Animal models of complement activation-related hypersensitivity

Dr. László Dézsi

Nanomedicine Research and Education Center, Institute of Translational Medicine, Semmelweis University, Budapest, Hungary

Biosafety assays for the diagnosis and prediction of drug-related complement pathology

Part I.

Development of CARPA models

The challenge: CARPA

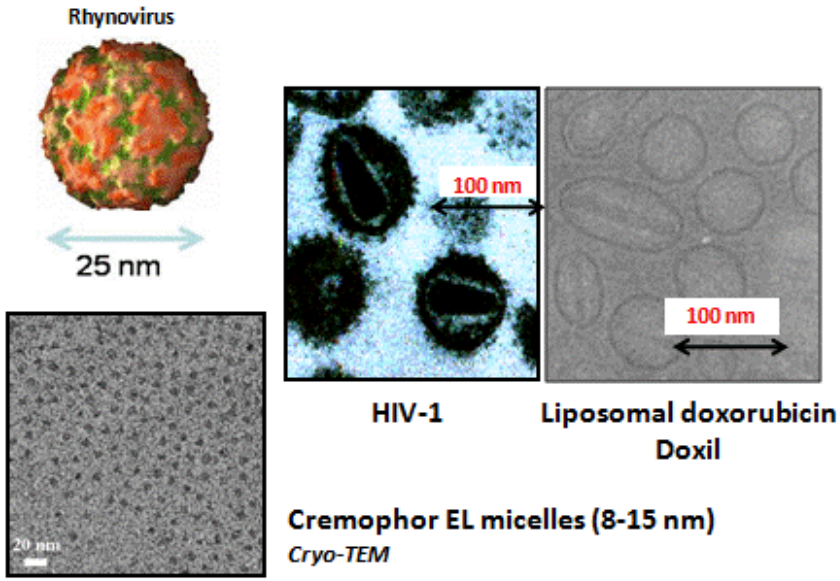
Complement Activation-Related Pseudoallergy

subcategory of Coombs & Gell's type I hypersensitivity reactions

Acute adverse effect of i.v. drugs manifested in an „infusion reaction"

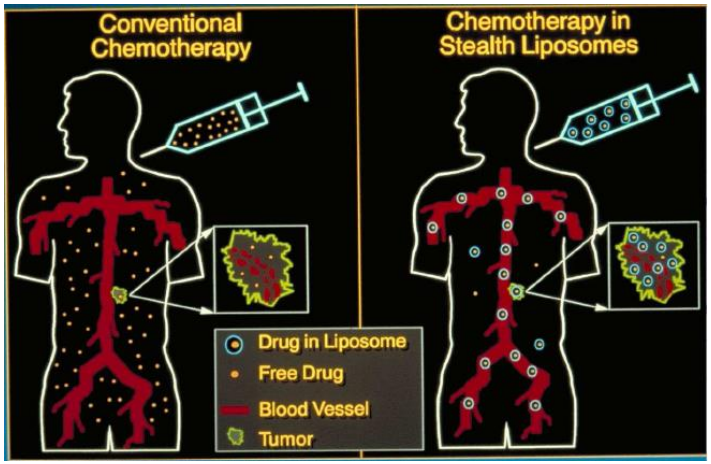
(anaphylaxis/anaphylactoid reaction)

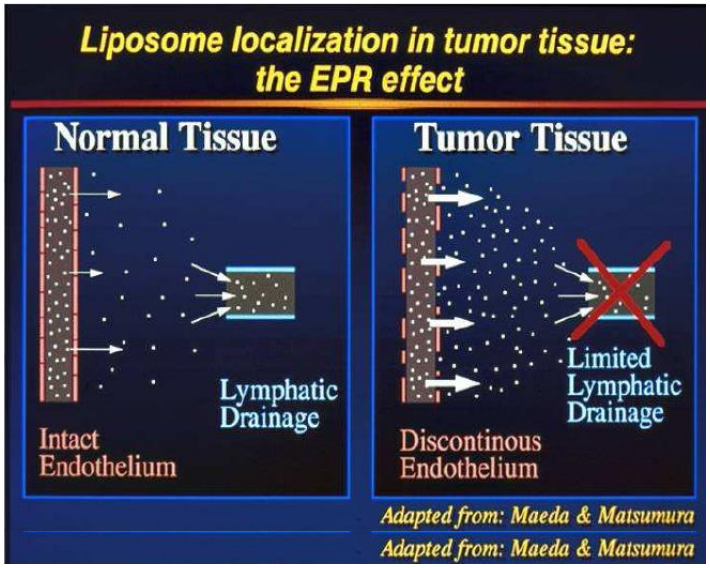
Size similarity between viruses and some (nano)medicines



Unexpected side-effects

Classical side-effects of cytostatic drugs disappeared





New side-effects revealed

Hand-foot syndrome

CARPA !!

Major symptoms of CARPA in man and in animals

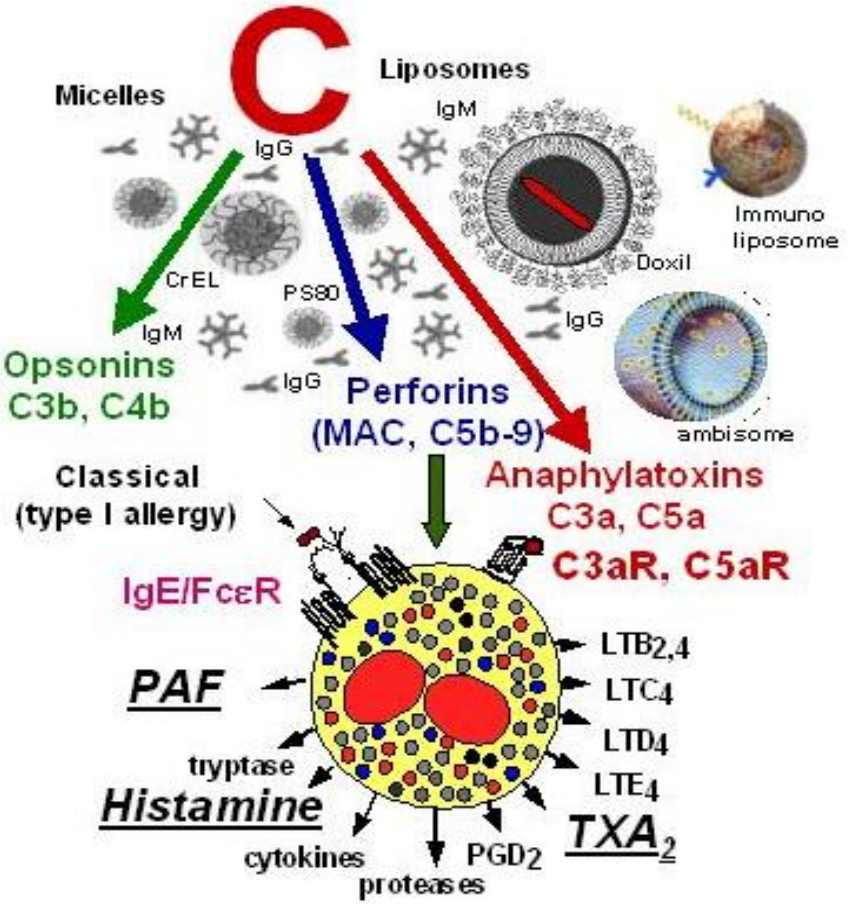
Organ System	Human	Animals
Hemodynamic	hypotension	pulmonary hypertension/systemic hypotension
Cardiopulmonary	dyspnea tachy-bradycardia, chest pain, back pain	tachy-bradycardia/ arrhythmia, Ischemic ECG
Skin	flushing, rash	
Blood	thrombopenia, leukopenia/leukocytosis	
Equivalence of reactogenic dose	**Hypersensitive humans and pigs**	

Mechanisms of CARPA

C activation

Release of primary mediators

Release of Secondary mediators



reactogenic nanoparticles

mast cells basophils leukocytes

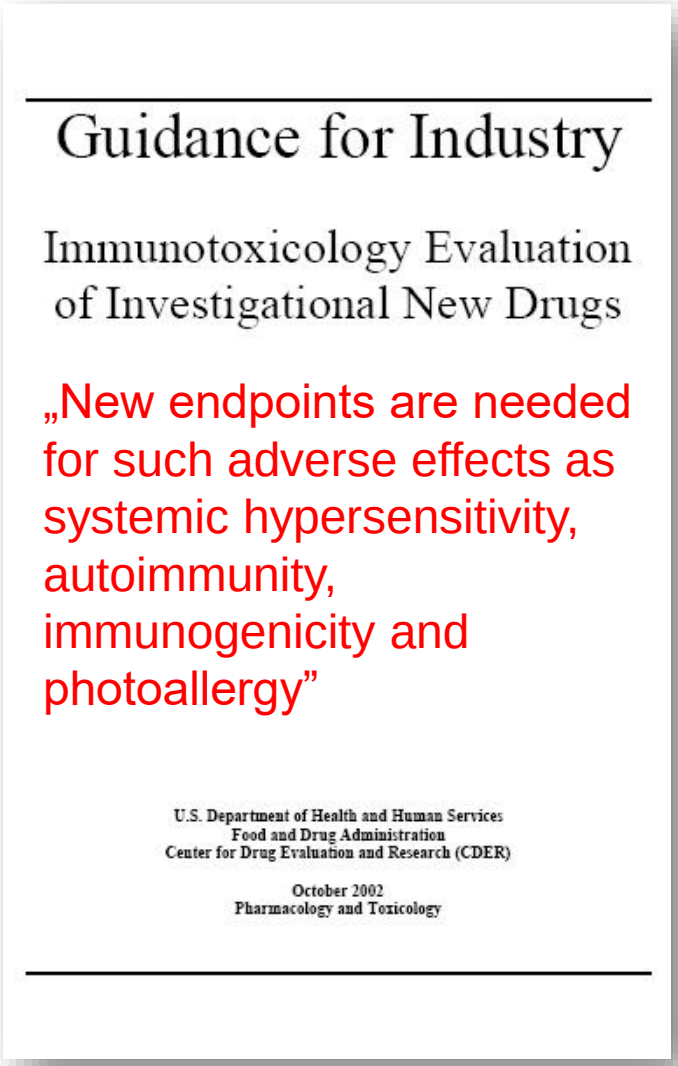
Drugs causing pseudoallergy in man

Liposomal drugs	Micelle-solubilized drugs	Antibodies	PEGylated enzymes	Radio-contrast media	enzymes/ proteins/ peptides	Misc.
Doxil, Caelyx	Fasturec	Avastin	Adagen	Diatrizoate	Avonex	Cancidas
Myocet	Elitec	Campath	Neulasta	Iodixanol	Actimmune	Copaxone
Abelcet	Taxol	Erbitux	Oncaspar, Pegaspargas	Iohexol	Abbokinase	Orencia
AmBisome	Cyclosporine	Herceptin		Iopamidol	Aldurazyme	Eloxatin
Amphotec	Vumon	Mylotarg		Iopromide	Activase	Salicilates
Amphocyl	Etoposide	Vectibix		Iothalamate	Zevalin	ACE inhibitors
DaunoXome	Taxotere	Rituxan		Ioversol	Neupogen	AR blockers
Visudyne		Remicade		Ioxaglate	Neulasta	aspirin
		Xolair		Ioxilan	Fasturtec	cyclofloxacin
		Infliximab		SonoVue	Plenaxis	corticosteroids
		Muronomab		Magnevist	ACH	opiates
				Metrizamide	urokinase	vancomycin
				Iodipamide	protamine	intralipid

Nanomedicines

(Frequency: 2-40 %)

Regulatory response


Guidance for Industry

Immunotoxicology Evaluation of Investigational New Drugs

„New endpoints are needed for such adverse effects as systemic hypersensitivity, autoimmunity, immunogenicity and photoallergy”

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2002
Pharmacology and Toxicology

2011. EMA guidance “Data Requirements for Intravenous Liposomal Products Developed with Reference to an Innovator Liposomal Product”

…. “specific comment on hypersensitivity on infusion”

.

Regulatory response

Another challenge…

Update on EMA's immuntoxicity regulation

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 February 2013
EMA/CHMP/806058/2009/Rev. 02
Committee for Human Medicinal Products (CHMP)

Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudoallergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event.

What model to be used?

Journal of Liposome Research, 17:107–117, 2007

ISSN: 0898-2104 print / 1532-2394 online
DOI: 10.1080/08982100701375118

informa
healthcare

Animal Models of Complement-Mediated Hypersensitivity Reactions to Liposomes and Other Lipid-Based Nanoparticles

JÁNOS SZEBENI,[1,2,3] CARL R. ALVING,[2]
LÁSZLÓ ROSIVALL,[3] ROLF BÜNGER,[4] LAJOS BARANYI,[5]
PÉTER BEDÖCS,[6] MIKLÓS TÓTH,[6] AND
YEZHECKEL BARENHOLZ[7]

Table 1
Agents and symptoms of hypersensitivity reactions to liposomes and other lipid particles in animals

Liposome/lipid complex	Animals	Biological and laboratory changes	References
MLV, LEH	Pigs	Hypotension or hypertension, pulmonary hypertension, cardiac arrhythmia, decreased cardiac output, flushing or rash, cardiac anaphylaxis, shock, death	Szebeni et al., 1999, 2000
Doxil			Szebeni et al., 2000, 2006
MLV, LEH	Rats	Hypo- or hypertension, tachycardia, tachypnea, thrombocytopenia, hemoconcentration, elevation of plasma TXB_2. Hypotension, anaphylactic shock with multiorgan failure	Rabinovici et al., 1989, 1990, 1992
MLV, LEH			Szebeni et al., 1994
"High-Chol" MLV			Baranyi et al., 2003
Doxil	Dogs	Hypotension, leukopenia with subsequent leukocytosis, thrombocytopenia, vegetative neural activation, histamine release	unpublished
Taxol			Poirier et al., 2004
CrEL			Lorenz et al., 1977

MLV, multilamellar vesicles; LEH, liposome encapsulated hemoglobene; CrEL, cremophos EL; TXB_2, thromboxane B_2.

Our goals

- To utilize well known porcine CARPA model to investigate biosafety of novel nanomedicines
- To develop new rodent (rat and mouse) CARPA models
- To compare CARPA models using appropriate test agents

Zymosan

direct complement activator

Doxil

the first nanomedicine

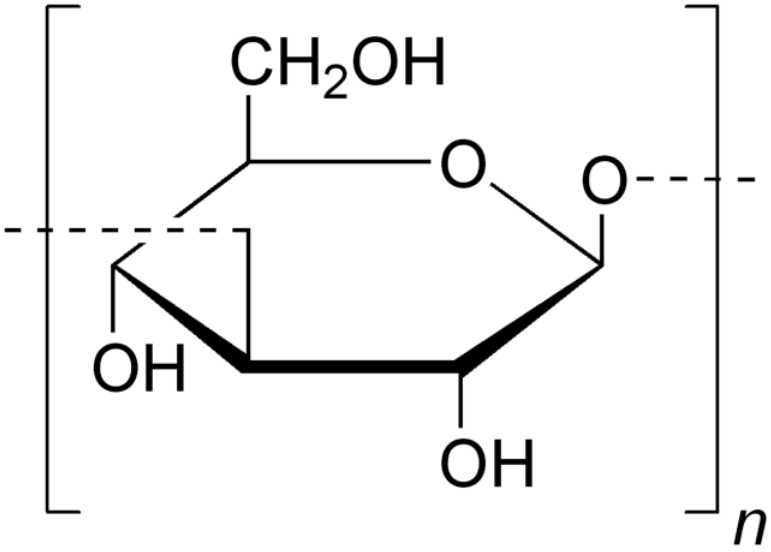
AmBisome, Abelcet

liposomal amphotericin-B

- CARPA inducers in human treatments
- strong C activator liposomes in in vitro and in vivo

Zymosan

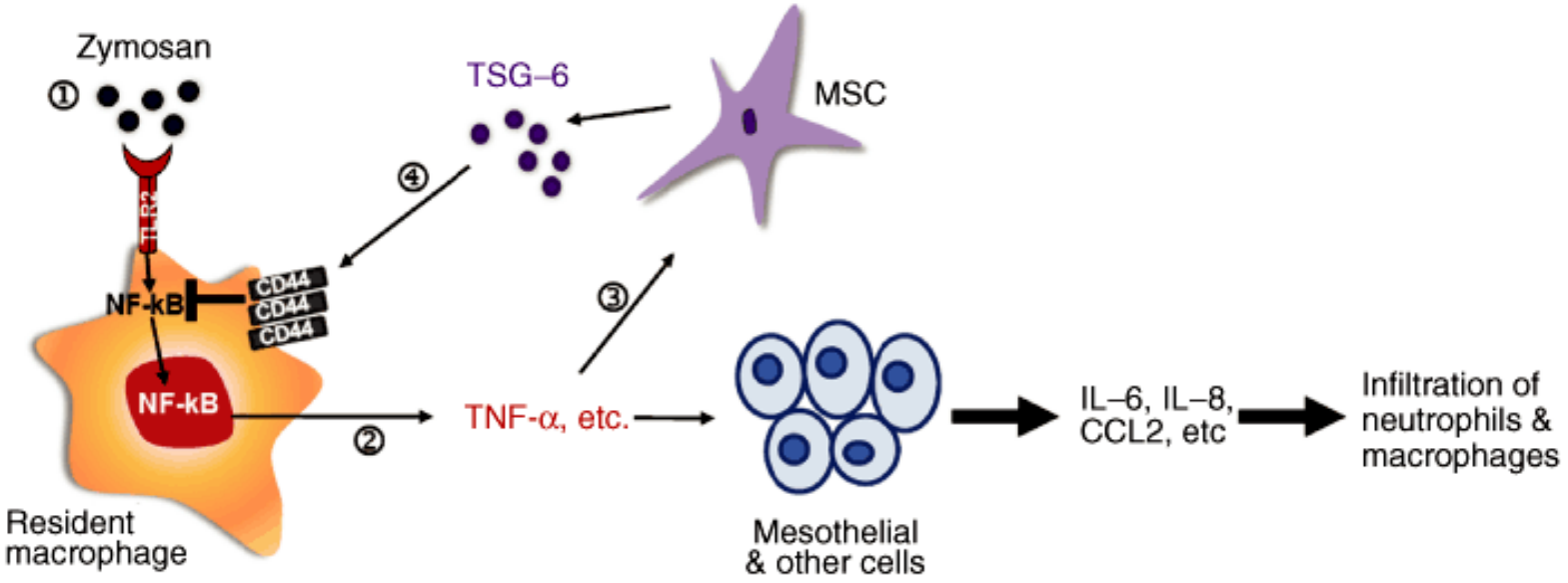
- **Zymosan is a glucan with repeating glucose units connected by β-1,3-glycosidic linkages**
- **Zymosan is a ligand found on the surface of fungi like yeast**



β-1,3

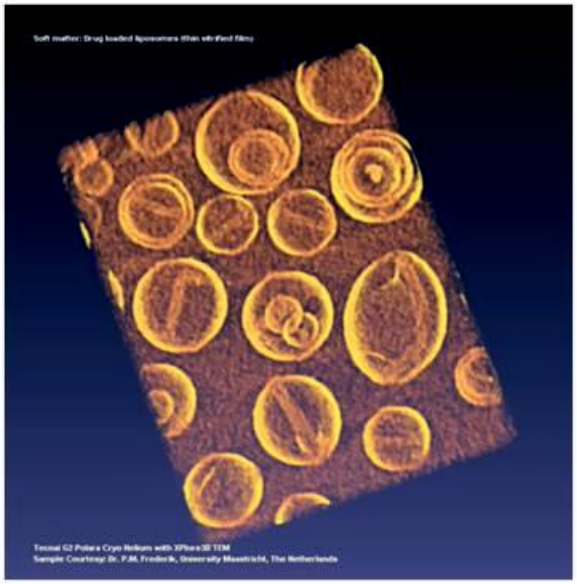
Zymosan-activated nuclear factor-κB (NF-κB) signaling in resident macrophages via Toll-like receptor

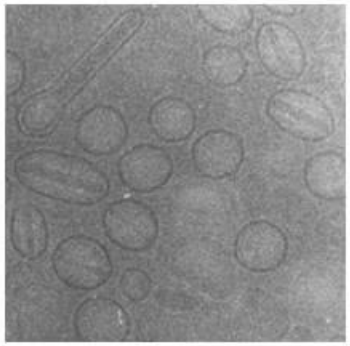
Zymosan binds to TLR2

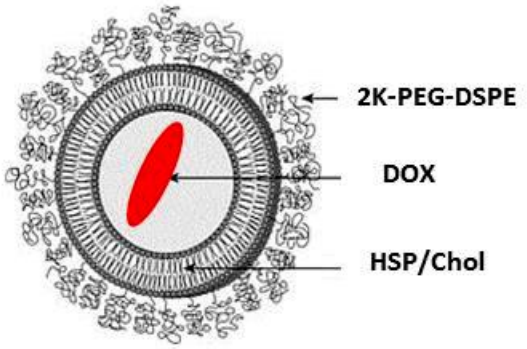


Prockop and Oh, *Molecular Therapy* (2012); **20** 1, 14–20.

