K, Forczig M, Szabó D, Anderlik P, Rozgonyi F (2006) The effect of different doses of cisplatin on the pharmacokinetic parameters of cefepime in mice. Lab Anim 40: 296–300.*

MÁRTA PÉNTEK (2008)

Health status and disease burden of patients with rheumatoid arthritis in Hungary

Supervisor: László Gulácsi

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory condition characterized by joint destruction. In Hungary, a comprehensive measurement of standardised disease characteristics did not spread widely in RA. Generalisability of international results is questionable due to differences in health care and social systems between countries. Introduction of costly new biological drugs have sped up the studies on health status, comorbidities and disease burden in RA.

A cross-sectional survey was performed involving 6 rheumatology centres to assess the health status, health related quality of life and health care utilisation of RA patients in Hungary. The National Heath Insurance Fund Administration's database covering the entire population was searched for fractures which is one of the most common complication in RA. Costs of RA was calculated based on the results of the cross-sectional survey. Our results highlighted that health status of RA patients is significantly worse than of the Hungarian population. A significant correlation was established between disease-activity, functional and health status and quality of life. Correlation between disease progression and health status did not differ significantly from international studies involving cohorts without biological drugs. Our results offer country-specific data for cost/QALY calculations in Hungary.

Fracture incidence in RA is about double compared to the general population in age group 50–100 years. Further studies are needed on vertebral fractures to assess their risk on a wider level. Costs of RA increase with disease progression, funcional disability is determining. Severe disease activity and decreased quality of life lead to higher costs. The amount of disease related costs in Hungary is much lower than in developed European countries. Health-economic and cost-effectiveness analysis of such countries cannot be adapted to Hungary without adjustments of costs. Our study offers baseline data regarding health status and costs of RA for further cost-effectiveness models.

- *Pentek M, Kobelt G, Czirjak L, Szekanecz Z, Rojkovich B, Polgar A, Genti G, Kiss CG, Brodszky V, Majer I, Gulacsi L (2007) Costs of rheumatoid arthritis in Hungary. J Rheumatol 34:1437.*
- *Lovas K, Kaló Z, McKenna SP, Whalley D, Péntek M, Genti Gy (2003) Establishing a standard for patient completed instrument adaptations in Eastern Europe: experience with the Nottingham Health Profile in Hungary. Health Policy 63: 49–61.*
- *Brodszky V, Czirják L, Géher P, Hodinka L, Kárpáti K, Péntek M, Poór Gy, Szekanecz Z, Gulácsi L (2007) A rituximab szerepe a rheumatoid arthritis kezelésében: irodalmi áttekintés. Orv Hetil 148: 1883–1893.*

PROGRAM 8/4.

PUBLIC HEALTH AND HEALTH SCIENCES

Coordinator:
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Program overview

The program includes issues and joint areas of public health, forensic medicine, hygiene and dietetics. The various subprograms sum up knowledge in the fields of organ damages caused by environmental injuries, endocrinological diseases and virology. A special Program deals with topics of health education and nursing. The various Programs have a common basic and following the branching-off there is an opportunity to select a special subjects.

Titles of research projects	*Supervisors*
Dietary epidemiology, physiology and pathophysiology	Magda Antal
Role of mission in health occupations	Péter Balázs
Prevention of pediatric obesity	Mária Barna
The relationship between childhood obesity and adulthood cardiovascular disease with special respect to comorbidity	Antal Czinner
Protein structures in oncology	Judit Fidy
Health history	Judit Forrai
Carcinogenicity of environmental chemicals, biological markers	Sarolta Gundy
Identity of profession and councelling in nursing	Kornélia Helembai
Development of teaching in higher education of health—paradigms in nursing and teaching at the 21st century	Sándor Hollós
Death caused by drugs (opiate and dopaminerg systems in heroin taking)	Éva Keller
Role of antioxidants in prevention of certain diseases	Andrea Lugasi
Statistical and clinical epidemiology to discover etiology of cancer	Gábor Makara
Comparative study on the asthmatic children taking part regulary in swimming programs	György Mezei
Analysis of effects of health education and nursing	Judit Mészáros
Epidemiology of adenoviruses in immunosuppressive conditions. Adenoviral therapy	József Ongrádi
Psychosocial characetristics of additive disorders	József Rácz
Toxic injury of the myocardium	Péter Sótonyi
The questions of the hygienic and medical right, contractual relationship in the Hungarian legal practice	Péter Sótonyi
Interaction of iodine and selenium supply in elderly ages	István Szabolcs
Quality of life in subclinical hypothyroidism	István Szabolcs

Effect of globalization on the onset of diseases	Anna Tompa
Unemployment and the environment (local environment and dietary habit)	György Ungváry

Ph. D. students		***Supervisors***
Domonkos Bán	pt	Judit Forrai
Adrienn Bartók	pt	Judit Forrai
Sándor Bollók	pt	István Vingender
Judit Bozóki	pt	Péter Balázs
Róbertné Csajbók	pt	István Szabolcs
Andrea Fogarasi-Grenczer	pt	Péter Balázs
Gábor Grósz	pt	Anna Tompa
Diána Kaldau	ft	Judit Forrai
László Kádár	pt	Péter Balázs
Zsuzsanna Balogh Kériné	ft	György Berencsi
Judit Kormos-Tasi	pt	Antal Czinner
Zsuzsanna Menczel	pt	István Vingender
Erika Nagy	pt	Kornélia Helembai
Ákos Novotny	pt	István Szabolcs
Mariann Csernus Raskovicsné	pt	Sándor Hollós
Ágnes Regős	pt	Endre Morava†
József Révész	pt	Judit Forrai
Péter Váry	a	Sándor Hollós