Doxil (liposomal doxorubicin)



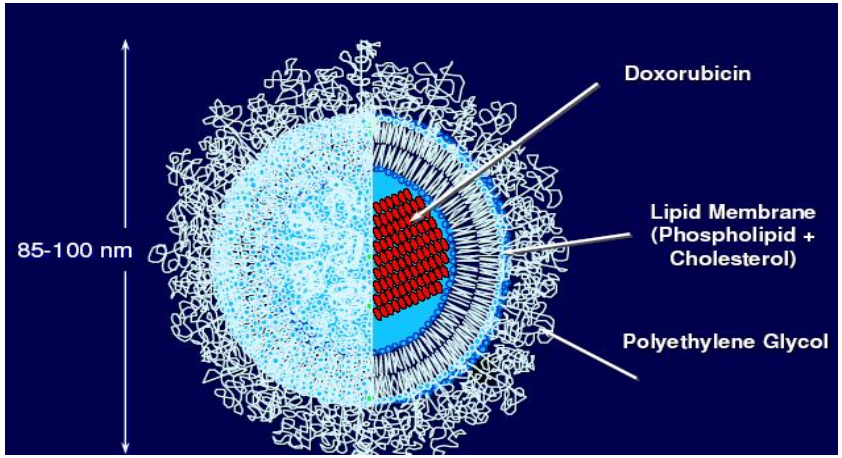




„**WARNING: Acute infusion-related reactions, sometimes reversible upon terminating or slowing infusion, occurred in up to 10% of patients. Serious and sometimes fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications/emergency equipment to treat such reactions should be available for immediate use ...**"

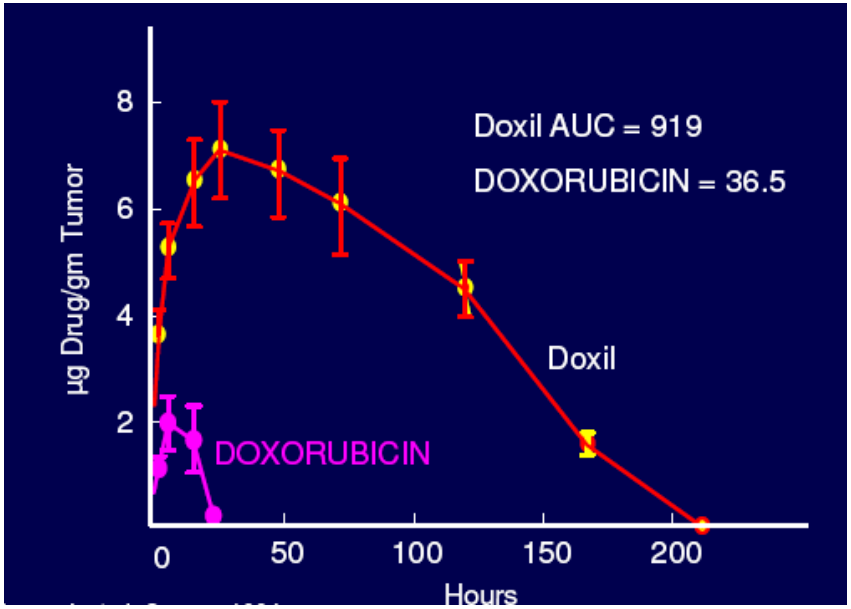
Source: www.doxil .com

The advantages of using Doxil

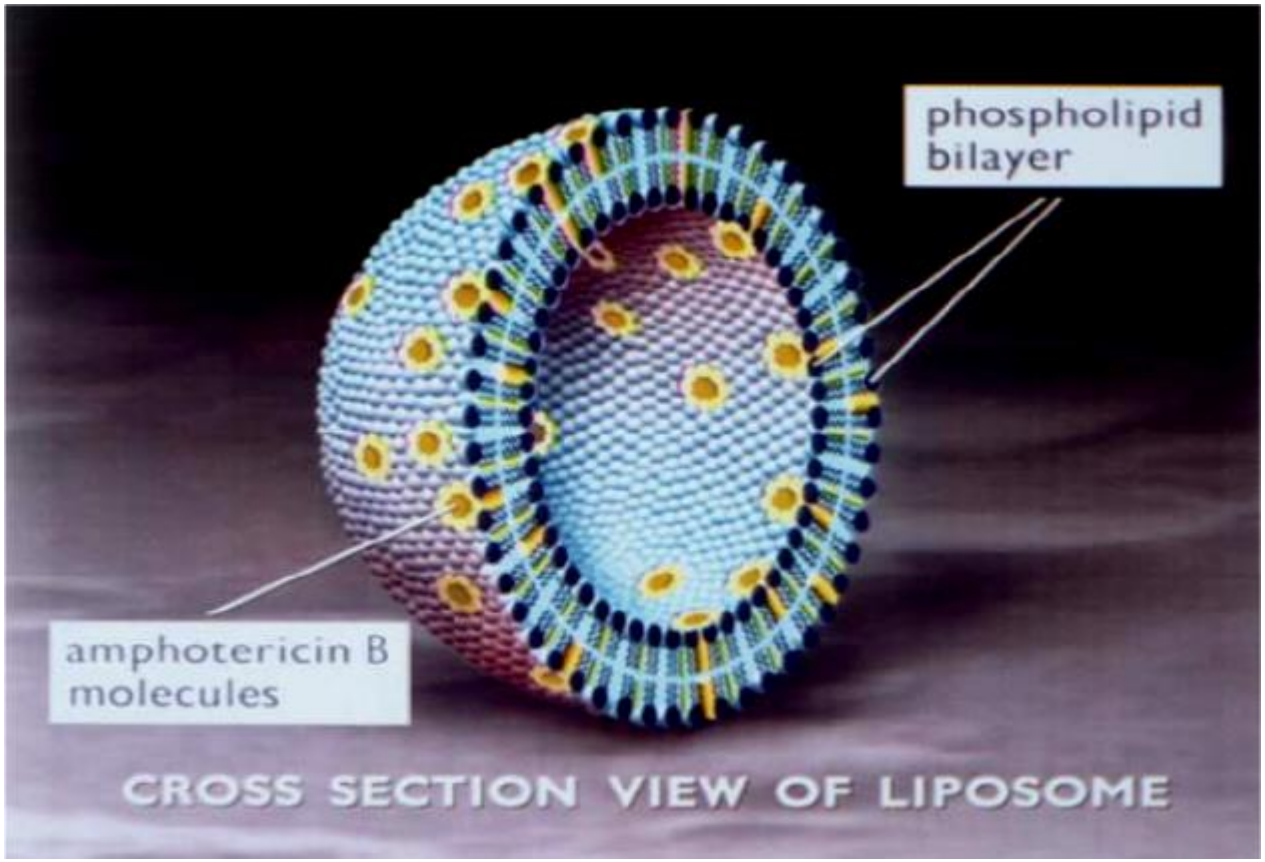


Structure

Activity



AmBisome®
(amphotericin B) liposome for injection

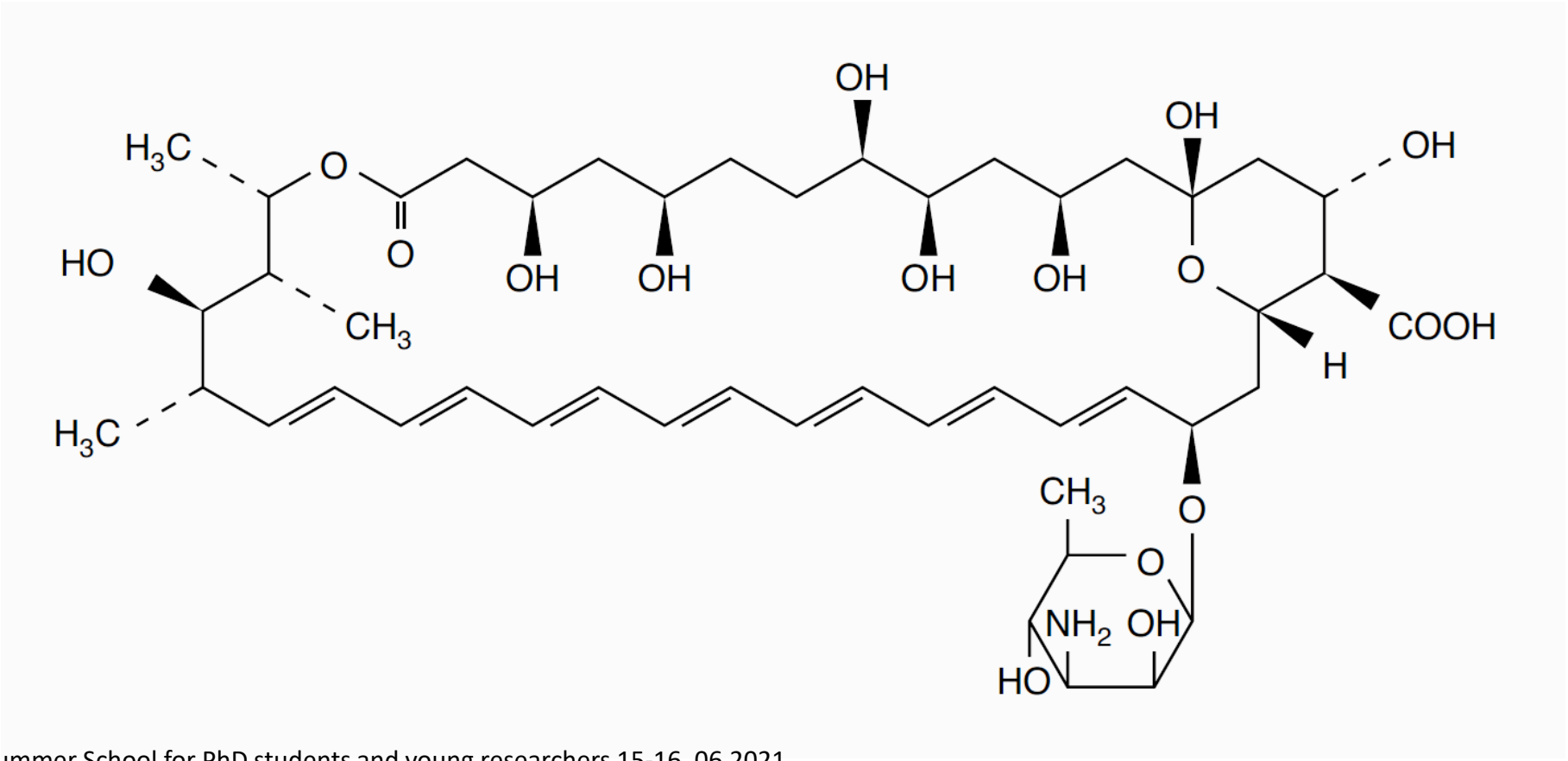


AmBisome for Injection is a sterile, non-pyrogenic lyophilized product for intravenous infusion. Each vial contains 50 mg of amphotericin B, USP, intercalated into a liposomal membrane consisting of approximately 213 mg hydrogenated soy phosphatidylcholine; 52 mg cholesterol, NF; 84 mg distearoylphosphatidylglycerol; 0.64 mg alpha tocopherol, USP; together with 900 mg sucrose, NF; and 27 mg disodium succinate hexahydrate as buffer. Following reconstitution with Sterile Water for Injection, USP, the resulting pH of the suspension is between 5-6.

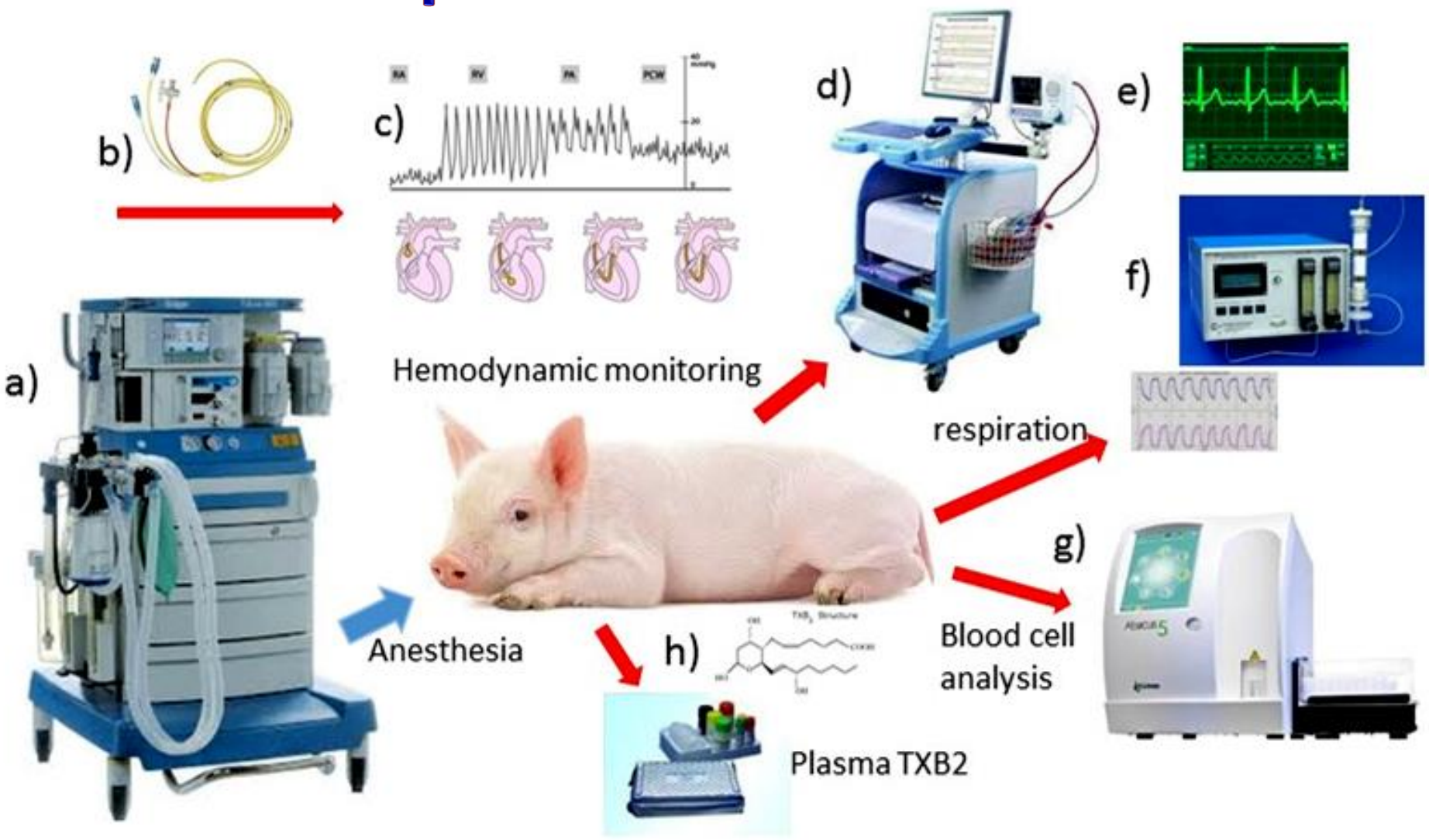
ABELCET®

(Amphotericin B Lipid Complex Injection)

ABELCET® is a sterile, pyrogen-free suspension for intravenous infusion. ABELCET® consists of amphotericin B complexed with two phospholipids in a 1:1 drug-to-lipid molar ratio. The two phospholipids, L-α-dimyristoylphosphatidylcholine (DMPC) and L-α-dimyristoylphosphatidylglycerol (DMPG), are presentin a 7:3 molar ratio. ABELCET® is yellow and opaque in appearance, with a pH of 5 - 7.

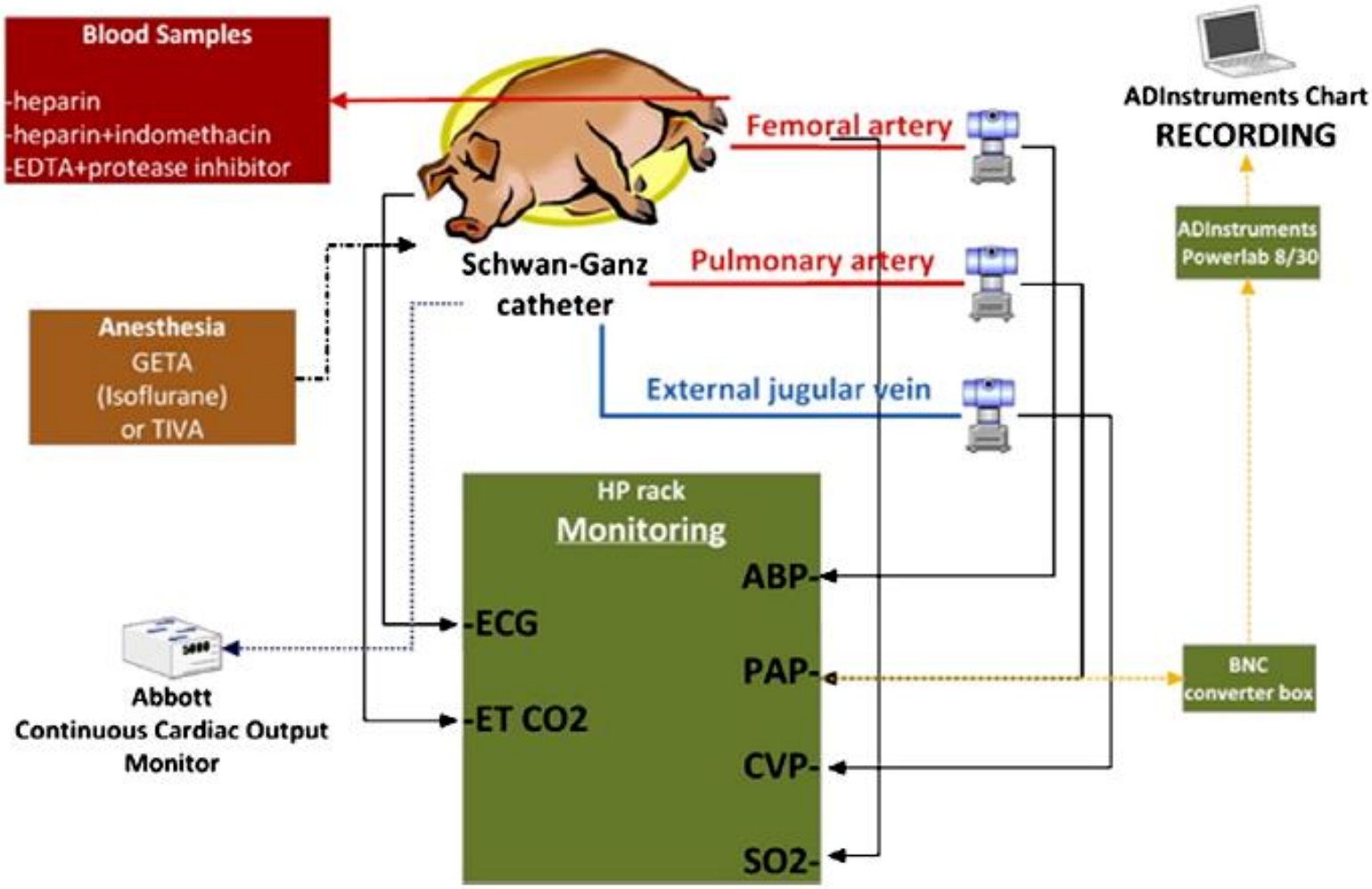


The porcine CARPA model



Szebeni J et al., A porcine model of complement-mediated infusion reaction to drug carrier nanosystems and other medicines ***Adv Drug Deliv Rev. 2012;64:1406-1416***

Flowchart of porcine CARPA model



Manifestations of porcine CARPA

Hemodynamic alterations

- rise of PAP
- rise or decline of SAP
- declince of CO and pCO_2

Cardiac abnormalities

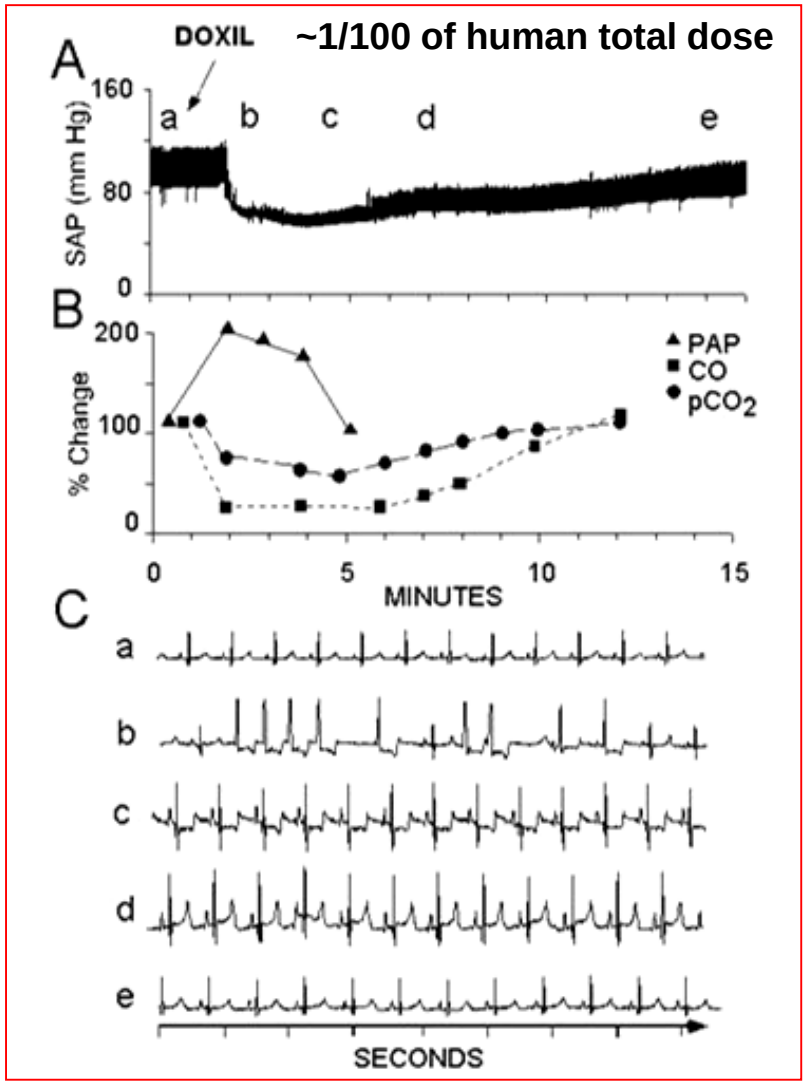
- tachycardia, bradycardia, arrhythmias
- ventricular fibrillation, arrest