Ph. D. candidates		***Supervisors***
Kinga Balla	pt	György Mezei
Éva Bocskay	pt	Péter Balázs
Gábor Borgulya	pt	Gábor Makara
Erika Erdősi (Csapóné)	pt	Kornélia Helembai
Sarolta Csatordai	pt	Sándor Hollós
Vera Feuer	pt	Judit Mészáros
István Hornyák	pt	Mária Barna
Katalin Kardos (Horváthné)	pt	István Szabolcs
Leonárd Janik	ft	József Ongrádi
Annamária Pakai (Karamánné)	pt	Péter Balázs
Júlia Kis	pt	Sándor Hollós
Erzsébet Mák	pt	István Szabolcs
Katalin Tátrai (Némethné)	pt	Mária Barna
Erzsébet Pálfi	pt	Mária Barna
László Papp	pt	Kornélia Helembai
Marcel Marius Pop	pt	Judit Mészáros
Younes Saleh Ali Saleh	i	György Berencsi
Ágnes Simek	i	Anna Tompa
Enikő Tátrai	ft	Péter Sótonyi
Tímea Tóth	pt	Judit Mészáros
Ibolya Tulkán	pt	Sándor Hollós
Márta Bálint (Veresné)	pt	István Szabolcs

Ph. D. graduates		*Supervisors*
Zoltán Balogh	pt	Judit Mészáros
Helga Judit Feith	pt	Péter Balázs
Péter Felkai	i	Péter Balázs
Péter Fritz	pt	Judit Mészáros
Monika Csilla Horváth	pt	Éva Keller
Zsuzsanna Kiss (Soósné)	pt	Mária Barna
Gábor László Kovács	ft	István Szabolcs
Ágnes Tóth (Kovácsné)	pt	Péter Balázs
Natália Lásztity	ft	Magda Antal
Szabolcs Péter	ft	Magda Antal
Gábor Pörzse	i	Judit Forrai
Emese Sipter	ft	Erzsébet Tátrai
Orsolya Szakács	ft	Péter Sótonyi
Imre Szerb	pt	Magda Antal

a, absolutorium; ft, full-time; pt, part-time; i, individual

Abstracts of Ph. D. theses successfully defended in 2005, 2006, 2007 and 2008

ZOLTÁN BALOGH (2008)

Developing nurse training in the field of community nursing

Supervisor: Judit Mészáros

The topic of the doctoral thesis is the development of nurses: directions of the development of the nursing education in community and home nursing based on the European and national directives and based on the results of the nursing research.

During the past seven years (1994–2001) the work team of the Healthcare Science Institute performed an investigation in the circle of bachelor degree nurses graduated from the day-school (target-group). The aim of the research was to reveal the professional progress of the graduated colleagues, to examine the professional and moral esteem of the nurses and to get to know their reasons for leaving and changing career. That was the first phase of the research work. We tried to find the answer to three groups of questions in the background of the research aims:

Which are the factors on the basis of which the graduating students chose working field, workplace? We suppose that the nurses graduated from the day-school select workplace on the basis of their professional experiences, field practices.

What circumstances, factors influence leaving, changing career? We suppose that leaving career is rather influenced by the lack of the professional and moral esteem than the rate of the financial recognition.

Where, in which field and why do the graduates find jobs here the most often? We suppose that most of them chose not state-owned health care or social fields.

As a result of the survey using questionnaires performed in the circle of nurses graduated during the past seven years it can be stated that the choice of the first workplace was highly influenced by the positive and negative experiences gained in the course of the field practices. All of the professionals teaching special subjects at the College have been making efforts for several years to organize the field practices on as high level as possible. This demand has been strengthened even more by the integration process taking place at the Semmelweis University and concerning also our College and at the same time it has established also a new opportunity by which the organization of the field practices can be organized and managed even more efficiently and realized in a more demanding way.
Therefore we have developed a new metodology (nurse mentor programme) to organise and follow-up the nurse sudents' clinical practice in the primary—community and home care—nursing. We have also developed a new educational startegy based on Bologna process and other European recommendations and the needs of those nursing professionals who are working in the home care system in Hungary. That was the second phase of the research work. The results of the study adapt to the educational strategy.