Skin reaction

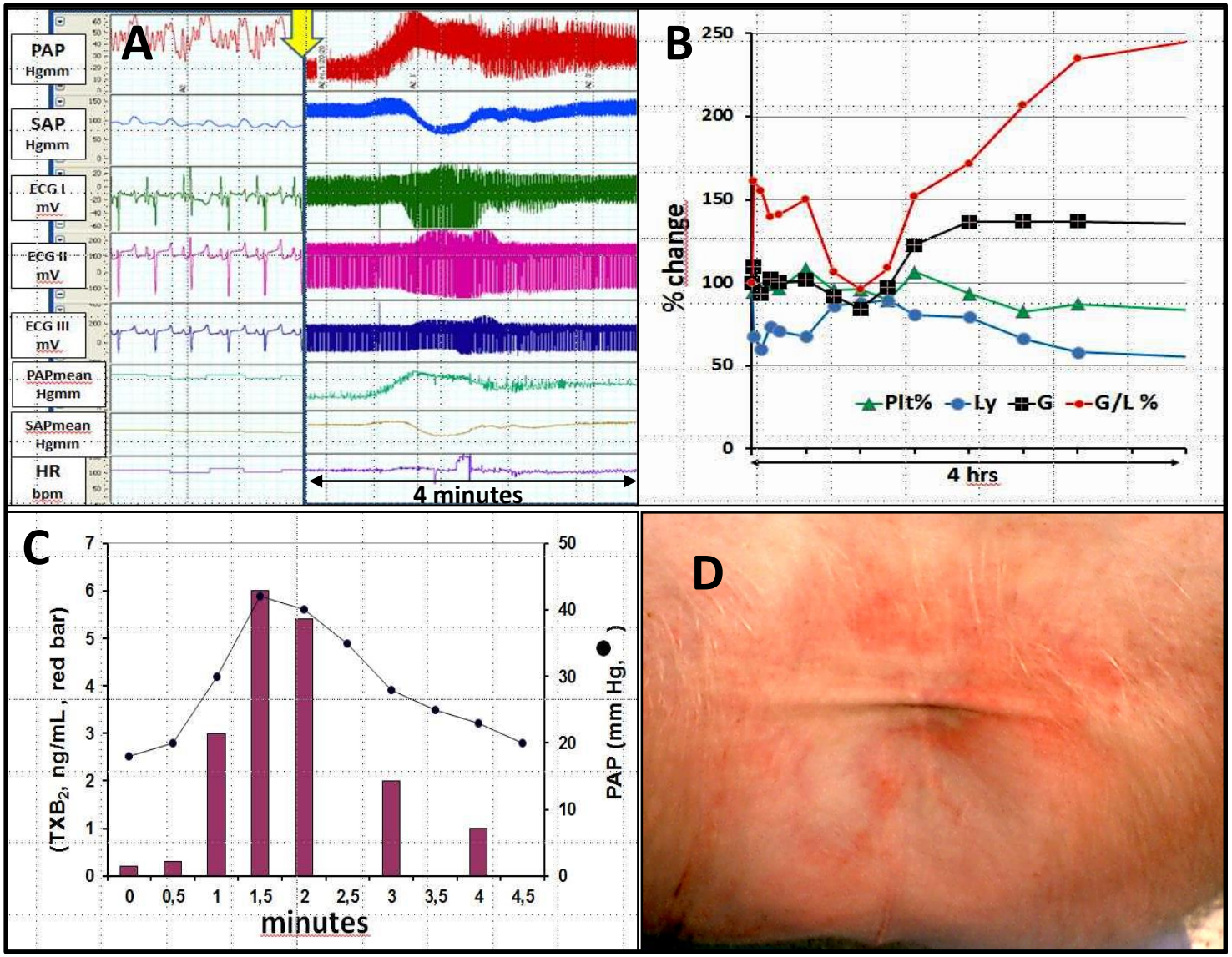
- erythema,
- rash

Blood abnormalities

- Leukocytosis
- leukopenia
- thrombocytosis
- thrombopenia

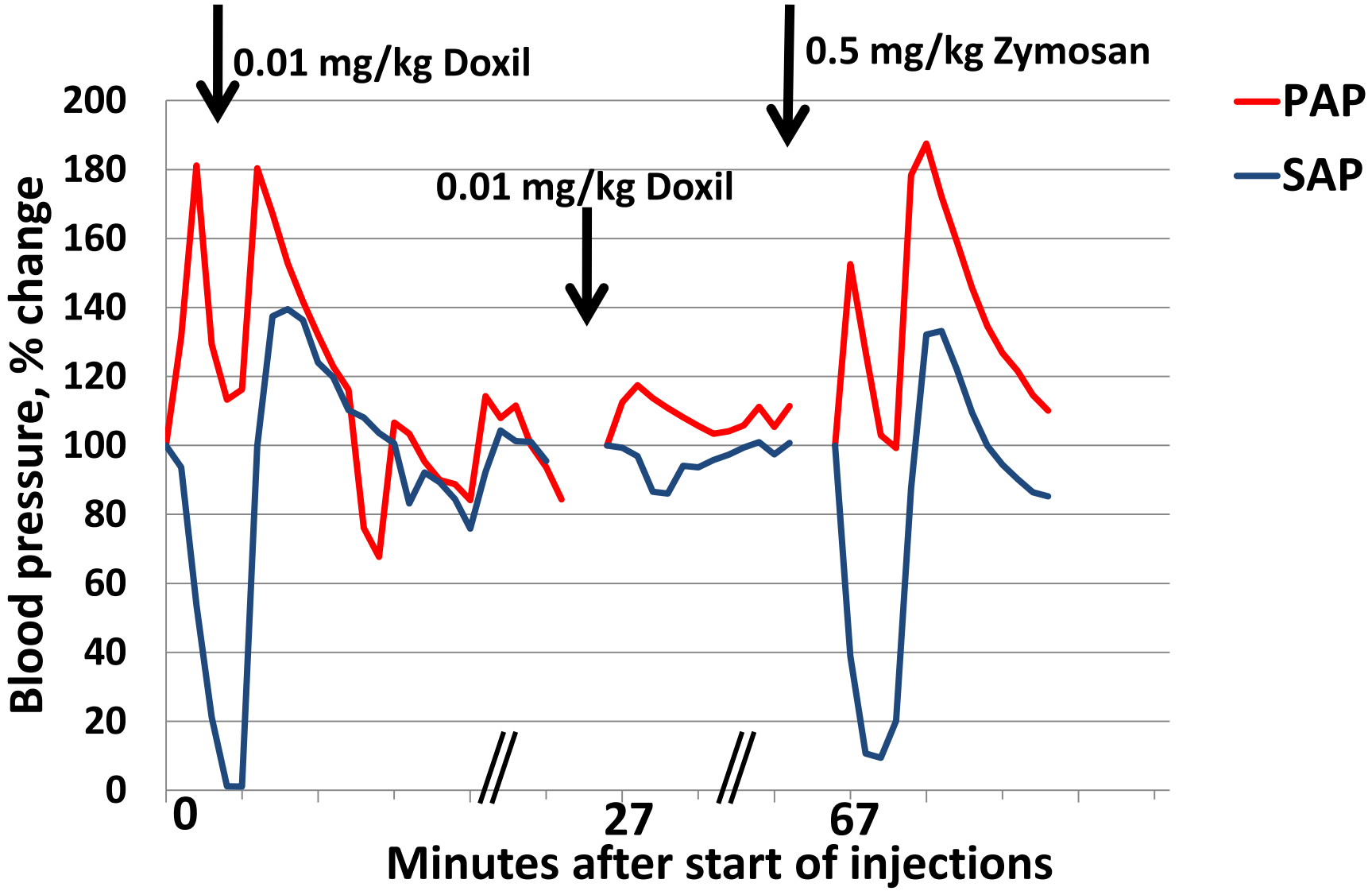


Reactions to liposomes in porcine CARPA



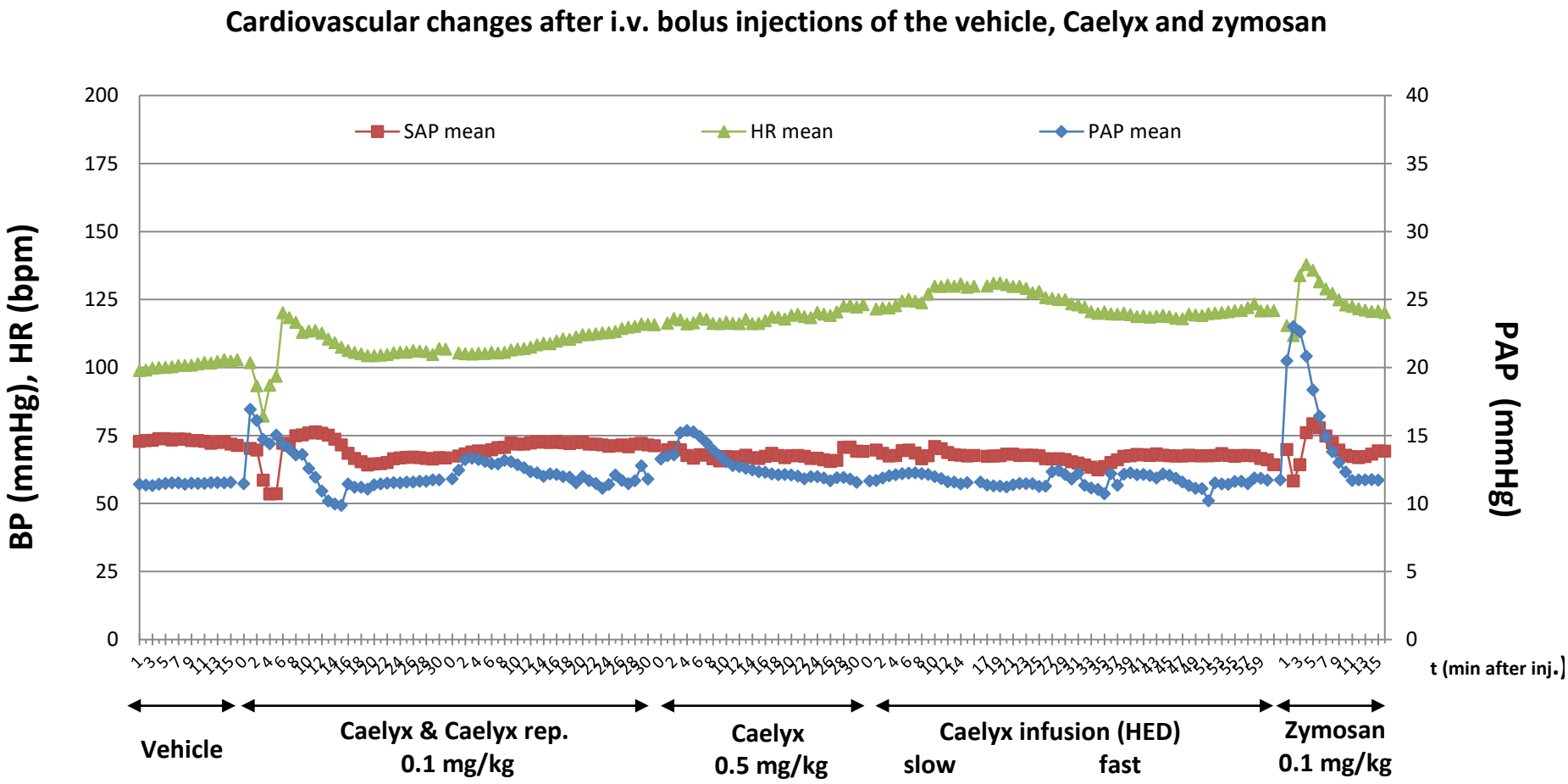
Urbanics et al. 2013

Doxil causes tolerance via tachyphylaxis



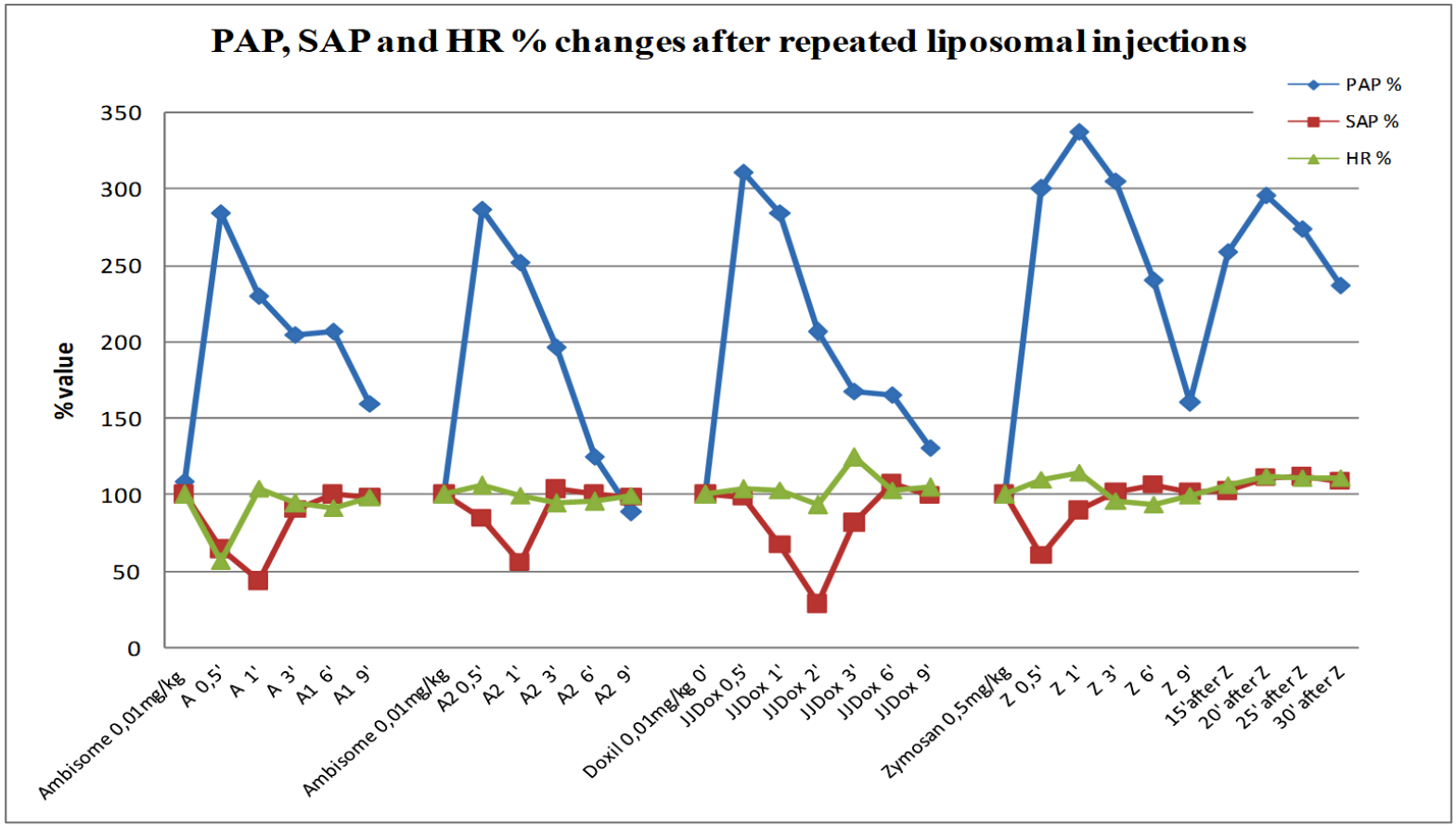
Szebeni et al. 2015

Doxil tolerance of injections and infusion



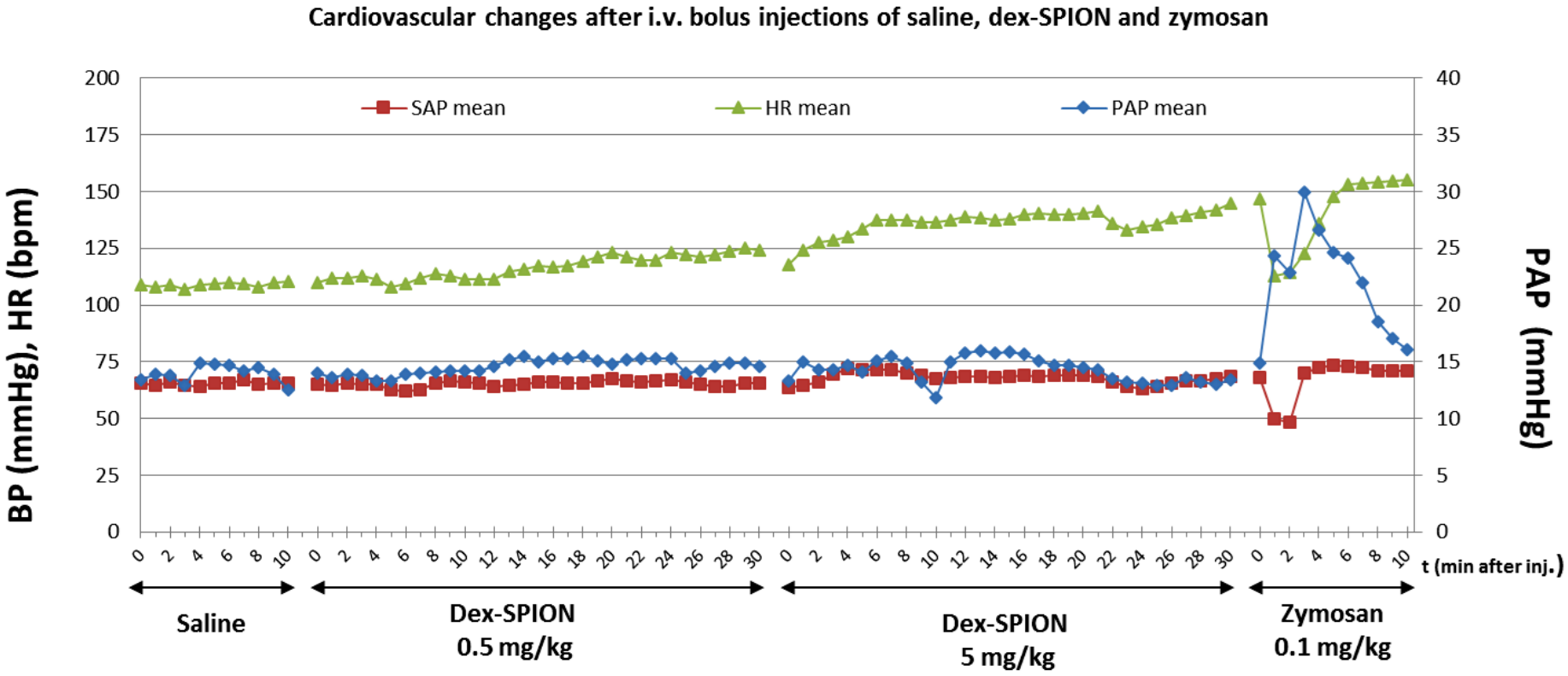
Dézsi et al. 2016

AmBisome does not cause tolerance



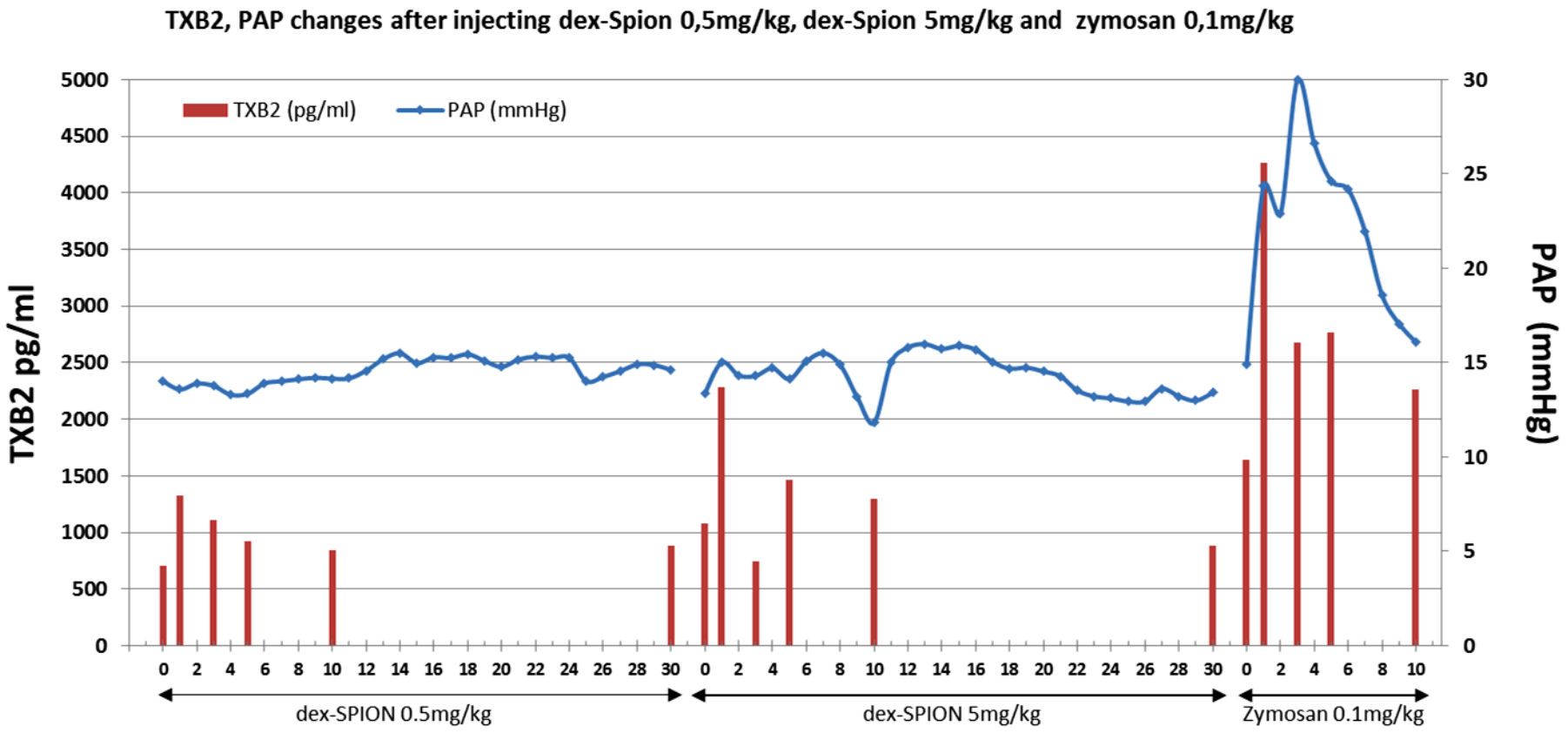
Urbanics et al. 2013

Dex-SPION does not cause CARPA



Dézsi et al. 2016

Dex-SPION does not cause CARPA



Dézsi et al. 2016

Rat CARPA model

Procedures

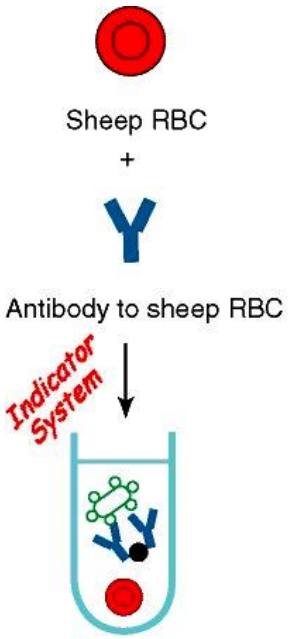
- Male Wistar rats (340-480 g) are anesthetized with thiobutabarbital (Inactin, 120 mg/kg i.p.)
- Left femoral a. & v., left carotid a., and right jugular v. cannulated
- Systolic, diastolic & mean blood pressure and heart rate are measured (ADInstruments, Experimetria)
- Test agents injected i.v.
- Blood samples collected from the carotid artery at:

  time 0 & 1 - 3 - 5 - 10 - 30 min after injection
- Blood cells counted by hematology analyzer (Abacus)

Assays

- Total complement activation by hemolytic complement assay (CH50/CHA)
- Plasma TXB2 measured by ELISA (Cayman)
- C3 measured after conversion to C5b9 using Pan-C3 kit (TECO-medical)

Methods to determine complement activation

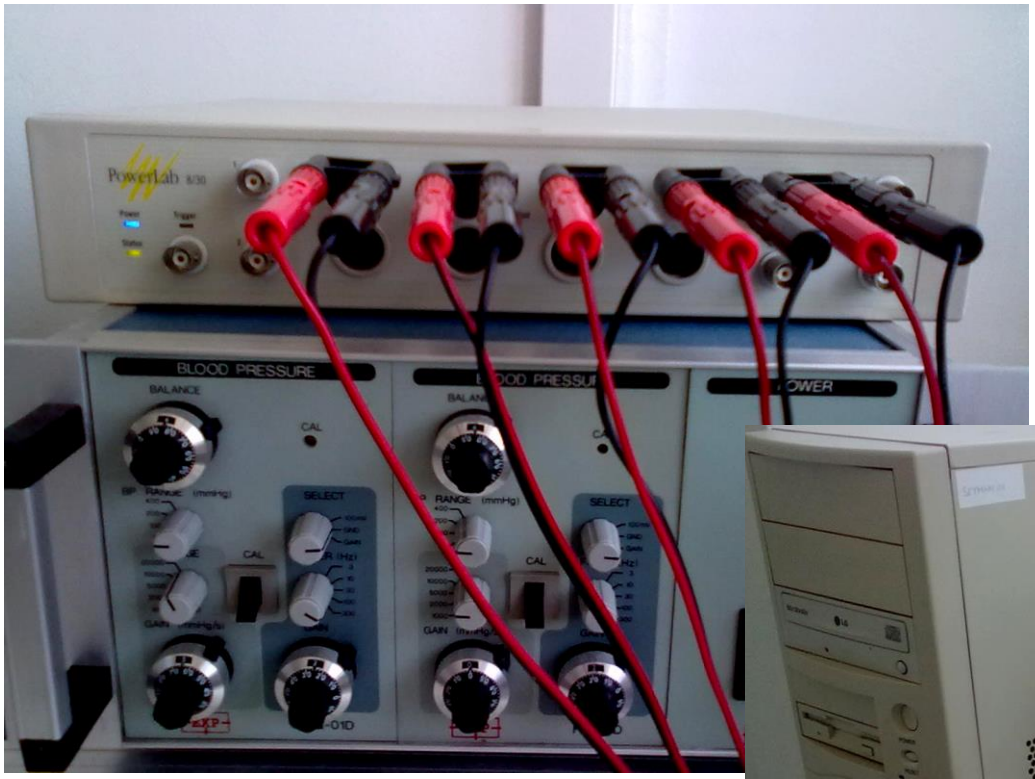


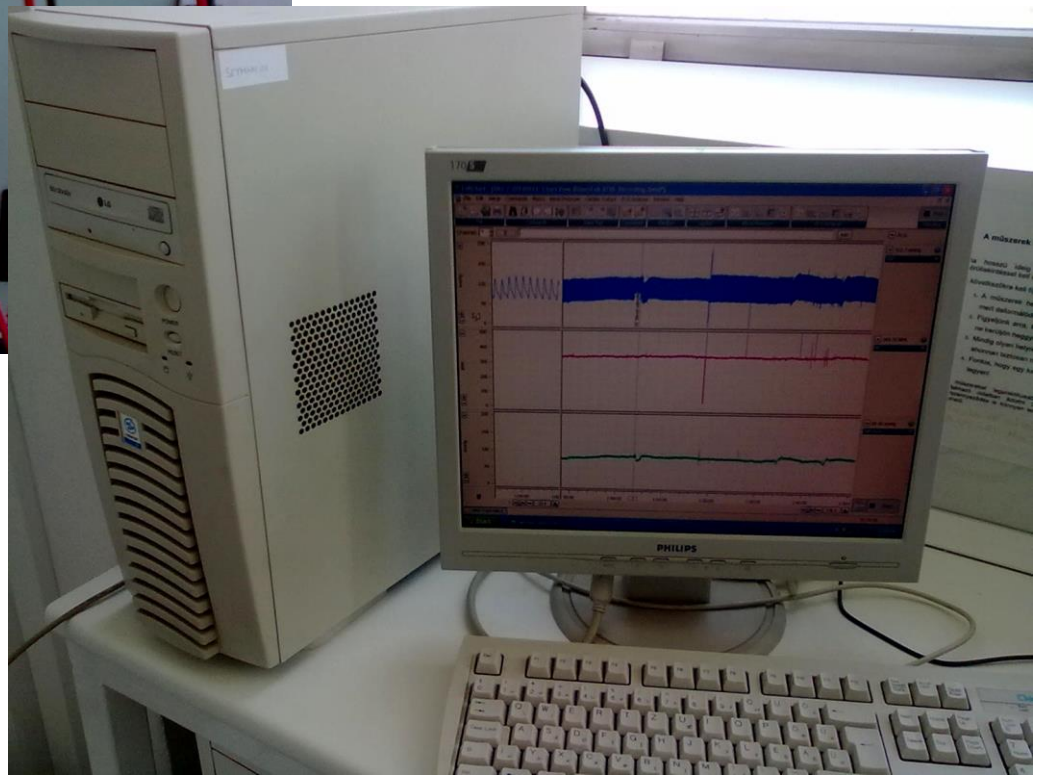
CH50/CHA

Pan-C3 reagent kit

Surgical procedures

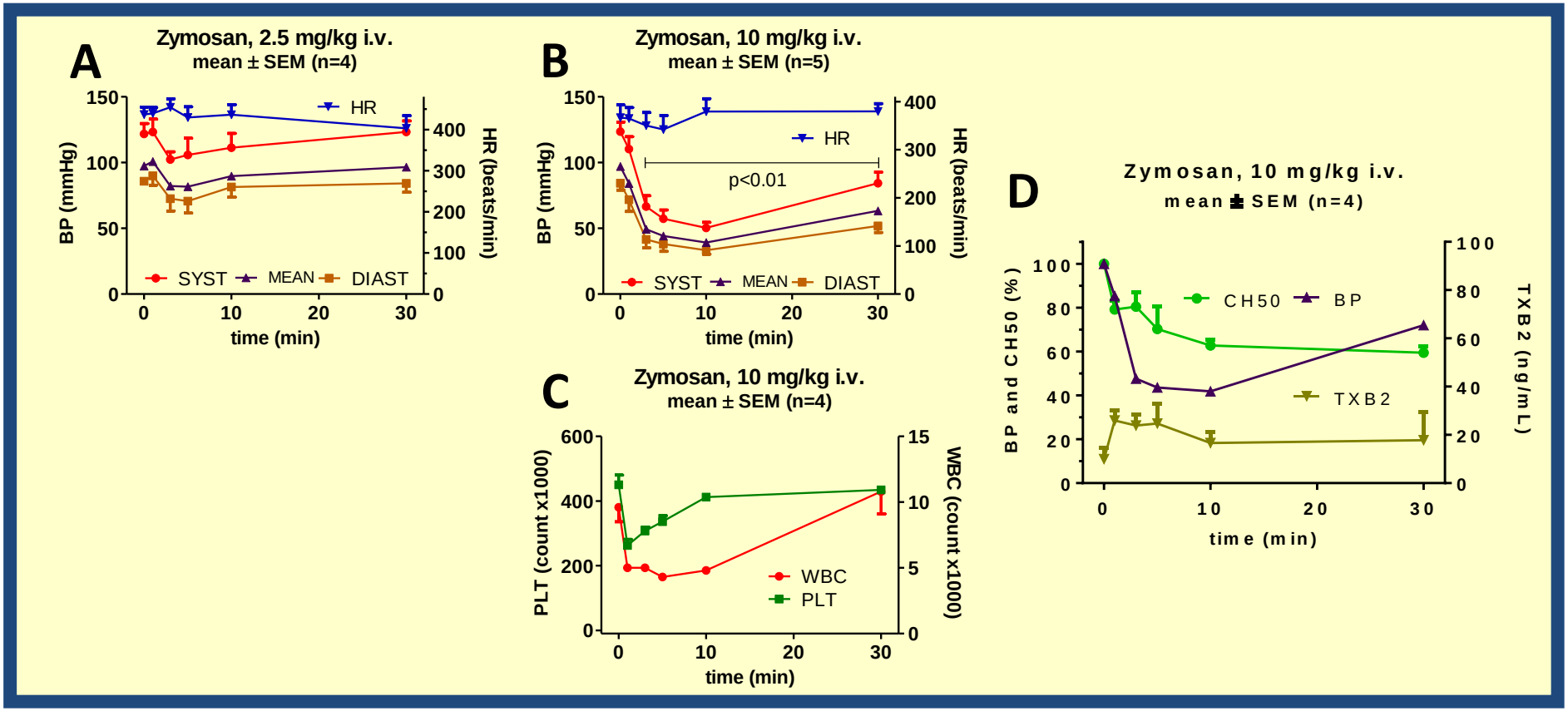
Experimental arrangement





Early data

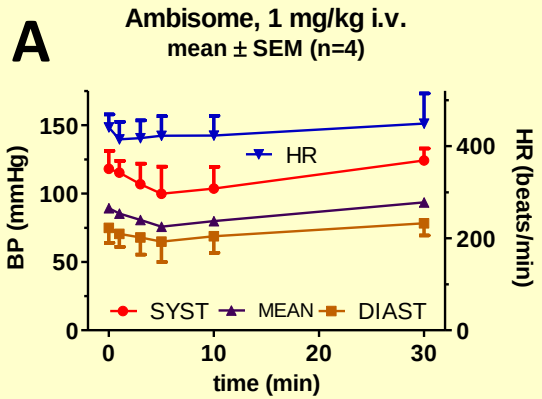
Changes in hemodynamic parameters following zymosan i.v. in rats

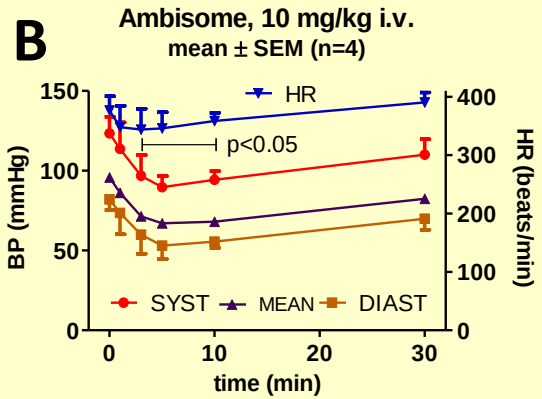


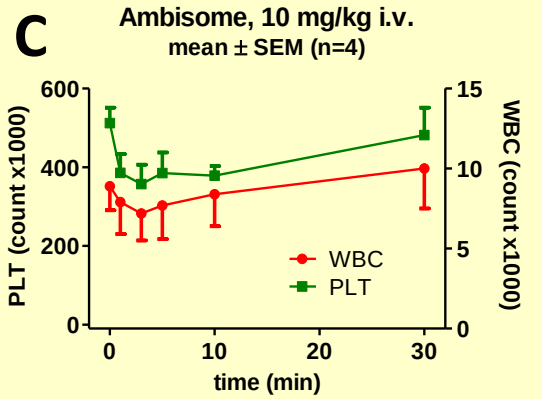
Dézsi et al. CLINAM 2013

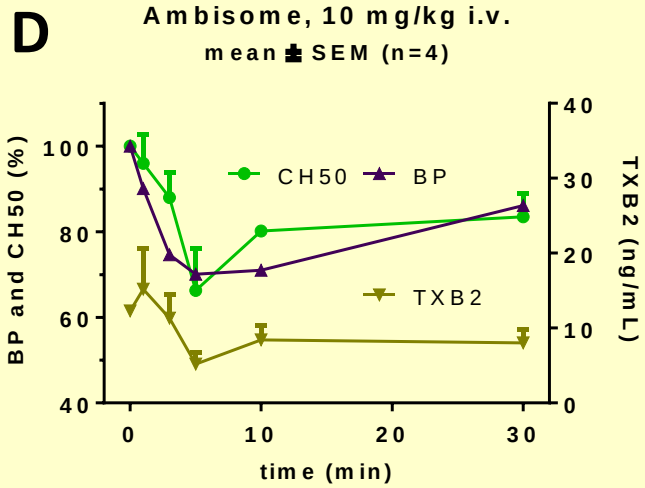
Early data

Changes in hemodynamic parameters following AmBisome i.v. in rats



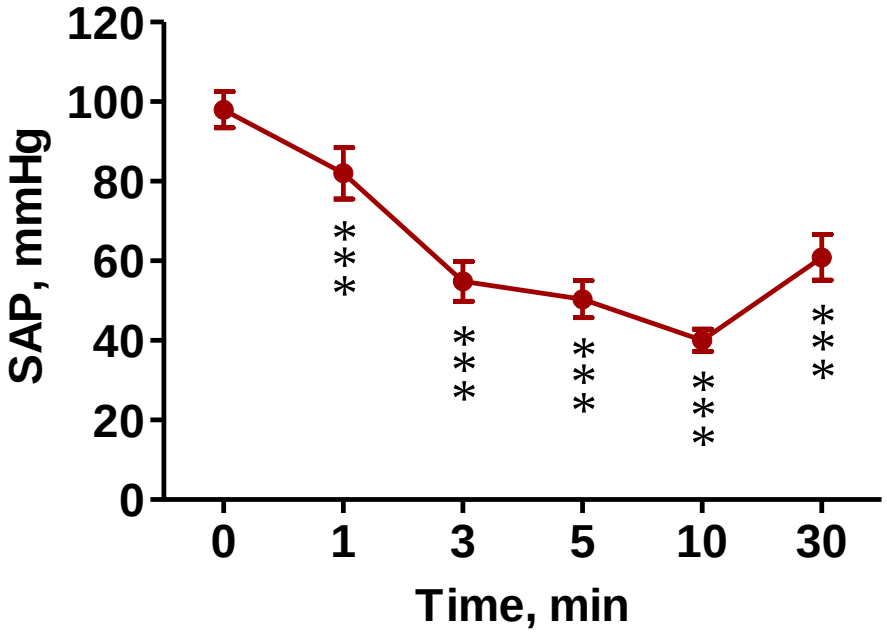


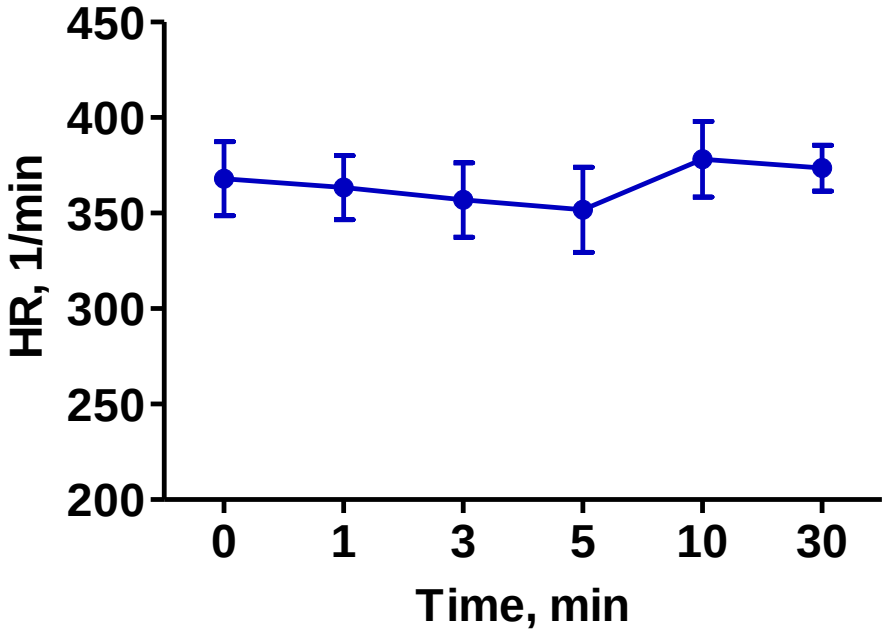




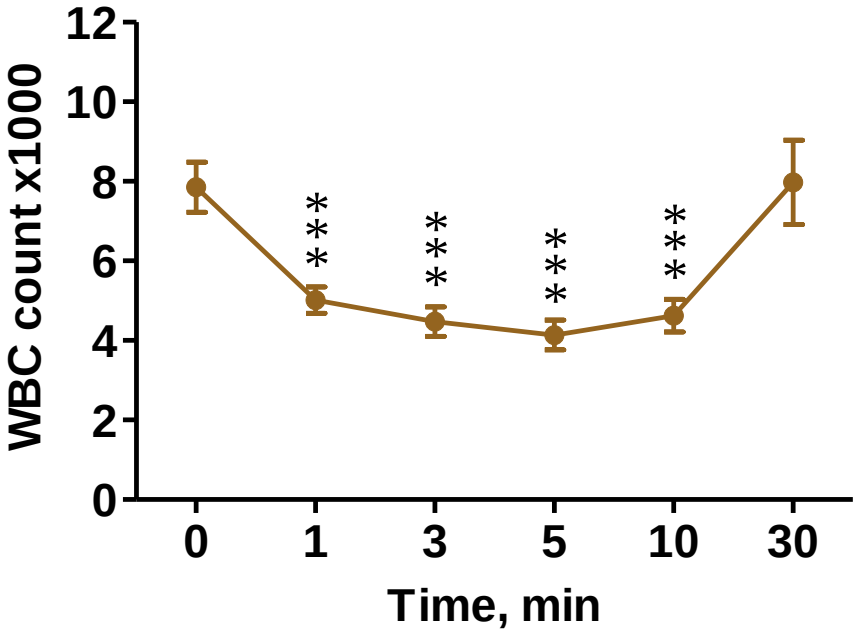
Dézsi et al. CLINAM 2013

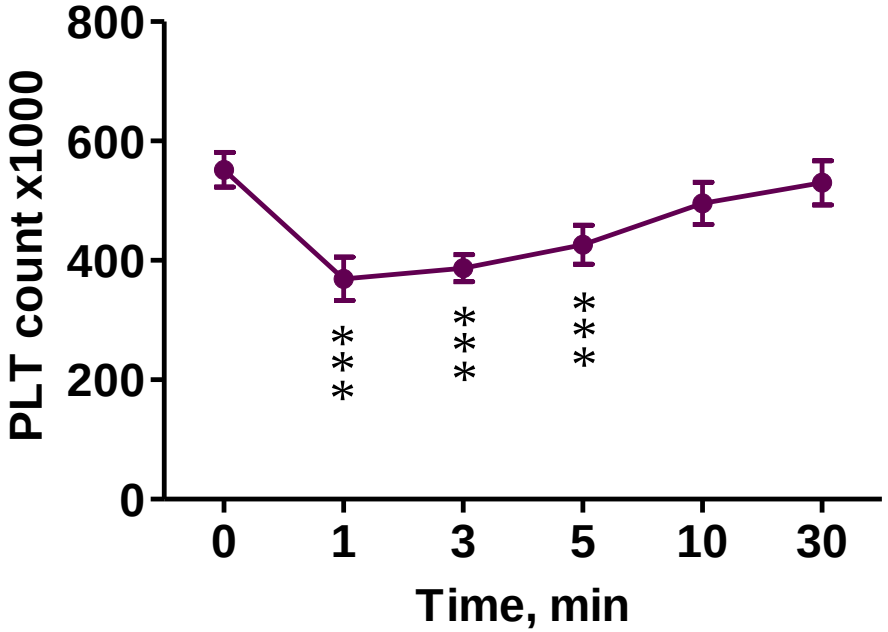
Effects of zymosan (10 mg/kg i.v.) on hemodynamic parameters in rats