- *Balogh Z (2001) Nursing literature in electronic form. Nővér 14: 24–27.*
- *Balogh Z (2003) Evidence-based practice guidelines for nursing and rehabilitation of stroke patients. LAM 13: 50–56.*
- *Zrínyi M, Balogh Z (2004) Student nurse attitudes towards homeless clients: a challenge for education and practice. Nursing Ethics 11: 334–348.*

HELGA JUDIT FEITH (2008)

Women's roles and conflicts in careers of healthcase professionals

Supervisor: Péter Balázs

The burdens of caring and curing professions are increased in the female workforce by duties becoming a child and serving a family as well. Working women in Hungary's healthcare system have to cope with further disadvantages, too: low income, inflexible time schedules, insufficient workplace conditions, lack of means, and job related mental stress factors. The objective of our research was to study and compare future and present family and career planes of students, respectively active professionals (nurses and female doctors), related to their physical and mental health and conflicts.
Our cross-section research was carried out among female nursing college students, female medical students, and among diploma nurses and female doctors in hospitals. According to the results, students consider parallel their future family and workplace roles, however diploma nurses prefer career-building in their professional activity. The number of children planned is the same as in the general population, but female medical students would like to have more children than the average. Their health and risk behaviours are at a substandard level, but nursing college students are more disadvantaged in this respect. Diploma nurses and female doctors estimate high both the family and workplace roles. Role conflicts are interrelating with their career and life satisfaction, health condition, and the prevalence of psychosomatic symptoms. Their roles as a social model in health promotion are rather questionable, for their insufficient health and risk behaviours.
In summary, we can state that there is a considerable tension and contradiction in planned and actual roles of future and present female workforce of Hungary's health care system.

In many cases they are unable to fulfil requirements based on their social engagement. Relevant handicaps of nursing college students and female diploma nurses are more prevalent, therefore we propose further analytic and comparative research in the future.

- *Feith HJ, Kovácsné TÁ, Balázs P (2005) Női szerepek diplomás ápoló hallgatónők jövőképében. Nővér 18: 3–9.*
- *Feith HJ, Balázs P, Kovácsné TÁ (2007) Female physicians' and nurses' self-rated general and psychosocial health status and health behaviour in Hungary. New Medicine 9: 55–59.*
- *Molnár R, Feith HJ (2000) Szerepkonfliktusok megnyilvánulásai orvosnők körében. LAM 10: 810–818.*

PÉTER FELKAI (2008)

Analysis of the prevention of travel related illness on the basis of the recent achievements of the travel medicine

Supervisor: Péter Balázs

When we survey the possibilities of the prevention in travel medicine, it is soon developed that the "classical" or "infectology-oriented" approach of the prevention (vaccination—chemoprofilaxis—pretravel advice) cannot respond to all peritravel medical problems. These newly arisen challenges require a new, complex outlook of the travel medicine, mainly in the field of prevention, treatment and management of the travel-related illnesses. Although travellers are still exposed to various infectious diseases, which are mostly preventable by the primary prevention methods (biomedical profilaxis), yet, the majority of the travellers suffers non-infectious diseases and fall ill or suffer accident by other, travel related factors. Moreover, the predominant destination is Europe for an average Hungarian traveller.

The travellers, whose destination is not any tropical or third world country, the adventure travellers, the people of extreme sports, the chronic patients all require the same-leveled peritravel (prevention-oriented) advices, too. Thus, besides the vaccination, the helmets, the salt-tablets, the medical kit, the mobile oxgenisator has to belong to the travel doctors' arsenal. We also have to mention the travellers' acute illness/accident abroad, travellers who require repatriation, and also the appropriate travel insurance policy, which should be worked out by evidence-based medical considerations.

The above mentioned complex task requires not only a broad-spectrum, multidisciplinary knowledge, but an appropriate co-operation between the travel medicine physician, the general practitioner of the patient, and the doctor of the insurance company. But, above all, we have to re-define the topic of travel medicine, its subspecialties, and their role in the three-level (primary, secondary and tertiary) prevention. We have to redefine the different prevention levels and the used methods related to the different subdisciplines of travel medicine. Upon the recent scientific researches, we can lay down four topics of travel medicine: the biomedical profilaxis, the assistance medicine, the wilderness medicine and the travel-insurance medicine.

This four-leaf partition is fit for the complex approach to the newly generated peritravel medical problems, both in Hungary and at the international level, too. This kind of approach makes the doctor (who contacted the travellers at any respect: travel medicine specialists, GPs, occupational medicine specialists, insurance physicians) able to partici-

pate in the peritravel treatment or pretravel advice of the travellers. The basic ideas of the prevention levels have to be established in each subdisciplines of the travel medicine.
Another urgent task is to establish a country-wide network of travel medicine facilities as well as the organisation of the gradual and postgradual education of the physicians and professionals. We have to implement new forms and methods during the training. The travel advice and the peritravel prevention requires well-trained and continuously educated doctors.

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PÉTER FRITZ (2008)

Elaboration of the method for complex improvement of health status and the respective efficiency analysis performed with the participation of college and university students

Supervisor: Judit Mészáros

The main aim of my dissertation was an efficiency analysis of the complex development of state of health. The research was done by the students of University of Science of Szeged, for whom it has been a 3-month period free health development programme. Out of the volunteers I could determine a 125 (29 men, 96 women) limit because of the research's budget.
Within the three-month health development programme I could measure and prove the effectiveness of physical and mental training comparing the state of health assessment before and after the trainings which I did with every group. I pieced together an investigational package determining state of health which is able to give a real picture of bio-psycho-social condition of the individual and determines the lifestyle and health behavior of the analyzed target group. The investigational procedure containing the control groups proved the health generative effectiveness of the health plan, in that group (B group) where apart from the health plan detrained by the examinations, they did not participate in the three-month period intervention programme. With this the justification and motivational effect of paper based health development was proven. The increase of occasions spent by weekly movements is observable in the group that possessed with simply health plan, which proved the effectiveness of health plan. On the basis of the test results I have managed to set up a multi-variable model, which reflects the coherence of factors affecting health status.
Aside from the enumerated examinations and methods the complex health development depended mainly on the analyzed persons and recreators I took into account specially. I chose the recreators who ran the personal training with the help of a special selectional system then I assured a special training for them appropriate to the programme.
It can be determined that in the execution of the training, the 7 men coach group complemented with 2 men consulting teacher proved the applicability of personal trainings in small group, building up on a matrix kind of structure which increased the effectiveness

of the personal training. One of our main aims of our examination has been realized, with the personal or small group training with a more complex matrix kind of organizational buildup and tried selectional, preparational and applied methods it can function in an effective form.

The programme package according to the aim of the examination can be applied to a wider social environment and can be available with the appropriate formal appearance to health development professionals and professional training.

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MONIKA CSILLA HORVÁTH (2008)

Drug-related deaths in Budapest and the neurobiology of heroin abuse

Supervisor: Éva Keller

Heroin abuse is a widespread phenomenon which in Hungary, just as in other parts of the world, has been slowly but gradually increasing in the past 15 years. Our work was aimed at characterizing the population of drug-related death cases, with a special emphasis on the demographic and toxicological evaluation of the subjects. We analyzed 309 drug related death cases registered between 1994 and 2006 at the Department of Forensic Medicine at Semmelweis University, Budapest. We found that the number of drug-related deaths is slowly but constantly increasing; the majority of drug-related deaths were caused by accidental heroin overdoses whose victims were usually male, aged between 20–30 years of age, with an approximately 3.5 years of drug-use history, almost all Hungarian citizens. We also analyzed the prevalence of some infectious diseases within the population; HIV was very rare (1 case during 6 years) while hepatitis C and B and syphilis had a prevalence of 23%, 7% and 7%, respectively.

In a subpopulation of the heroin overdose and control cases we collected human brain samples for further scientific assessment. In our attempt to understand the neurobiology of human heroin abuse, we examined the major brain regions linked to the "reward pathway": the ventral tegmental area (VTA) in the midbrain and the nucleus accumbens (Nacc) within the striatum. The systems most implicated in the neurobiology of heroin abuse are the dopaminergic and opioid systems; for this reasons we examined the major markers of these within the midbrain and the striatum. As a result of our experiments, a consistent alteration of the VTA paranigral nucleus (PN) subdivision was revealed for most of the DAergic markers which strongly suggests that there is a more profound disturbance of the mesolimbic reward circuit in heroin-dependent individuals. The paralleled disturbance of dopamine transporter (DAT) mRNA and protein levels in the PN and Nacc, respectively, emphasizes the continuum of disturbances throughout the mesolimbic circuit. In our population of opioid receptor subjects, 90% of individuals with an A118G

polymorphism of the gene (OPMR1) were heroin abusers. Down-regulation of opioid neuropeptide genes detected in the heroin users were exaggerated in 118G subjects and were most prominent for preproenkephalin in the Nacc shell. Reduced opioid neuropeptide transcription was accompanied by increased dynorphin and enkephalin peptide concentrations exclusively in 118G heroin subjects, suggesting that the peptide processing is associated with the OPRM1 genotype. Overall, the findings from these studies emphasize a prominent mesolimbic disturbance in the brain of heroin abusers.

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ZSUZSANNA KISS (SOÓSNÉ) (2007)

Providing professional support to parents expecting their first child

Supervisor: Mária Barna

Expecting and the birth of the first child and partners becoming parents and family will always be a topical issue for society, the social system and competent experts. With the birth of the first child, the family has to establish a new "family pattern", and at the same time must become able to fulfil the role of parent and caretaker. Apart from the natural socialization process, conscious preparation must also play a role in the process.

The obligatory services provided by legal provisions, which aim to facilitate the preparation for parenthood serve to guarantee that the parents will be prepared. The professional assistance systems operating outside the framework of such services provide alternatives, enabling parents to choose between them according to their demands, ambitions, needs and possibilities.

The aim of my research—of nearly 10 years now—is to create a new program for preparing those concerned for the changes in family life, for parenthood, as well as for taking care of and nurturing the baby. The program has a multidisciplinary approach, and builds on the active participation of the attendants, and their working as a pair. The speciality of the program is that it undertakes to prepare the parents without the presence of an expert.

My research shows that the preparation book, based on the principles of programmed education, as well as my self-made questionnaires and reply letters provide the mothers and fathers expecting their first child with a new possibility of preparation. At the same time my thesis is a professional and methodological recommendation to the experts engaged in providing professional support to parents expecting their first child.

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GÁBOR LÁSZLÓ KOVÁCS (2006)