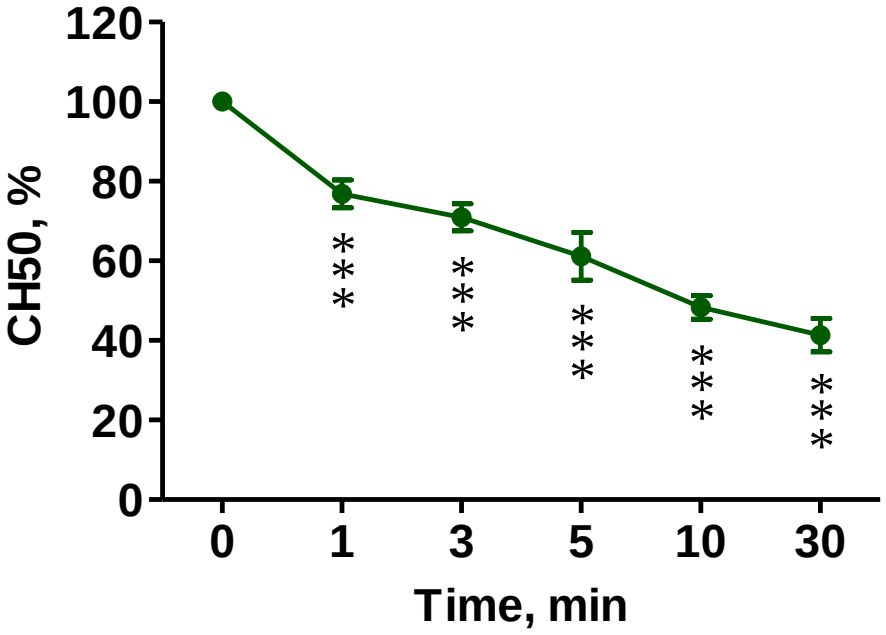


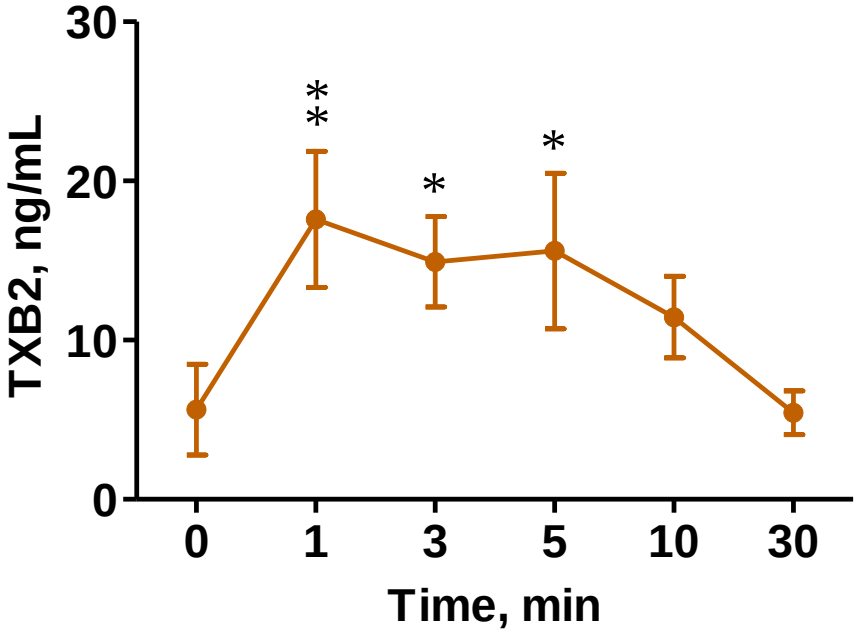
Effects of zymosan (10 mg/kg i.v.) on blood cells in rats



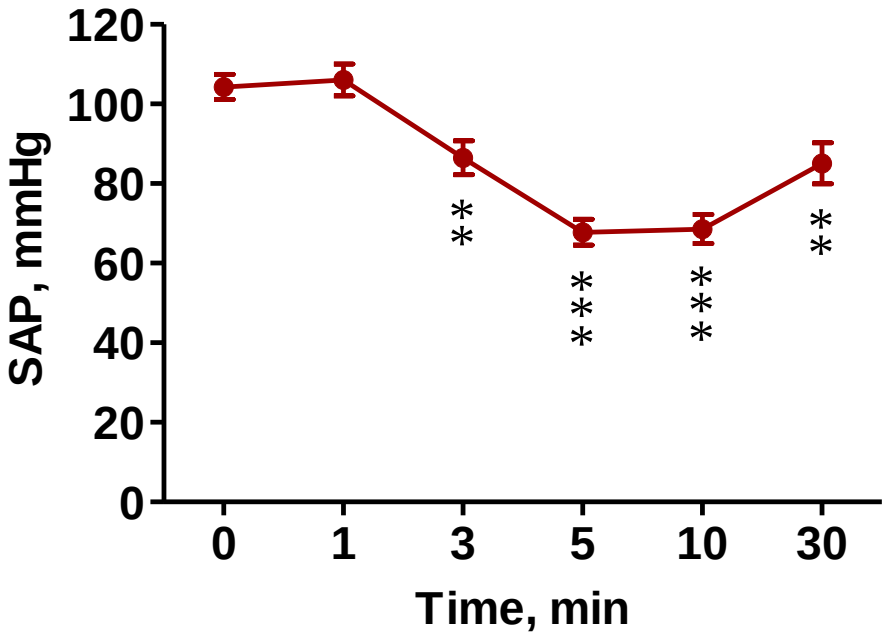


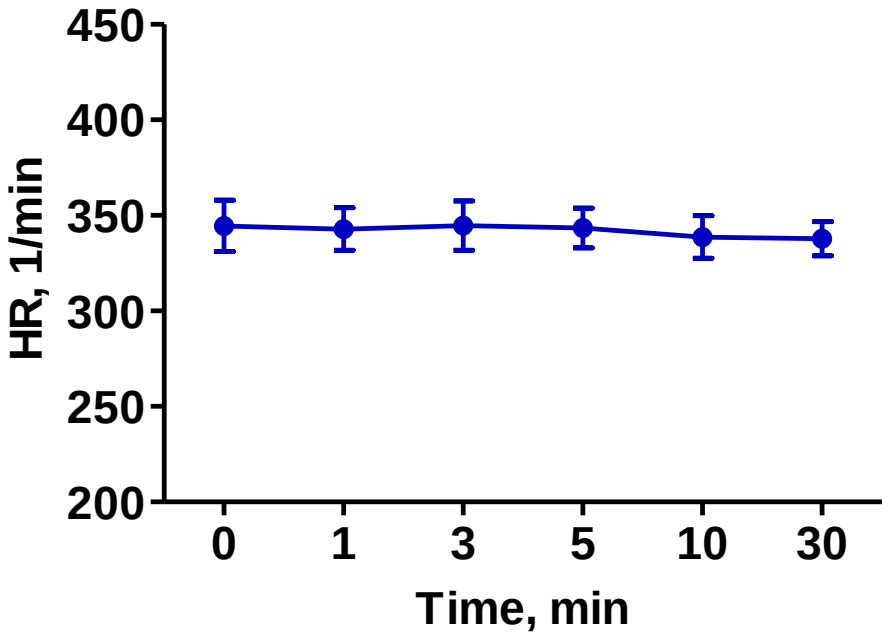
Effects of zymosan (10 mg/kg iv.) on complement activation and TXB2 production in rats



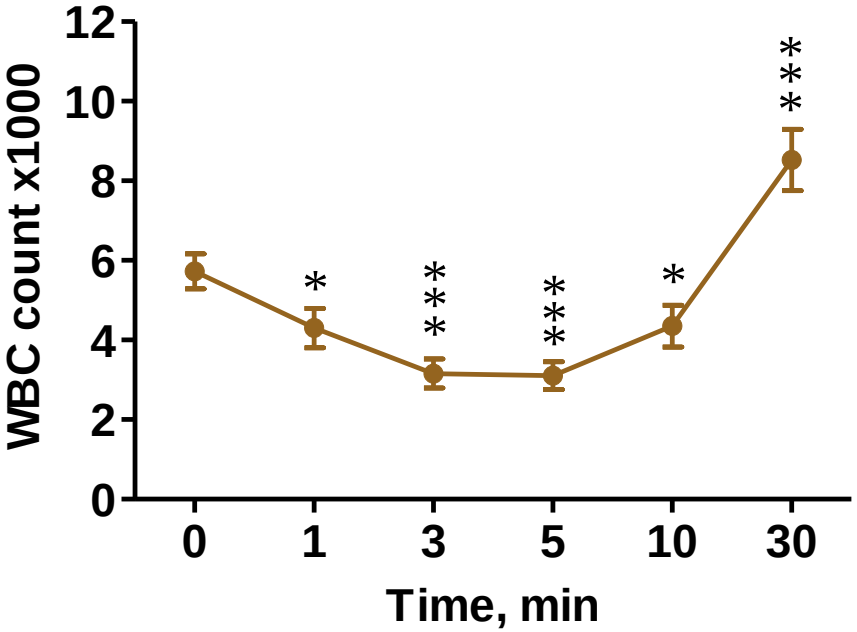


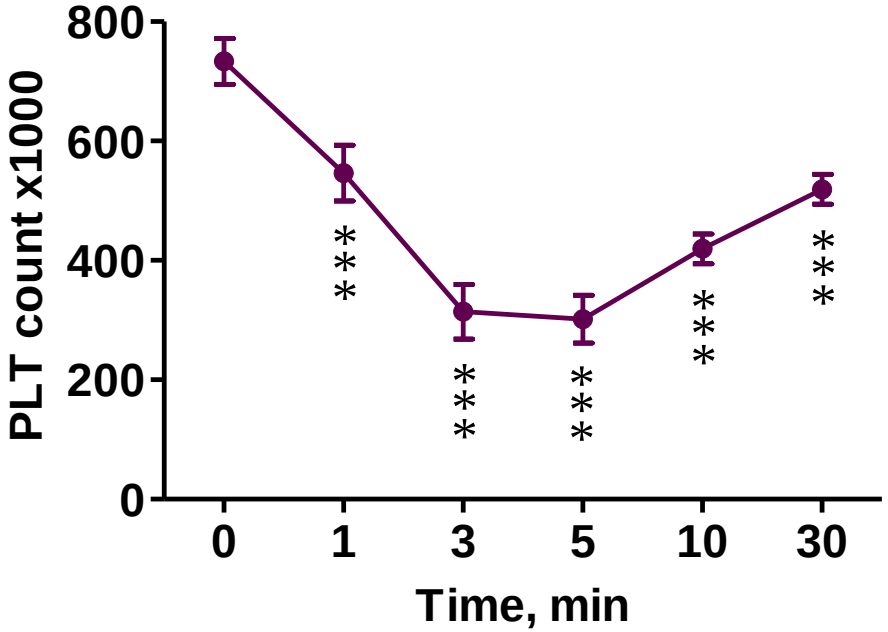
Effects of AmBisome (22 mg PL/kg i.v.) on hemodynamic parameters in rats



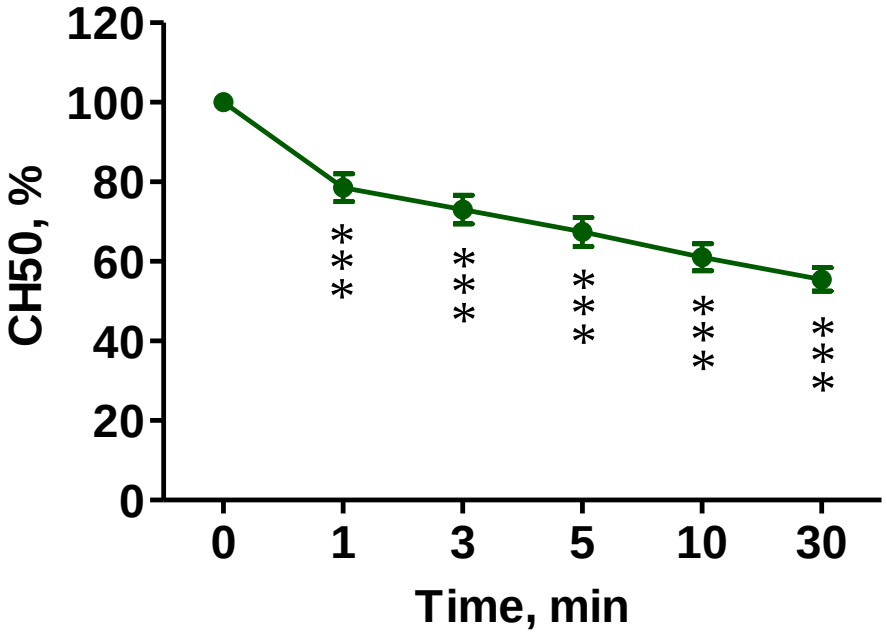


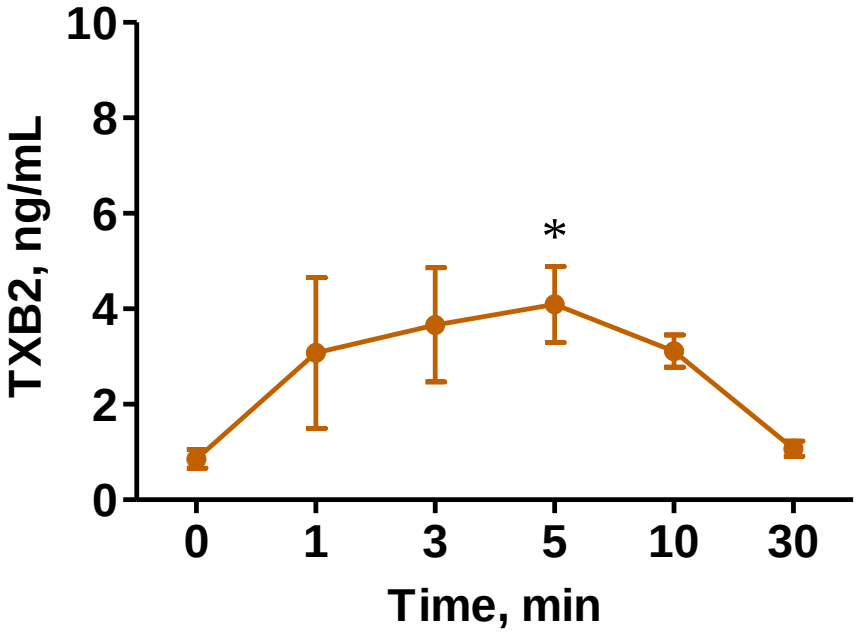
Effects of zymosan AmBisome (22 mg PL/kg i.v.) on blood cells in rats



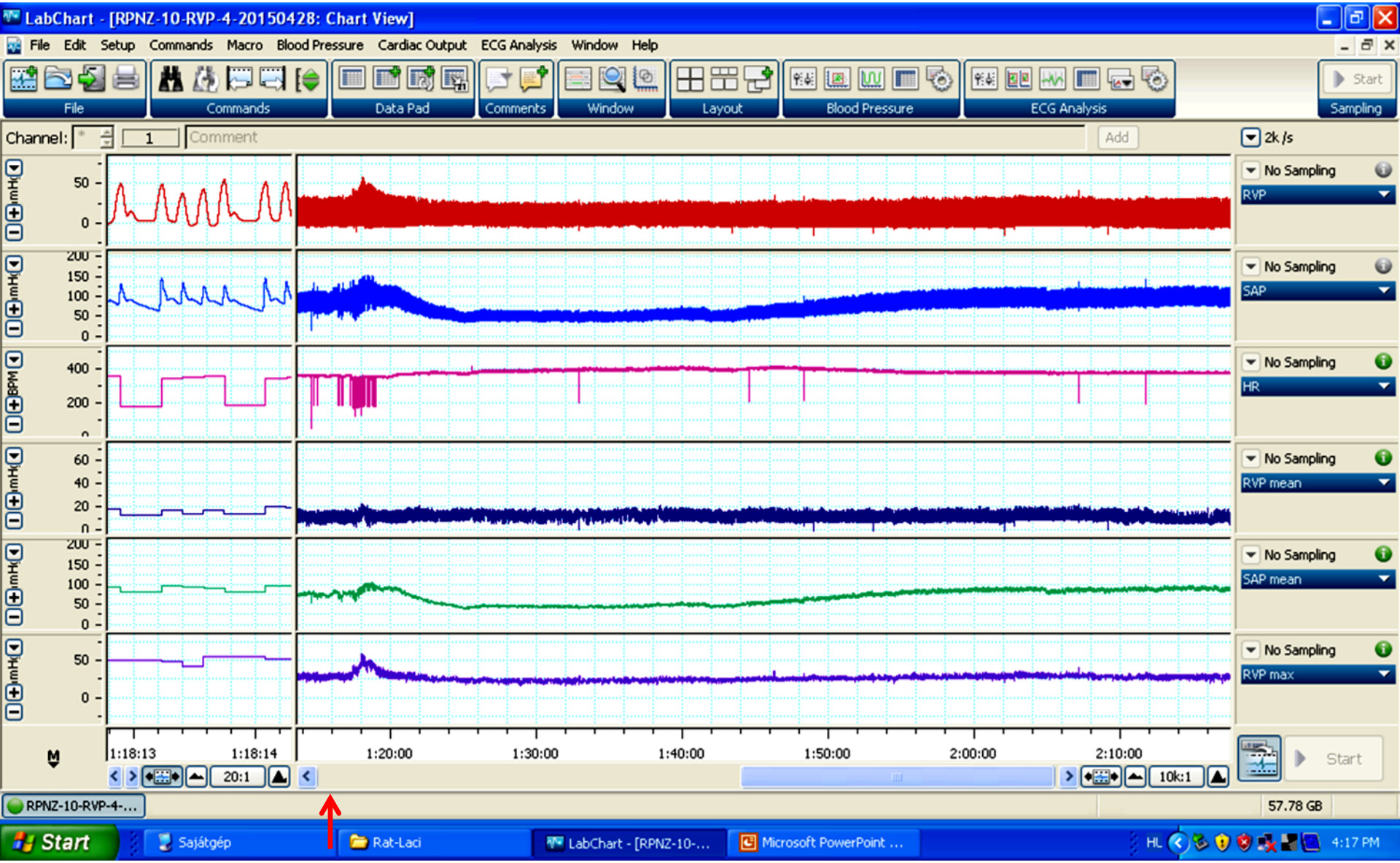


Effects of AmBisome (22 mg PL/kg i.v.) on complement activation and TXB2 production in rats

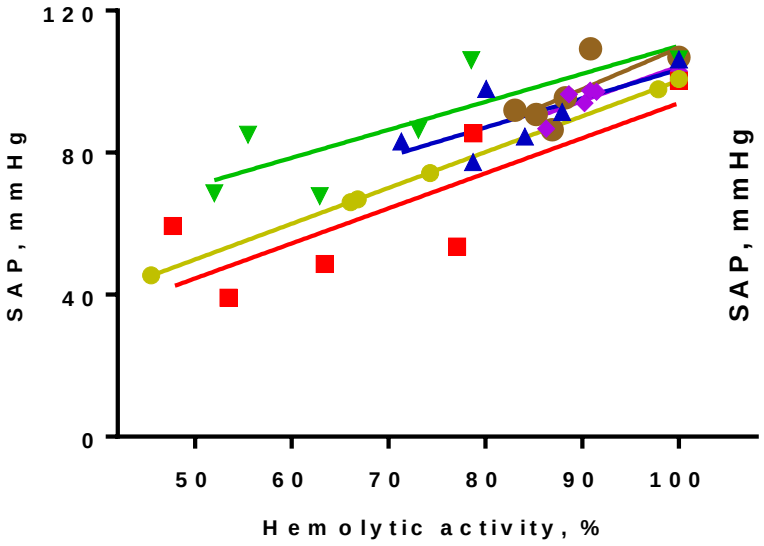


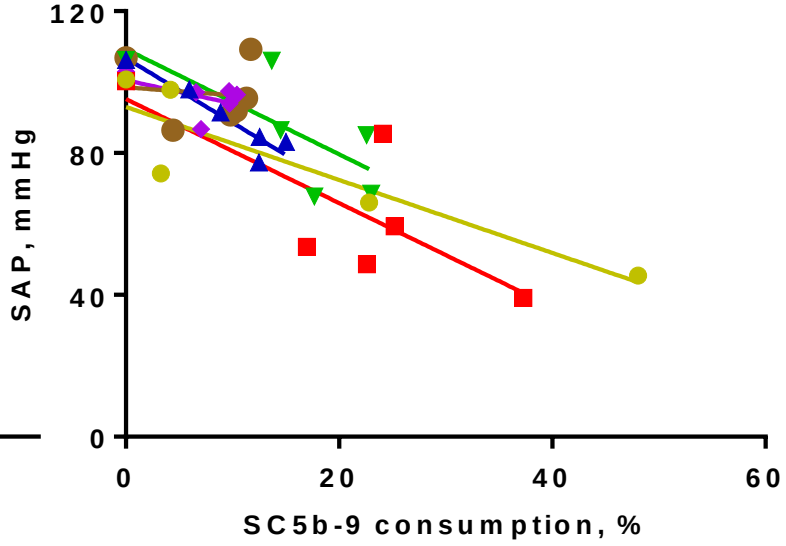


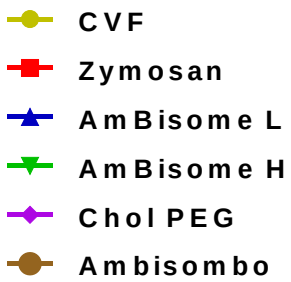
Effects of zymosan i.v. on SAP and RVP in rats

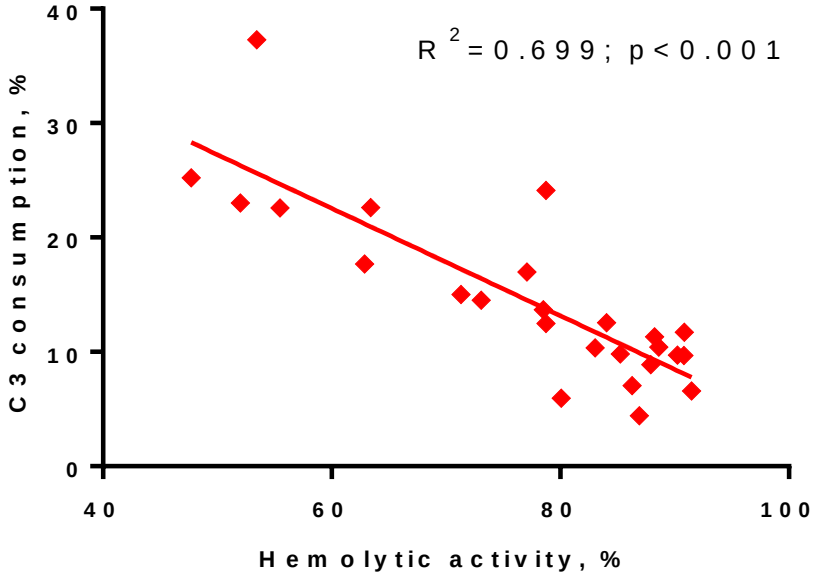


Correlation between CHA and Pan-C3 assays in rats









Mouse CARPA model

Protocol 1.