Epidemiology and biological features of thyroid microcarcinoma

Supervisor: István Szabolcs

The prevalence of thyroid microcarcinomas at autopsies is 1000 times higher compared to clinical cancer. We investigated the epidemiological and histological characteristics of microcarcinomas, in consecutive autopsy series from areas of different iodine intake. At iodine deficient area (ID): median iodine excrection (MIE) was 72 μg/g creatinine, median age 74–76 yrs, n=109/113 (males/females). At iodine sufficient area (IS): MIE: 200–513 μg/g creatinine, median age 68 yrs, n=132/89. In ID as compared to the IS area a higher thyroid weight (mean 27.75 g±18.43 g vs 16.5 g±9.6 g, $p<0.0001$), more goitrous glands (50/222 vs 5/221, $p<0.0001$) were found. 21 microcarcinomas were found (4.74%) without an iodine intake or gender related difference (ID: 4.95%, n=8/3; IS: 4.52%, n=6/4. Microcarcinomas seemed to be more prevalent at the ages of 40–59 (p=ns). All microcarcinomas were of the papillary type. The results suggest a different and benign behavior of MCs as compared to clinical cancer. The role of cyclin D1 overexpression in the pathogenesis of thyroid tumors is not known clearly, however overexpression of this protein was reported in well differentiated papillary cancers and in incidentally found metastasizing MCs. To date, cyclin D1 expression has not been investigated in autopsy derived thyroid MCs. Eight MCs were available for immunostaining and comparison with 15 clinically detected papillary thyroid cancers. Fourteen of the 15 clinical carcinomas expressed cyclin D1 (93.3%), while in the MCs this ratio was 1 out of 8 (12.5%) (p=0.0001). The only cyclin D1 positive MCs was multifocal (both lobes of the gland were affected). We concluded that the benign behavior of the most autopsy derived MCs may be associated with the lack of cyclin D1 overexpression.

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ÁGNES TÓTH (KOVÁCSNÉ) (2008)

The bases of values in the motivation of the choice of career among professional nursing students and students learning at teachers' training college

Supervisor: Péter Balázs

Education, training are the motives of modern societies, they serve as a means of transmitting knowledge, they reproduce human capital and are motive powers of economy. The aquired school qualification and the obtained profession have an influence on one's career. Simultaneously with the highest school qualification there is an increase in the possibility to be employed for the individual and also an increase in the number of potential jobs to be fulfilled.

Our cross section analysis was done among professional nursing students learning in higher education at the faculty of health, and also among teacher students. According to our results among caring professions the strongest professional motivation seemed to be helping people and being interested in the profession. In the respect of population samples we attributed the bigger role for parents' joint learning in the choice of a profession rather than for the different qualification of the father or the mother. It is typical that jointly lower qualified parents' children usually choose the profession of a nurse or a teacher, as college means for them the channel of becoming intellectuals, joining the cultural values of the world of intellectuals. Those participating in the education of health science and teacher training find the social honour of their aquired profession definitely low. Despite all these, the majority of nursing students, if they were given the chance to make a decision again, without any negative judgement and despite their professional practice, would choose the same profession.

In summary: altruism can be attached to the essence for caring professions, but the representatives of professions define it as a value. According to our results during the studies of students there is no change in their values, so those values aquired through an earlier socializing can be further confirmed. The correspondence in the values of the two student groups is due to generation factors and the influence of school qualification, and at the same time it contains a typical sample characteristic for the intellectuals. There is a constant need for teachers and nurses, they are the symbols of understanding and attention, unselfish help felt towards others. Their job is such a mission which teaches us to deal not only with ourselves but we should also notice other people as well. It is worth practising it only as a mission, otherwise it will lose its real value.

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NATÁLIA LÁSZTITY (2005)

Changes of oxidative stress, inflammatory mediators and malnutrition in acute and chronic pancreatitis

Supervisor: Magda Antal

The pathogenesis and dietotherapy of different forms of pancreatitis has been studied. In the framework of the experimental part the acute pancreatitis was induced in rats by administration of excessive doses of lysine and arginine, and the secretory and morphological changes were investigated. A direct non-specific membrane damaging and cell-necrotizing effect of these amino acids has been confirmed. These experimental models may be useful for investigating different factors playing role in the development of acute necrotising pancreatitis.

In the clinical examinations, effect of early enteral nutrition was studied in patients with acute pancreatitis treated at the Department of Gastroenterology of MÁV Hospital. A correlation between the early jejunal feeding and changes of inflammatory mediator release, oxidative stress and nutritional status was examined. The antioxidant and nutritional status were also measured in patients suffering from chronic pancreatitis. In spite of early enteral nutrition the levels of cytokines remained high with slow recovery in patients with severe and complicated acute pancreatitis. Measuring the level of primary antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPOX), the total antioxidant status (TAS) of the serum, a reduced antioxidant defense was found in comparison to control group already at admission and during the treatment. The reduced SOD activity significantly correlated with the severity of the disease and with the frequency of complications. The antioxidant status of patients did not fully normalize in spite of clinical recovery. The deterioration of acute protein-energy malnutrition induced by catabolic stress and inflammation was prevented by jejunal feeding. First in the literature, we studied the effect of enterally supplemented n-3-PUFAs (3,3 g/day) in acute pancreatitis. The n-3 to n-6 PUFA ratios increased significantly in serum lipids of the patients. As a result of the treatment the length of jejunal feeding and hospital stay was significantly reduced. At the examination of patients suffering from chronic pancreatitis, a significant decrease of SOD activity was observed, however, this decrease was milder in comparison to acute pancreatitis. In chronic pancreatitis 24% of patients had moderate or high risk of PEM. The frequency of protein malnutrition was significantly higher in patients with alcohol induced chronic pancreatitis. The final part of the thesis—based on the results of experiments discussed above—summarizes the principles of dietary treatment of patients, suffering from acute and chronic pancreatitis, emphasizing the supplementation with antioxidants and n-3-PUFAs.