- Male NMRI mice (29-35g) are anesthetized with Na-pentobarbital (Nembutal, 60 mg/kg i.p.)
- Left carotid a. and right jugular v. were cannulated.
- Arterial blood pressure (ABP) and heart rate (HR) are recorded (ADInstruments, Experimetria)
- Test agents injected i.v. (jugular vein)

Mouse CARPA model

Protocol 2.

- Substances are administered into the tail vein in awake mice
- Blood was collected under isoflurane anesthesia in one time point only, either from sham treated mice (time 0), or at 1-3-5-10-20-30 min after treatment
- Blood cells counted by hematology analyzer (Abacus)

Assays

Total complement activation by hemolytic complement assay (CH50/CHA)

Plasma TXB2 measured by ELISA (Cayman)

Early data

Surprise, surprise!

DE GRUYTER

Eur. J. Nanomed. 2015; 7(3): 257–262

Short Communication

Tamás Mészáros, Gábor Szénási, László Rosivall, János Szebeni[a] and László Dézsi[a,*]

Paradoxical rise of hemolytic complement in the blood of mice during zymosan- and liposome-induced CARPA: a pilot study

Early data

Effects of zymosan (0.5 mg/kg iv.) on complement activation and tromboxane production in BL6 and ApoE mice

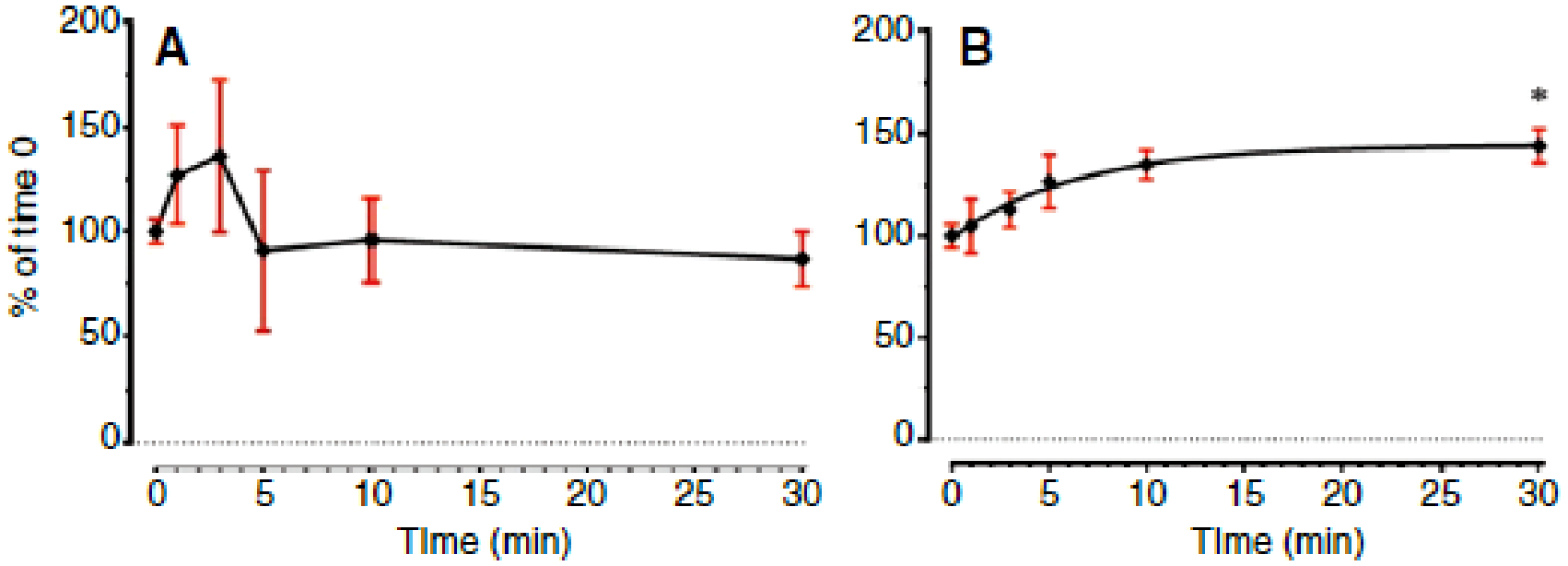
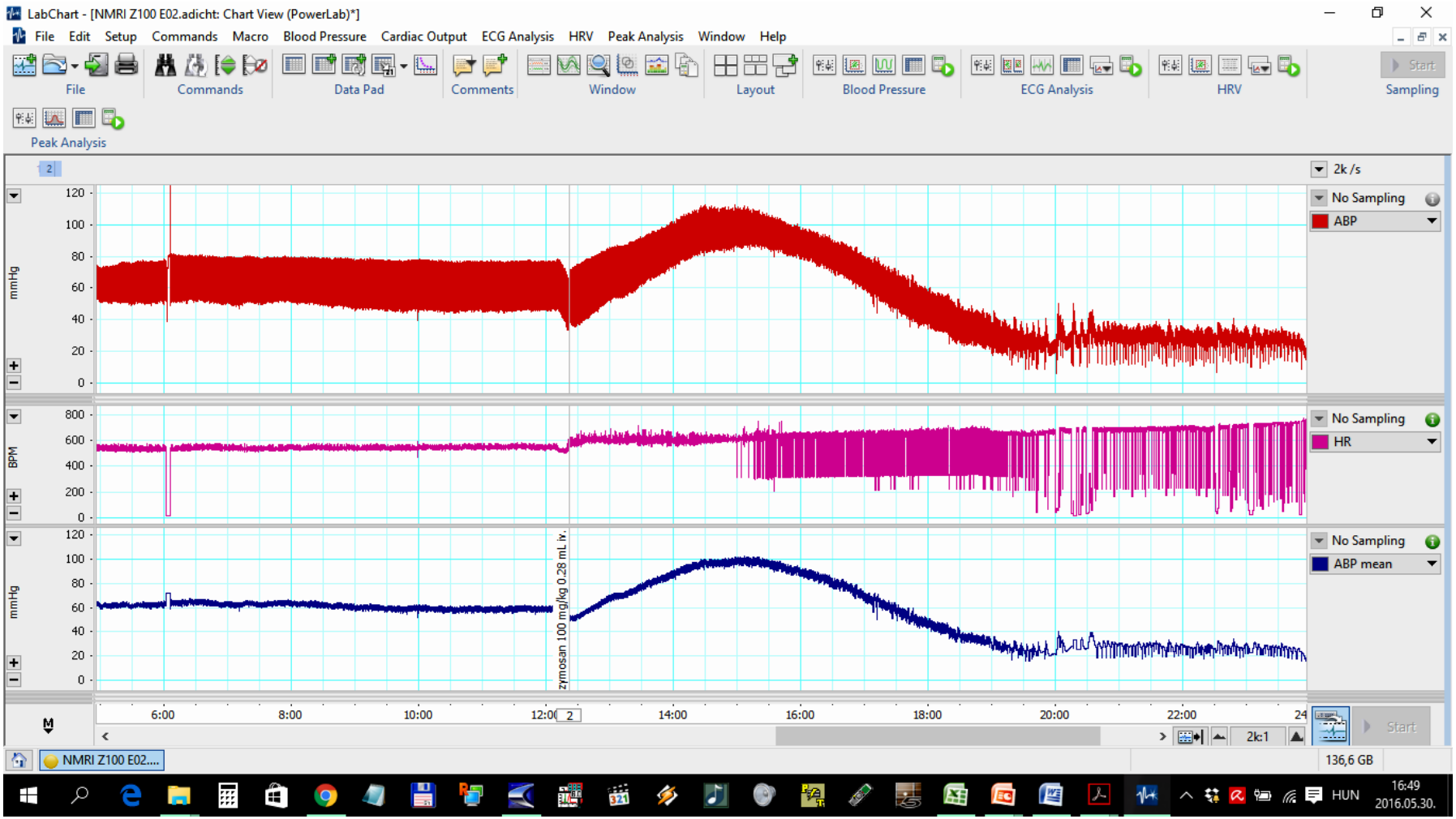


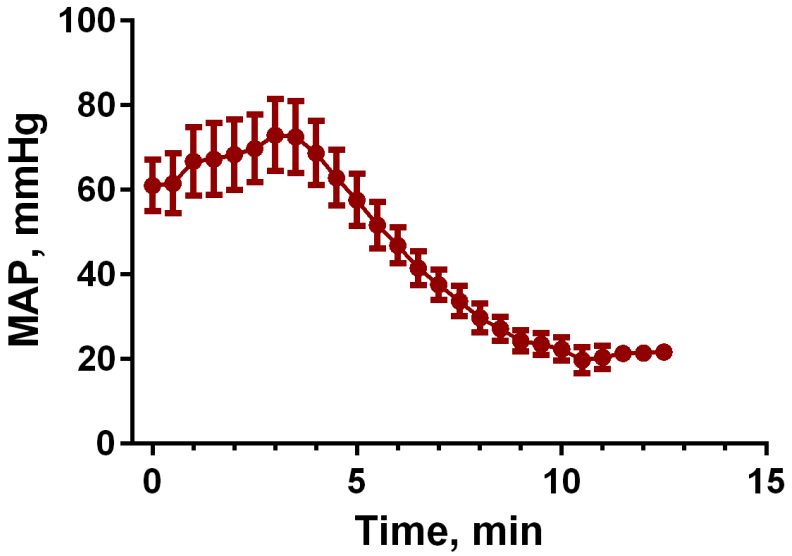
Figure 1: Effect of zymosan (0.5 mg/kg) on hemolytic complement activity (HCA) in BL6 (A) and ApoE (B) mice. The data are expressed as hemoglobin OD_{541}, % of saline control (0 min), mean±SD for n=3–4 mice at each time point. The 0 min value (OD_{541}: 0.51±0.13, n=4) represents immediate sampling after injection of saline at a volume (50 µL/10 g) that corresponds to the volume of zymosan and liposome administrations. HCA was determined by the SRBC assay, as described in the methods. For statistical analysis one way ANOVA was used. *$p<0.05$.

Effects of zymosan (100 mg/kg) i.v. on SAP in NMRI mice

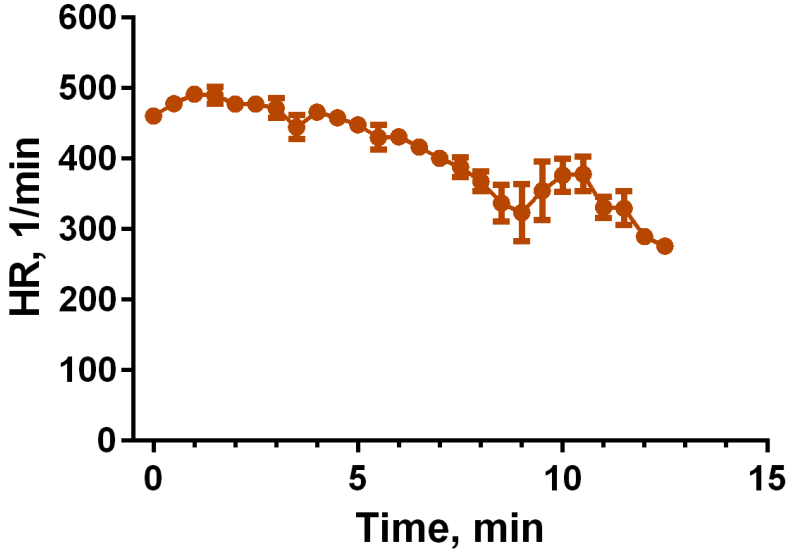


CARPA in mice

Mean Arterial Pressure
Zymosan 30 mg/kg i.v.

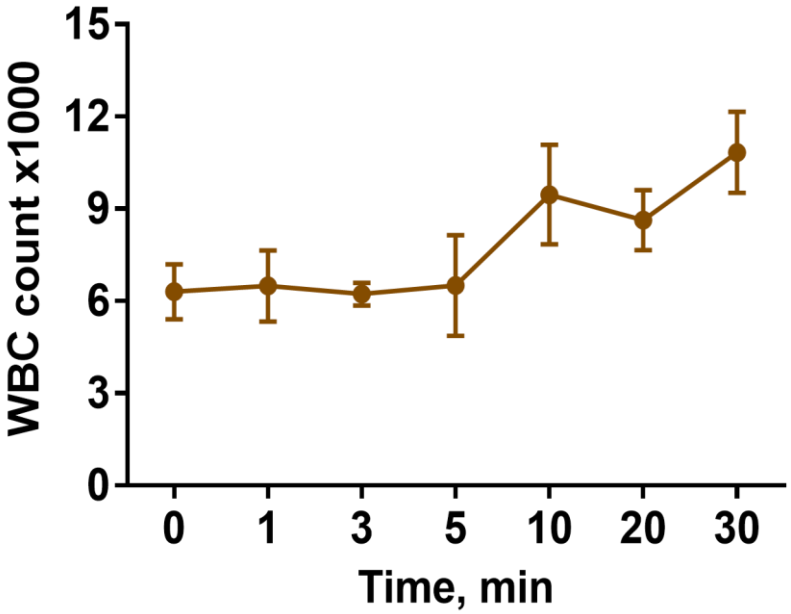


Heart Rate
Zymosan 30 mg/kg i.v.

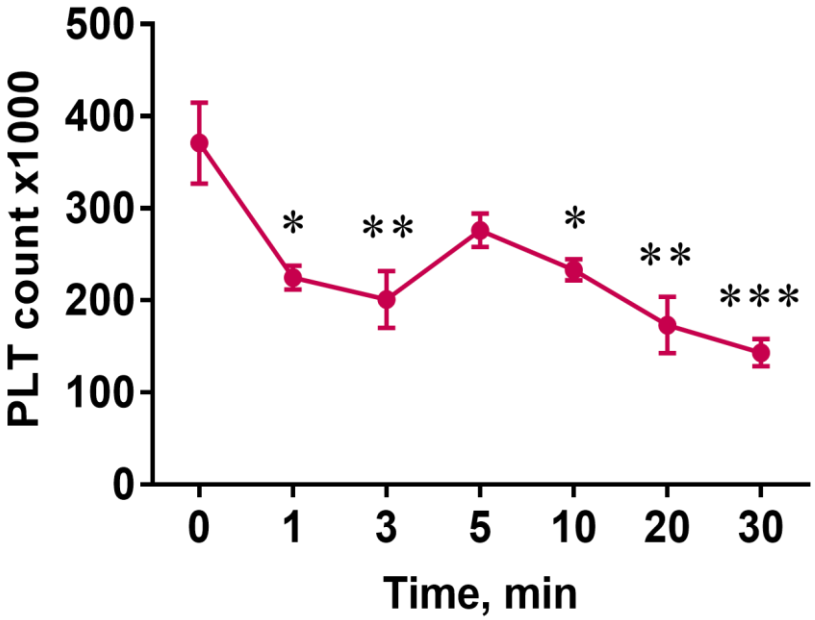


CARPA in mice

White Blood Cells
Zymosan 30 mg/kg i.v.

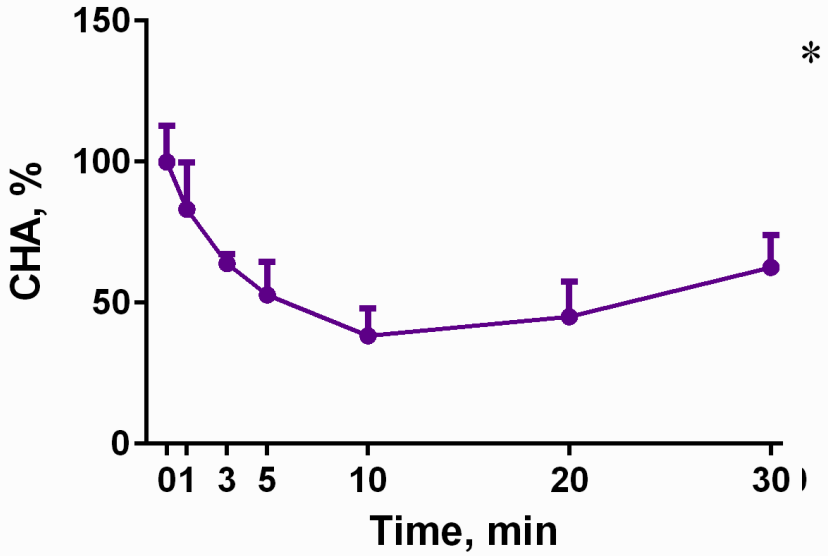


Platelets
Zymosan 30 mg/kg i.v.

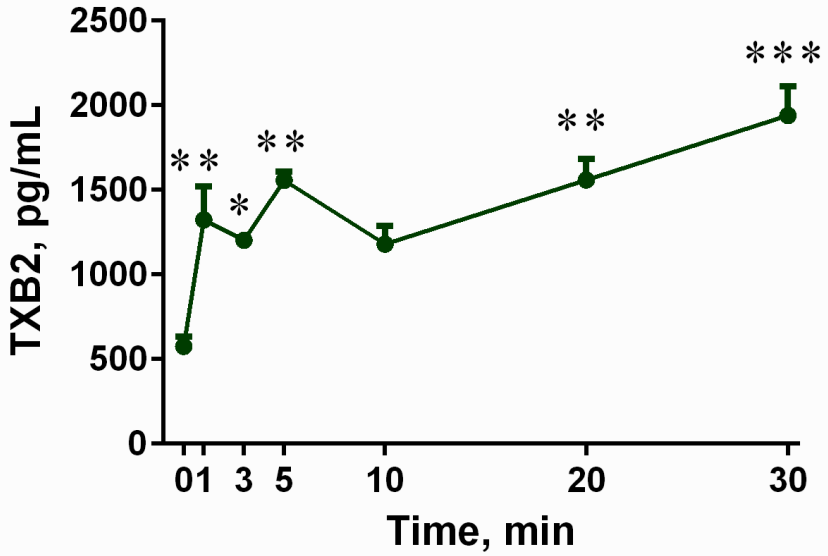


CARPA in mice

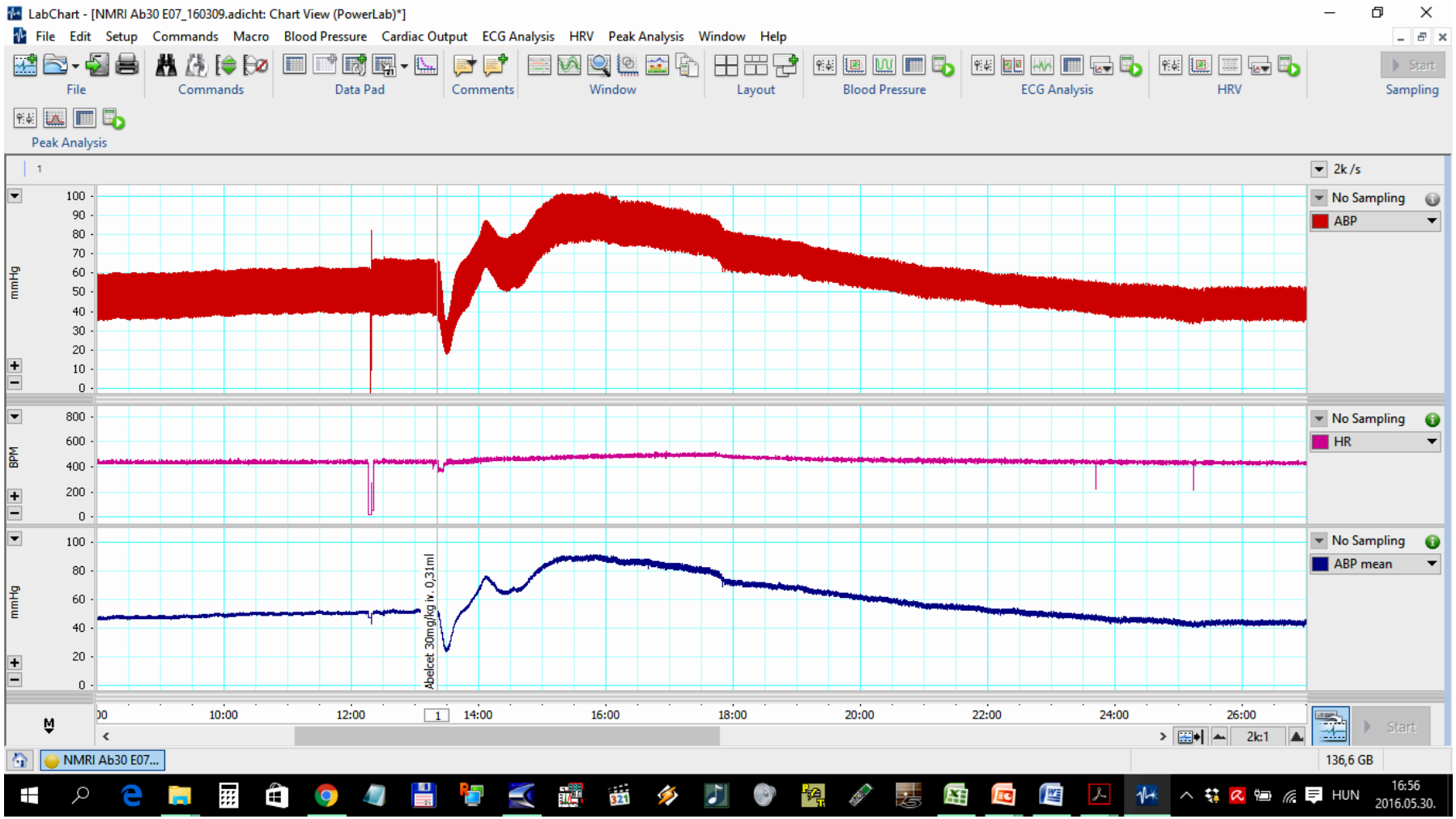
Complement hemolytic activity
Zymosan 30 mg/kg i.v.