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SZABOLCS PÉTER (2008)

Role of lifestyle in obesity prevention

Supervisor: Magda Antal

The prevalence of overweight and obesity among children and adolescents is increasing dramatically and requires early prevention. Obesity is multifactorial and depends on lifestyle as well as genetic components. The purpose of this cross sectional study was to investigate the lifestyle of school children and students attending elementary and secondary schools in Budapest and to estimate the prevalence of thinness, overweight and obesity in this group. The lifestyle part of the research was questionnaire-based. Many children and adolescents live a sedentary life and are dissatisfied with their body weight. Among school children one in five boys and one in three girls, in the secondary schools more than 40% of the girls have been on a slimming diet at least once. School children in the upper classes and students did not have a regular daily eating rhythm. More than 5% of the 11–14 year-old boys, more than 10% of the girls and nearly 60% of the secondary schoolleavers smoked regularly. For the anthropometric investigations height and waist circumference were measured, other data were analyzed by eight-polar bioelectrical impedance procedure. On the basis of age and gender specific diagnostic criteria for body mass index, prevalence of thinness, overweight and obesity among school children was 5.1, 18.1 and 7.4% for boys and 6.8, 19.6 and 6.3% for girls. Altogether the prevalence of overweight and obesity was 18.8 and 6.9%, respectively. About 49.1% of overweight boys and 28.0% of girls were obese according to %BF. On the basis of body fat percentage, prevalence of obesity was 17.9 and 12.7% for boys and girls, respectively. On the basis of body mass index, prevalence of leanness, overweight and obesity among students in the boy group were 6.7, 15.9 and 4.1%, respectively. In the girl group the respective figures were 7.2, 7.9 and 1.9%. The rate of overweight and obese students altogether was 12.2% and 3.0%. About 10.5% of overweight boys and 77.0% of girls were obese according to %BF. On the basis of body fat percentage, 5.1% of boys and 16.2% of girls were obese, whereas on the basis of data for waist circumference, visceral obesity occurred in 10.5% of boys and 7.9% of girls. Among macrosomic babies the rate of overweight and obesity is higher than among normal-birth-weight babies, particularly in childhood. According to these results, coordinated cooperation is necessary among stakeholders to support the aim of changing nutritional and exercise behaviour of Hungarian children and adolescents.

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GÁBOR PÖRZSE (2008)

The development of European Union common research and development policy and programmes with special regard to life sciences

Supervisor: Judit Forrai

In the present doctoral dissertation, I aim to deal with the scientific Research and Development (R&D) that plays a decisive role in common EU policy, paying special attention to the role of life sciences R&D within the Framework Programmes (FP).
The aim of the dissertation is to (1) outline the development of the R&D policy; (2) evaluate the role and importance of the FPs in the community R&D system; (3) introduce in detail the role and development of Life Science Research (LSR); and (4) analyse the participation and effectiveness of the Hungarian LSR community in the FPs, in the light of the statistical data collected and processed. In the dissertation I would like to demonstrate that (1) R&D, with the progression of integration, has became a motor of competitive capability and sustainable development and a basis for other community policies; (2) The FPs have contributed to the harmonisation of national R&D activities and reinforced the whole of the EU research system; (3) current challenges may be addressed only with the joint cooperation of nations, economic sectors and scientific disciplines; (4) the priorities of LSR are changing significantly due to development; (5) Hungarian researchers—in so far as their resources allow—have successfully joined the R&D FPs in life sciences. The dissertation is based on the processing of professional literature, on the analysis of the statistical data, on personal interviews and on previous experiences. My hypotheses were confirmed by the results of my research. (1) I have certified that the community R&D has significantly contributed to the strengthening of EU competitive capability, although it has not made up significant ground on the continent's main competitors. (2) I have indicated that the FPs have strengthened the whole of the EU research system, but have been less effective in the area of innovation. (3) I have shown, using several examples, the importance of international cooperation, and collaboration between the government and private sector, and certain fields of science. (4) I have also pointed out those challenges that have led to important priority changes in the field of LSR. (5) I have confirmed with the statistical data that the Hungarian researchers have successfully joined the FPs; their effectiveness with respect to their activity and participation in projects stands up to international comparison as well. My conclusions referred also to the future direction of the present research.

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EMESE SIPTER (2008)

Human health risk assessment of toxic metals

Supervisor: Erzsébet Tátrai

The environmental research carried out in the village of Gyöngyösoroszi has documented increased concentrations of arsenic and heavy metals in the soil of vegetable gardens. The differences between the metal contents of flooded and non-flooded vegetable gardens were significant, which confirm that the floods of Toka creek spread the contamination over the flood-plain of the village. The source of pollution is the tailing dump and the flotation plant. The metal contents of vegetables were low and the arsenic content was under the detection limit in every case. The sorrel cultivated in flooded gardens has the highest metal content. Significant differences were also found between the flooded and non-flooded vegetable gardens. The vegetable concentration results further support the view that soil metals are not always absorbed as well as soluble forms, therefore use of default bioconcentration factors in assessing human health risk may overestimate the hazard. In our study, all of the bioconcentration factors were under 0.25 and the mobile elements were cadmium and zinc. Generally, the bioconcentration factors of non-flooded vegetable gardens were higher; the sorrel was the most accumulating vegetable. In the pot experiment the BCFs were higher, but the tendencies were the same. The most accumulating vegetable was sorrel and the most mobile element was cadmium and zinc. The arsenic contents were also immeasurable.