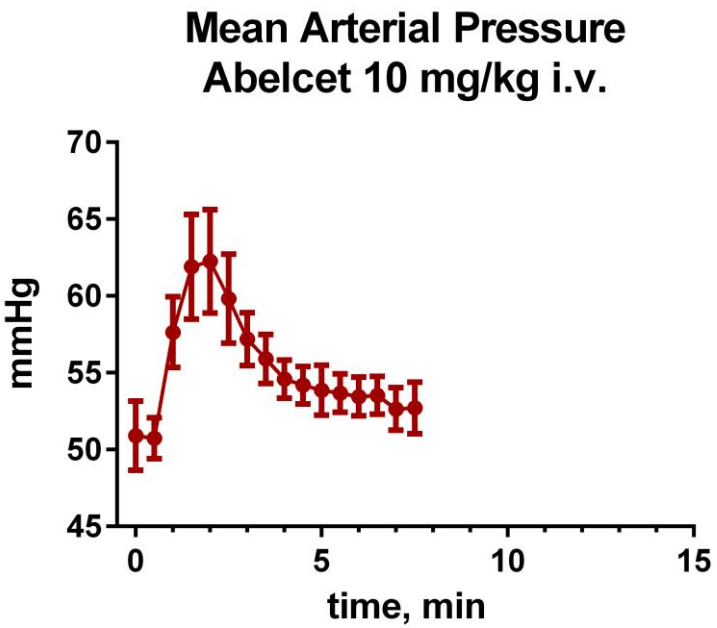
Thromboxane B2 production
Zymosan 30 mg/kg i.v.

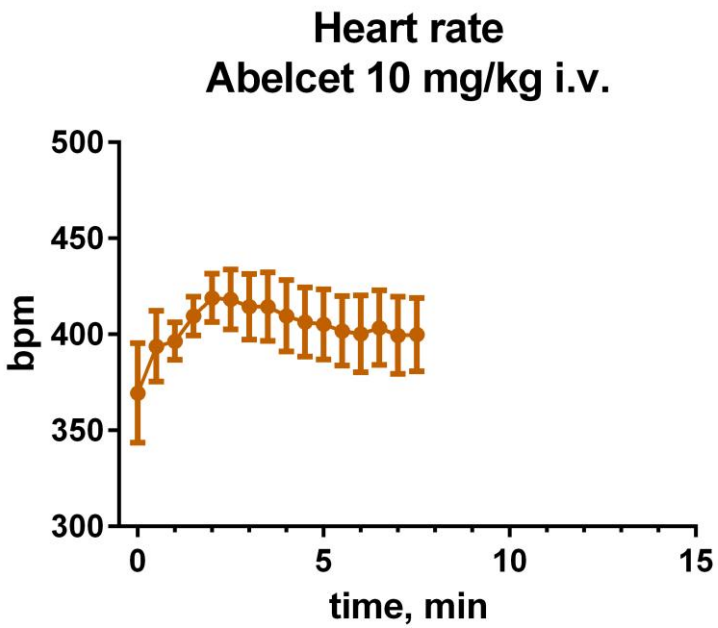


Effects of Abelcet (30 mg/kg) i.v. on SAP in NMRI mice

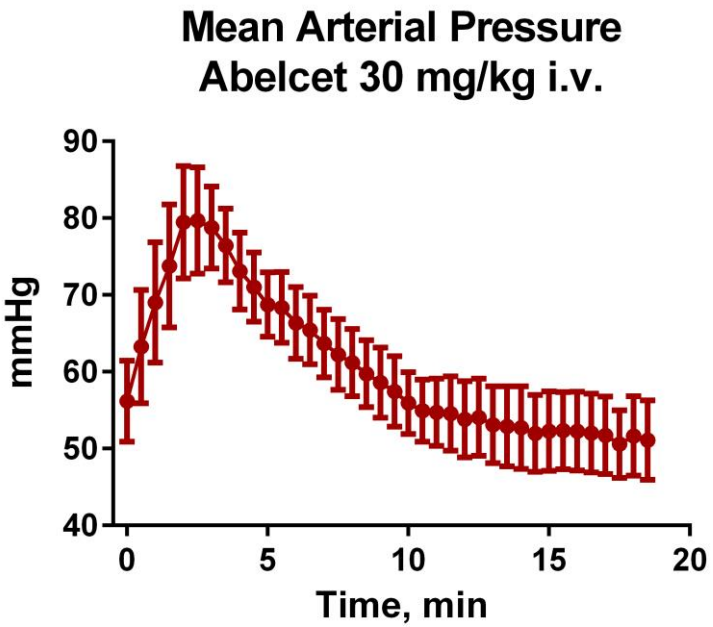


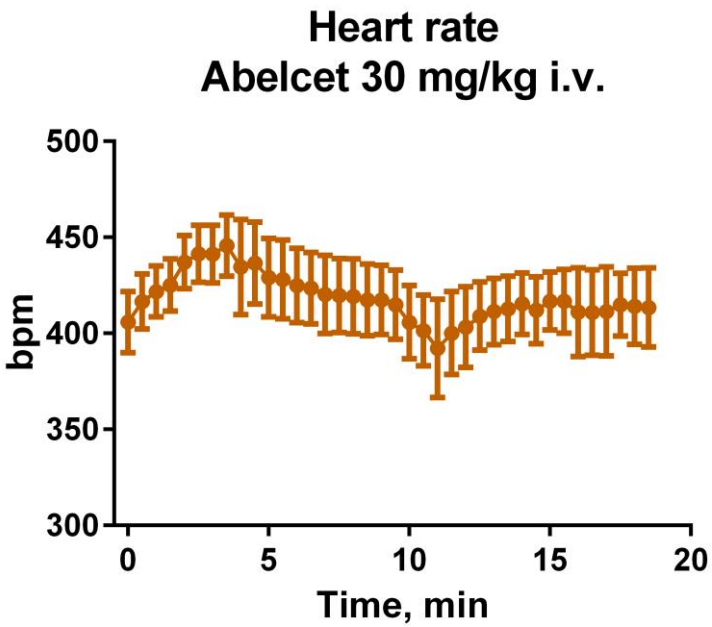
CARPA in mice





CARPA in mice





Comparison of CARPA models

Porcine model

- Advantage: it is a good model of CARPA of most sensitive humans
- Disadvantage: cost- and labor-demanding
  not suitable for screening a number of molecules

Rodent models (rat and mouse)

- Advantage: suitable for screening of a number of molecules
- Disadvantage : a relatively insensitive model
  substances must be applied in high doses

Table 2
Lipid dose dependence of hypersensitivity reactions in man and three animal species

	Pigs	Rats	Dogs	Reactive man
Trigger dose (mg phospholipid/kg)	0.01–0.3	5–25	0.05–0.1	0.01–0.2

CONCLUSIONS

- Currently porcine CARPA model is the most appropriate to test features of nanomedicines
- Tolerance induction could be a method to prevent CARPA symptoms
- We demonstrated the appearance of CARPA in rats and mice upon injections of liposomal and other nanomedicines
- CARPA was demonstrated by changes in cardiovascular, hematological and functional parameters, i.e. changes in C hemolytic activity, and TXB2 production
- Although rats and mice are not sensitive species, 100 to 1000 x less sensitive compared with pigs that represent most sensitive humans
- Therefore, using appropriate dosage they are applicable for
  - ➢ immune toxicity screening, and
  - ➢ investigating the mechanism of action of CARPA

Part II.

A porcine model of hypersensitivity reactions during hemodialysis

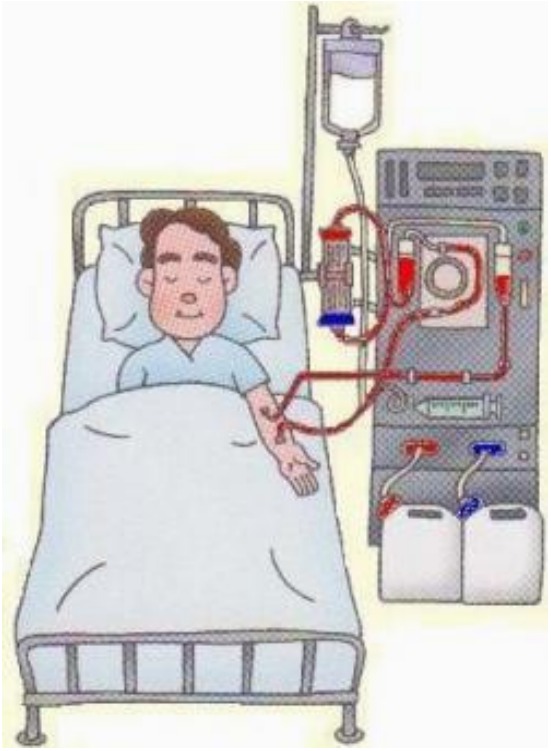
Introduction

- A number of medical conditions can play a role in the development of acute or chronic kidney damage
- In the US, treating chronic kidney patients claimed 52 billion dollars in 2014
- No exact data are available in Hungary, but at least 1 million people suffer from chronic kidney disease

United States Renal Data System. 2016 Annual Data Report. Vol 1, Ch 6: Medicare expenditures for persons with CKD. https: //www.usrds. org/2016/view/v1_06.aspx. Accessed January 21, 2017.

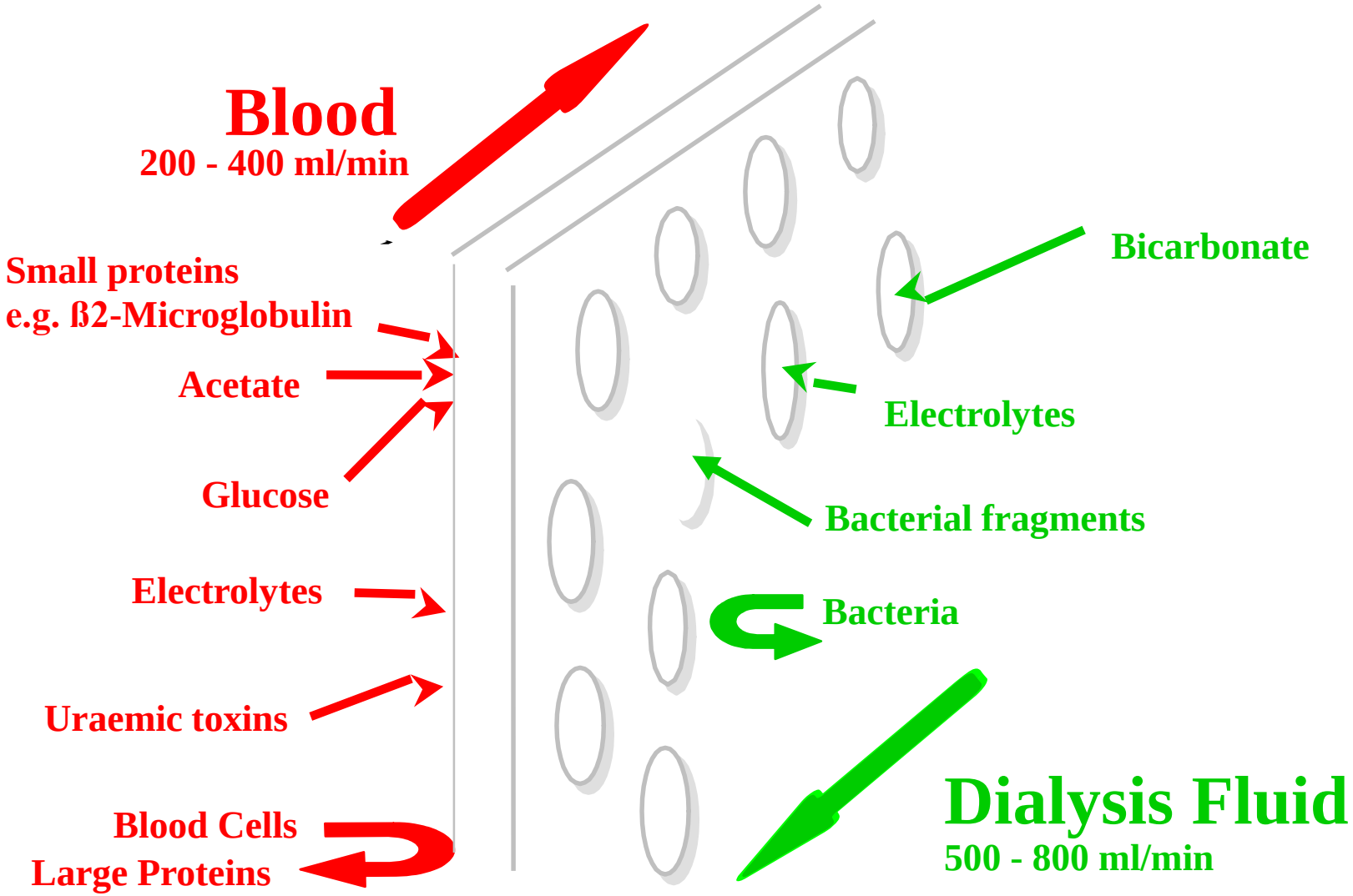
Extracorporeal treatment procedures

- ➢ Renal replacement therapies
- Hemofiltration
- Hemodialysis
- Hemodiafiltration
- Chronic Ambulant Peritoneal Dialysis (CAPD)
- ➢ Other treatments
- Hemoperfusion (toxicological cases)
- Ultrafiltration (therapy resistant edema)
- FPSA (Liver Replacement Treatment)



ubisafe.org/explore/dialyze-clipart-hemodialysis

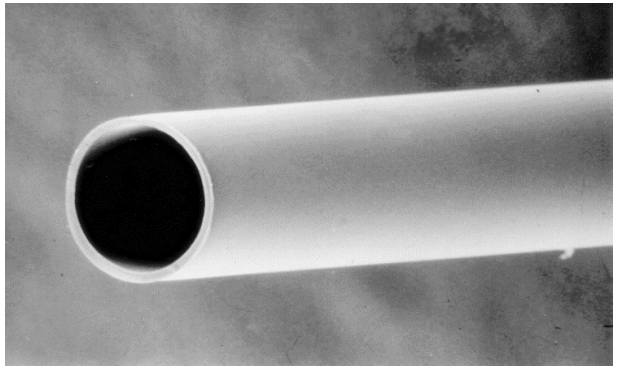
What happens on both sides of the dialysis membrane?



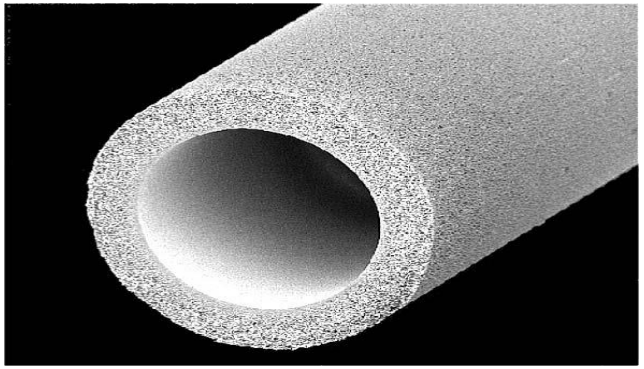
Types of dialysis membranes

- Regenerated cellulose (cuprophane-tetra-amine-copper-hydroxide)
- Cellulose acetate
- Polyacrylnitrile (PAN)
- Polysulfone (w. different pore sizes)
- Polypropylene (transmits all blood proteins - plasma filter)

Cellulose and synthetic membranes

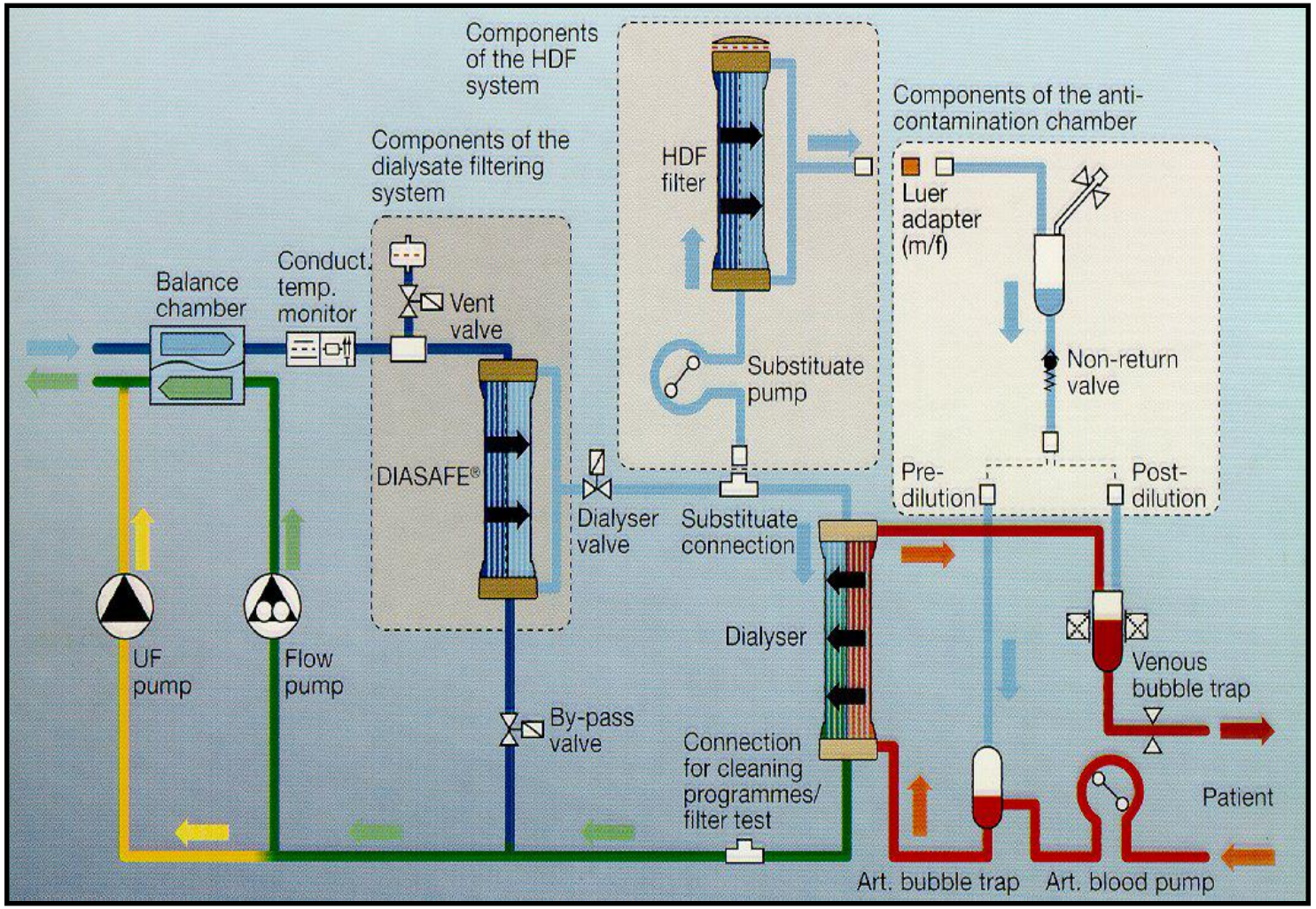


- cellulose membrane
- 8 μm dry and 20 μm moist



- Polysulfone membrane
- 40 μm

Dialysis device



Source: Fresenius Medical Care

Water purification system

softener / ion exchange

Fine Filter 50 - 10 um

activated carbon filter

sand filter

raw water inlet unit

Fine Filter 5 - 1 um

RO-system AquaSafe 08

permeate storage tank

Source: Fresenius Medical Care

Dialysis reactions

- Ethylene oxide, formaldehyde (sterilization), medications (e.g. erythropoietin, intravenous iron, heparin)
- Dialysis membrane precipitation

  Polyalkylnitrile reaction (HSR):

  surface charge = > complement activation
- "A" type (IgE mediated): 5-20 minutes after dialysis start, pruritus, urticaria, bronchospasm, larynx edema, anaphylaxis, shock
- "B" type (complement-mediated, IgG): in later stages of dialysis, moderate intensity symptoms, chest, back pain

Hemodialysis Emergencies, Saha et al.
Clin J Am Soc Nephrol 2016. doi: 10.2215/CJN.05260516

Complications of dialysis reactions

- Incompatibility in patients leads to
  - inflammation
  - thrombosis
  - fibrosis
- During hemodialysis
  - C3a peaks in the first 10-15 min
  - C5b-9 formation occurs at later stages of dialysis

Chenoweth DE, Cheung AK, Henderson LW. Anaphylatoxin formation during hemodialysis: effects of different dialyzer membranes. *Kidney Int* (1983) 24:764–9. doi:10.1038/ki.1983.225

Polysulfone reaction

- It can activate GpIIa-IIIb receptors of the platelets = > platelet activation
- Different proteins e.g. ficolin-2 may be bound to it = > complement activation
- Due to pore structure "back-filtration" may develop (dialysate = > blood)
- MBL (mannose-binding lectin) and ficolin-2 protein binding to membrane => lectin pathway activation
- Polysulfone membranes absorb factor-H and clusterin => dysregulation of the alternative pathway

L. Butani and G. Calogiuri / Ann Allergy Asthma Immunol 118 (2017) 680-684
dx.doi.org/10.1016/j.anai.2017.04.006

THE IDEA:

Let's combine porcine CARPA model with hemodialysis!