Site-specific exposure parameters and a newly created equation for ingestion of vegetables were applied in risk assessment process. The site-specific exposure factors were generated from the results of questionnaire survey in the village, while the equation was based on the cultivation habits of homegrown vegetables. The outcome of risk assessment has indicated acceptable risk in the village of Gyöngyösoroszi, both in flooded and non-flooded vegetable gardens. The risk assessment process with default exposure parameters overestimated the risk. In contrast with previous study in the area, home gardening does not increase the risk for inhabitants at present. The most relevant exposure route was ingestion of homegrown vegetables. It is possible to further reduce the risk of human exposure to soil metal contamination by selecting leafy vegetables such as sorrel in home gardening.

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ORSOLYA SZAKÁCS (2006)

Molecular analysis of urinary bladder tumors

Supervisor: Péter Sótonyi

Cancer of the urinary bladder is one of the most common tumors and urologic diseases. The most prevalent form of bladder cancer in the Western world is transitional cell carcinoma (TCC), which makes up about 90% of all bladder tumors. Urothelial cancers of the bladder (UC) comprise biologically heterogeneous group of tumors.The majority of urothelial cancers (UC) are diagnosed as noninvasive tumors (Ta), whereas 20% to 25% of the cases show an invasive growth (T1–4) at the time of first presentation.

At present, grade and stage are the best prognostic indicators in TCC. These parameters are also the main factors in deciding the type of treatment. However, this staging and grading schema may not always adequately reflect the true biologic nature of the individual tumor. Conceivably, a more accurate representation of the biology of bladder cancer might show a variety of distinct but interrelated pathways by which different forms of bladder cancer might develop and progress. This concept is supported not only by clinical observations but also by recent findings of molecular distinctions between various types of bladder cancer. We completed a detailed allelotyping on many chromosomal regions of 123 UC's. We delineated three regions of LOH at chromosome 2q, the region at chromosome 2q21.2 was restricted to the LRP1B gene. The genetic data and pathologic parameters suggest that inactivation of the LRP1B gene is associated with high grade, invasive growing UCs.

We induced the Bayesian network model from the LOH data of UCs. The model contained the most reliable dependencies between the losses of heterozygosity. This enabled us to extract patterns of DNA losses in bladder cancer and to show primary and secondary abnormalities associated with the tumor development. We suggested a possible flow of progression of allelic losses in bladder cancer as heterogeneous, distinct and converging genetic pathways.

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IMRE SZERB (2006)

Examination of the viability of human articular cartilage according to nourishment and biomechanical factors

Supervisor: Magda Antal

According to the data in the literature, the prevalence of cartilage defects on the weight bearing surfaces of the knee joint may reach 40% during open surgeries or arthroscopies. The regeneration capacity of the articular hyaline cartilage is minimal. Articular cartilage damage occurs more often among obese patients in daily orthopaedic practice. These three factors induced me to define my theses. My first thesis was: there must be a relationship between obesity and the prevalence of knee chondropathies. On the basis of this correlation, the prevalence of cartilage defects could be decreased with nourishment intervention and body weight reduction. With the evaluation of the results of 200 randomly selected patients, the relationship between obesity and the prevalence of knee chondropathies was unambiguously proven. Significantly higher body mass index (BMI) values (32.8) and waist circumference divided by hip circumference values (male: 1.07, female: 0.91) were found in the chondropathic patient subgroup. The next aim was the mapping of the stiffness of the cartilage covering the healthy knee joint to provide an etalon for further measurements and new data to understand the biomechanics of the human knee joint. I managed to achieve this with cartilage stiffness measurements in 87 patients who underwent diagnostic and therapeutic arthroscopies at our department. 696 measurements were performed in total with the internationally accepted Finnish Artscan 1000 arthroscopic articular cartilage stiffness tester. I established that cartilage stiffness varies at different sites even in the intact knee joint. The stiffest cartilage was found on the lateral femoral condyle (5.2 N), and the cartilage covering the femur is always stiffer than the cartilage on the tibia or patella.

My further aim was to objectify—with a biomechanical marker— the degree of cartilage degeneration detected during arthroscopic operations. The stiffness of the articular cartilage could be described with objective data provided by the arthroscopic tester and expressed in N.

My fourth thesis was to prove the relationship between the *in vivo* measured cartilage stiffness and the histological stage of cartilage degeneration. I took 196 biopsies from the above-mentioned 87 patients. Histological analyses were performed at two different centres, at the University of Debrecen and the University of Bristol, UK. Prof. László Módis and Prof. Anthony Hollander were the leaders of the working groups. The stage of the histological degeneration of the biopsies was determined by the Mankin-Shapiro score system. A linear regression analysis was performed to find a correlation between cartilage stiffness and the Mankin-Shapiro scores. The high values of the coefficient of determination yielded an unambiguous, reciprocal correlation. The value of the coefficient of determination was $r^2=0.82$ for the lateral femoral condyle, while it was $r^2=0.79$ for the medial femoral condyle. The same parameter was found for the lateral tibial condyle, $r^2=0.75$, while $r^2=0.72$ for the medial tibial condyle. By proving the existence of the above reciprocal correlation, it became possible to indirectly observe cartilage degeneration during arthroscopies, through alterations in cartilage stiffness. The verification of the second and fourth theses enabled the early diagnosis of cartilage degenerations. This early diagnosis may contribute, through the

indication of preventive operations, to the prevention of osteoarthritis. This was my fifth goal with my Ph. D. work.

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- *Hangody L, Kish G, Szabó Zs, Kárpáti Z, Szerb I, Gáspár L, Módis L (2001) Mosaicplasty for the treatment of osteochondritis dissecans of the talus: two to seven year results in 36 patients. Foot Ankle Int 7: 552–558.*

PROGRAM 8/5.

CLINICAL AND EXPERIMENTAL TRANSPLANTATION

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Program overview

The Ph. D. School for Transplantation was founded in 2007. Transplantation represents an interdisciplinary area of medicine, which is a quite young discipline. It was only 50 years ago that the first successful operations were performed and the number of these was quite low at the beginning. The surgical technique has not changed much since then, but our knowledge of immunology and the evolution of intensive care changed the scenery enormously. The introduction of new immunosuppressive drugs and problems of the follow-up of the immunosuppressed patient represent a big everyday challenge. The background for these challenges is unimaginable without the lab work, the experimental and big clinical studies and the dialogue between basic science and clinical science. Our Ph. D. School is the answer for these question and gives possibility for the academically interested professionals.

Titles of research projects	*Supervisors*
Isotope diagnostics in transplanted patients	Gabriella Dabasi
Significance of renal transplantation from living donors in Hungary	Jenő Járay
Anatomical and chirurgical basis of partial liver transplantation from living donor	László Kóbori
Diagnostic method to study drugmetabolizing capacity in transplanted patients—the future of individual immunosuppression	László Kóbori
Tolerance after renal transplantation	Róbert Langer
Hepatitis C before and after liver transplantation	Balázs Nemes
Monitoring redox-homeostasis, graft function and therapeutic drug level in transplanted patients	Enikő Sárváry

Complications after renal transplantation with special attention to malignant diseases	Éva Toronyi
Connection between immunosuppression and oncogenesis—immunosuppression as an oncogenic factor	András Tóth
The concerns of organ transplant and the immunsuppressive oncology treatment (experimental and/or clinical research)	Gyula Végső

Ph. D. students		***Supervisors***
Mátyás Kiss	pt	László Kóbori
Gergely Zádori	ft	Balázs Nemes

Ph. D. candidates		***Supervisors***
Tamás Benkő	i	László Kóbori
Imre Fehérvári	i	László Kóbori

Ph. D. graduate		***Supervisors***
János Fazakas	i	László Kóbori

pt, part-time; ft, full-time; i, individual

Abstract of Ph. D. thesis successfully defended in 2008

JÁNOS FAZAKAS (2008)

The role of procalcitonin in the perioperative phase of liver transplantation

Supervisor: László Kóbori

Liver transplantation is an indispensable part of the the end-stage liver diseases therapy. Because of the surgical intervention and the reperfusion syndrome, a systematic inflammatory response occurs in every case. The molecular patterns produced by the injured cells, tissues or the splanchnic bacteria are recognised like external and internal offence signs during the inflammatory response. The parallel evolving of the acute phase reaction reason is to moderate the inflammatory response, which typically depend from the liver function, after liver transplantation. The excessive inflammatory response leads to tissue lesion and organ failure, which are associated by infectious complications in the case of unsuccessful treatment. The procalcitonin measuring can be applied to identify incompatible donors, recipients, to monitoring the early postoperative inflammatory complications and for differential diagnosis of late complications: rejection, sepsis. Usually the procalcitonin level of the multiorgan donors and compensated liver recipients is normal. The slightly higher serum levels are related with the cause and severity of the liver disease. In spite of this the repeated research for focal is recommended. The removal of the grafts preservation solution, is recommended with 5% albumin solution, because the application of own blood intensifies the inflammatory response, caused by the reperfusion. The serum level during the surgery starts to rising after reperfusion in the absence of sys-

temic infection and lasts until the second postoperative day. Frequently, the source of the rising procalcitonin levels after the reperfusion is the graft itself, the process of this is unexplained yet. The peaks after surgery indicate the development of non-specific complication with good sensitivity and specificity. In the early phase after the liver transplantation, the sensitivity and specificity of the procalcitonin threshold, used at the diagnosis of the sepsis is low-built, because of the severe organ failure, caused by the inflammation, which can by themselves sustain higher procalcitonin levels. From the second postoperative day, the repeated measurements, the procalcitonin level fluctuation parallel with the result of culture helps to differentiate the cause of the systemic inflammation. The decreasing kinetics of procalcitonin serum level give information about the moderation of the systemic inflammatory response, which is proportional with the classic procalcitonin half-life time after single ischemic reperfusion induction, rarely after multiple inductions is less than this and it indicates infectious complication. Infrequently, the procalcitonin serum levels decrease ultra rapidly; the background is an tubular injury, in the course of the patient lose procalcitonin by urine, in such circumstances the close monitoring of these patients is recommended.

- *Fazakas J, Gondos T, Varga M, Sárváry E, Horovitz P, Perner F (2003) Analysis of systemic and regional procalcitonin serum levels during liver transplantation. Transpl Int 16: 465–470.*
- *Fazakas J, Mándli T, Ther G, Árkosy M, Pap S, Füle B, Németh E, Tóth Sz, Járay J (2006) Evaluation of liver function for hepatic resection. Transplant Proc 38: 798–800.*
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