The hemodialysis device

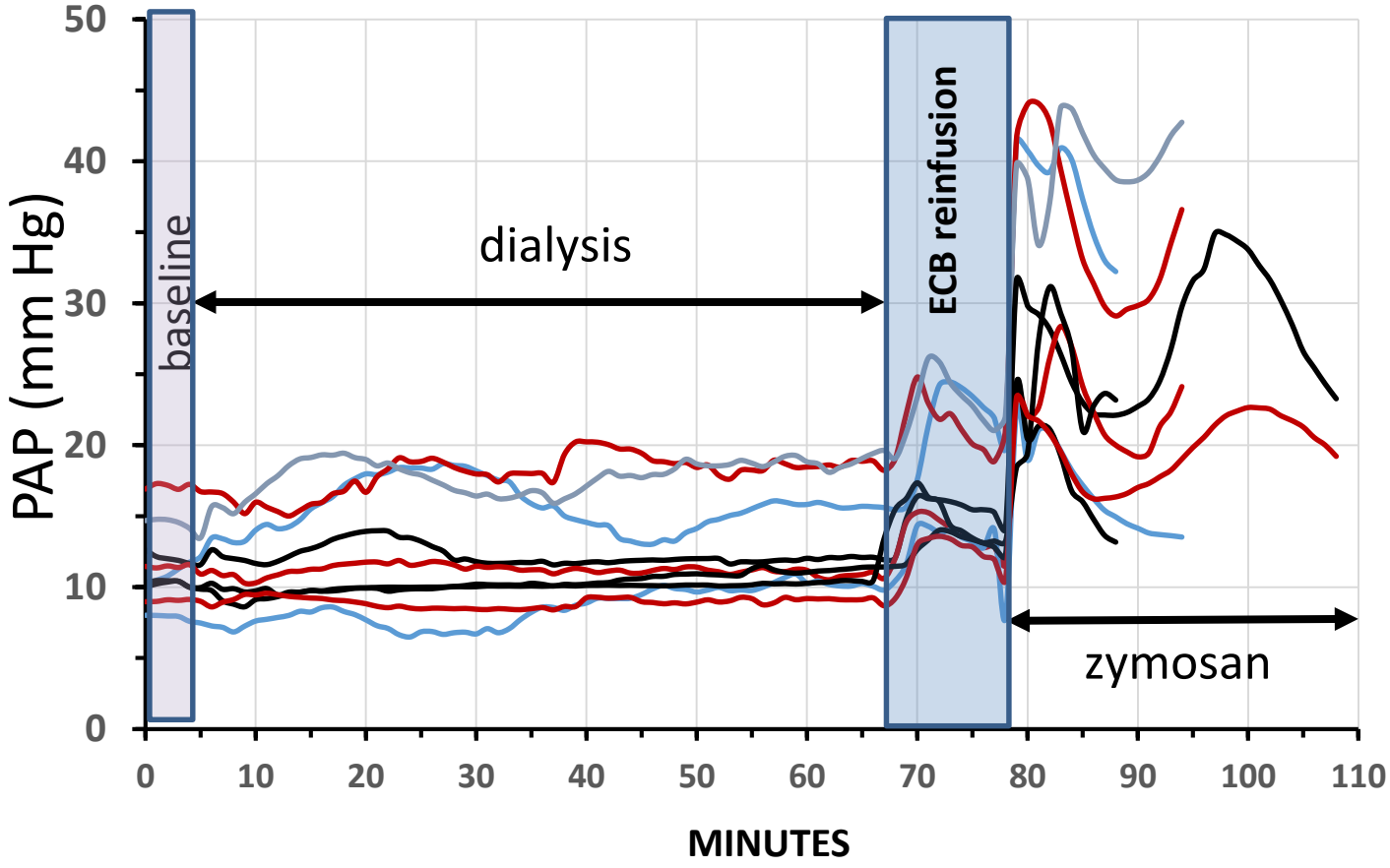
RO-based water purification system

Fresenius 4008 S

Experimental setup (CARPA pig + hemodialysis)

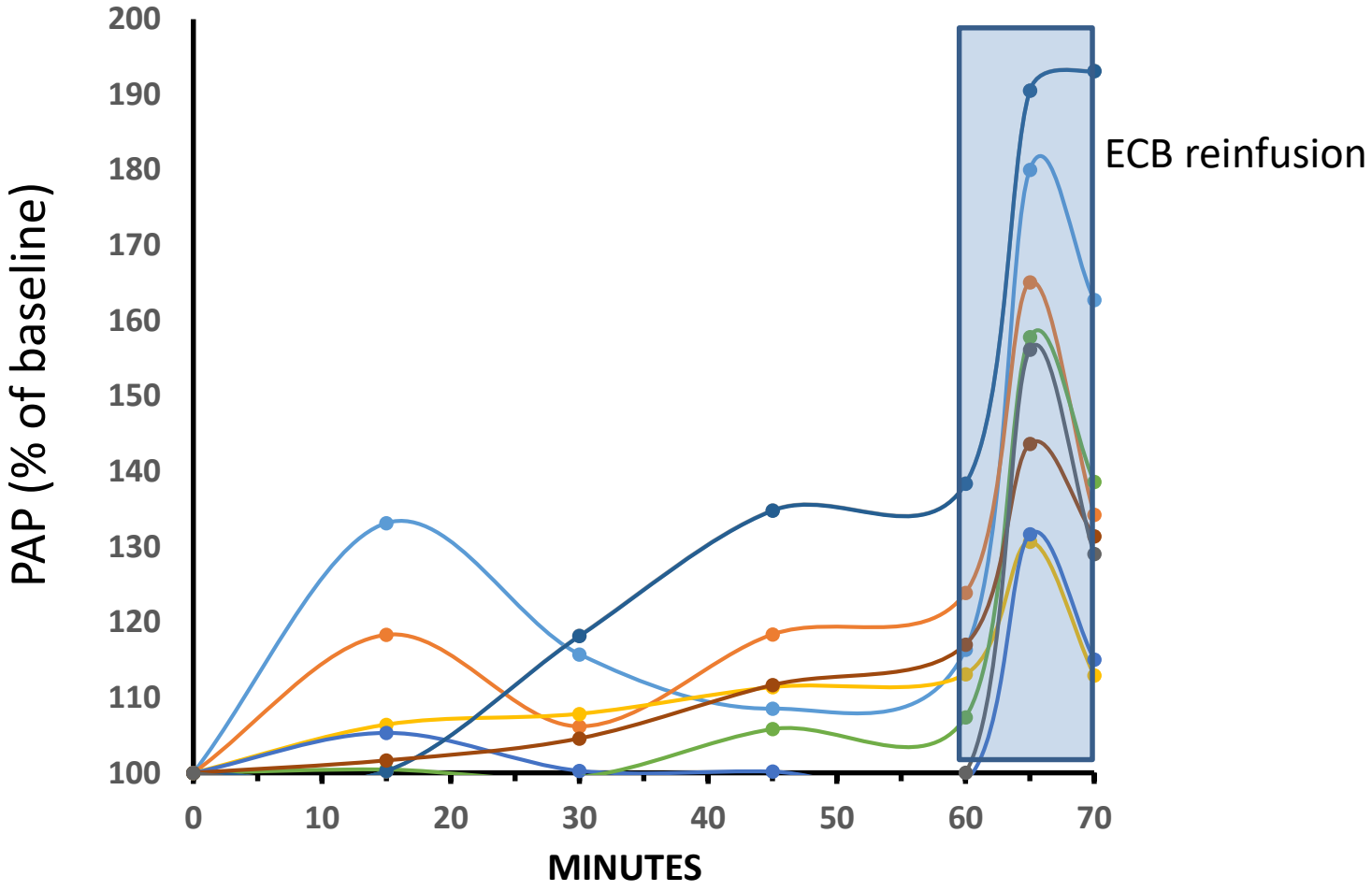
Results

Pulmonary arterial pressure changes during hemodialysis, reinfusion of extracorporeal blood (ECB) and zymosan



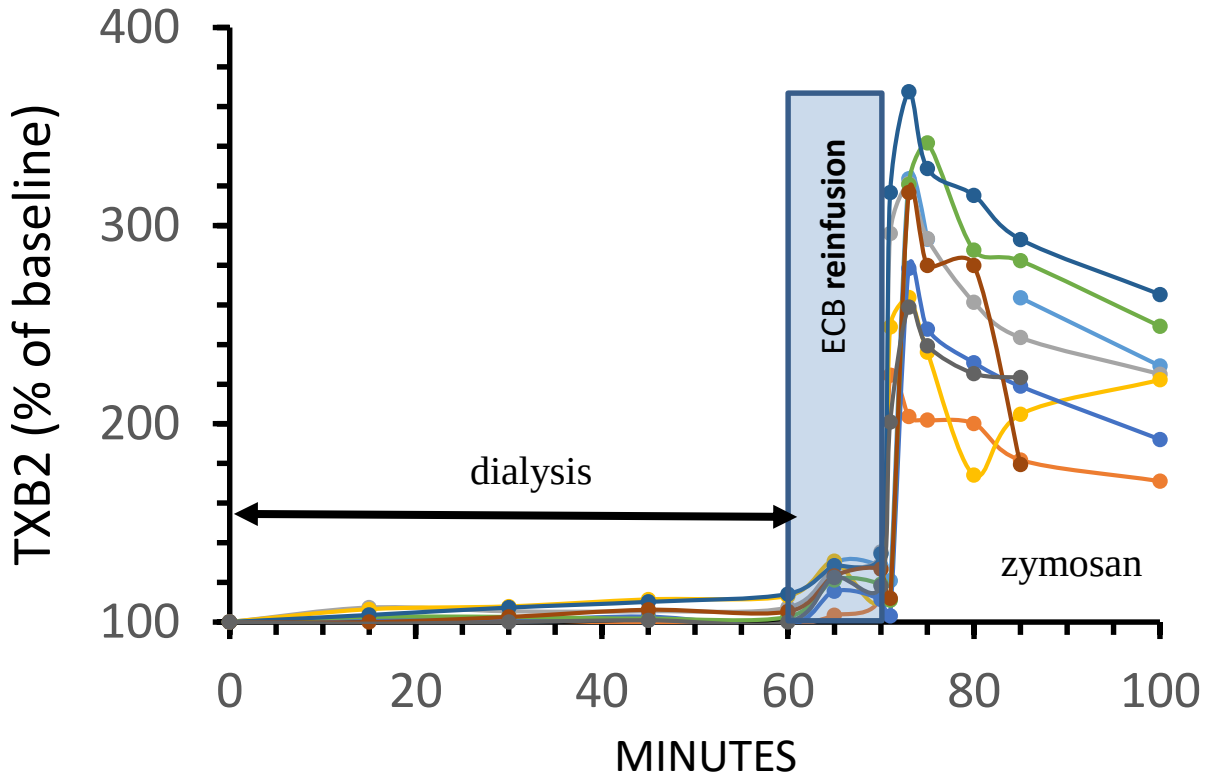
- **In all pigs ECB and zymosan caused significant pulmonary hypertension**

Pulmonary arterial pressure changes during hemodialysis, reinfusion of extracorporeal blood



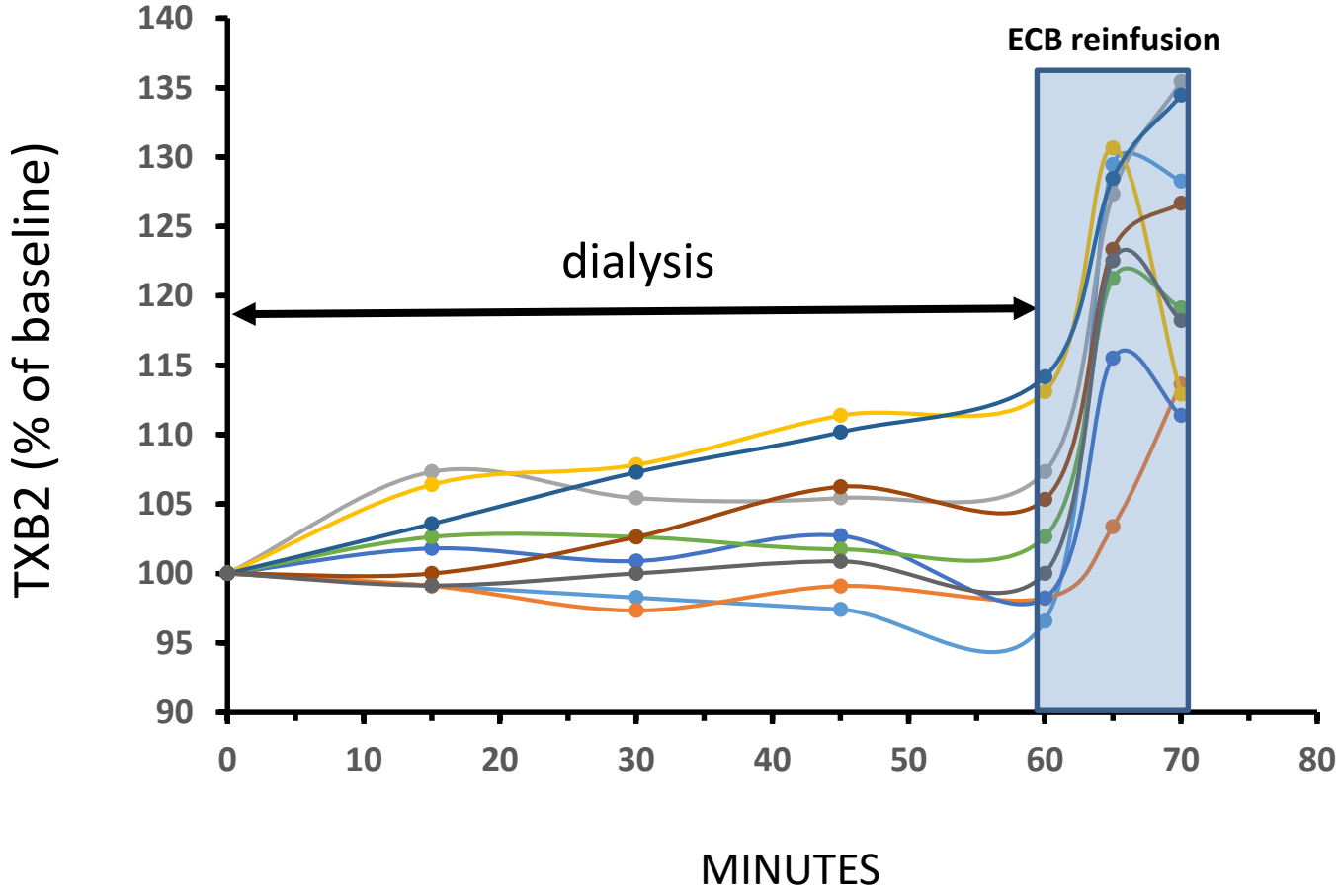
- In 3 of 9 pigs minor, variable PAP rises are also observed

Blood thromboxane A2 changes during hemodialysis, reinfusion of extracorporeal blood (ECB) and zymosan



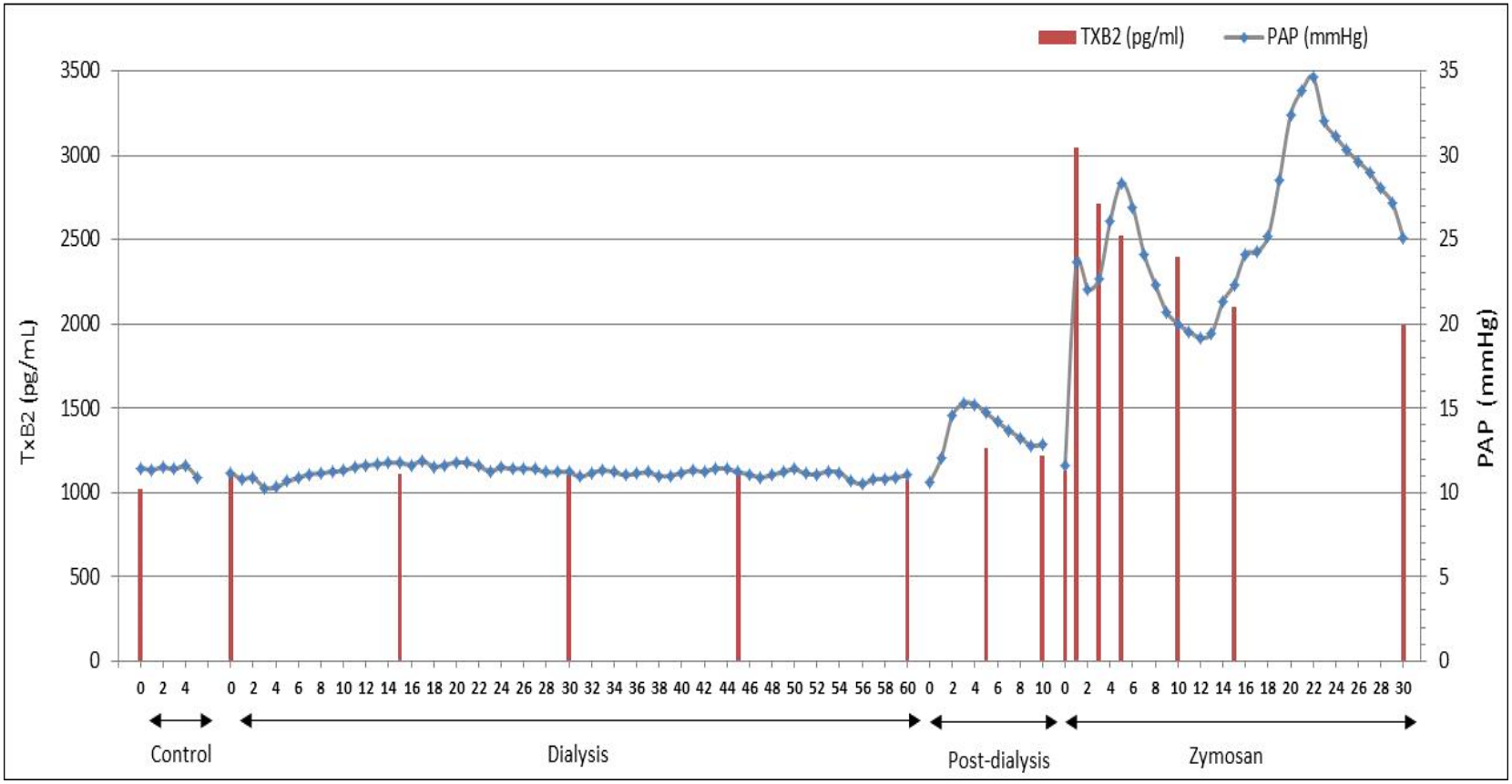
- **In all pigs ECB and zymosan caused significant rises of TXB2 at the same times when pulmonary hypertension was also observed**

Blood thromboxane A2 changes during hemodialysis, reinfusion of extracorporeal blood

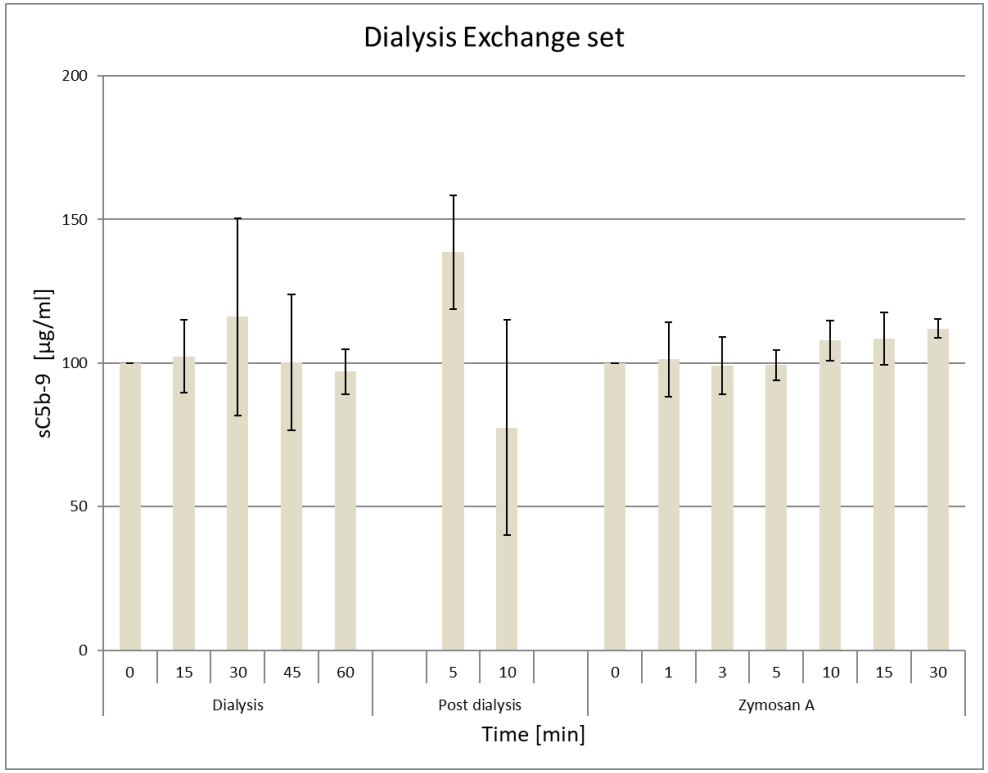


- **In 3 pigs (of 9) TXB2 also increased during hemodialysis (although mild increase)**

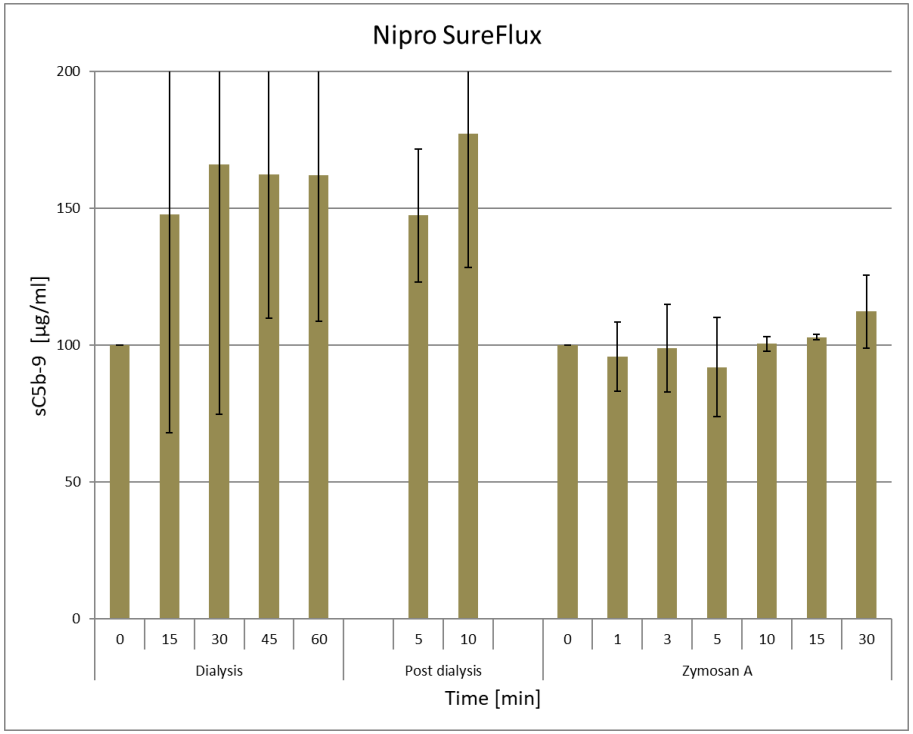
Relation of PAP and TXA2 changes during hemodialysis, reinfusion of extracorporeal blood and zymosan

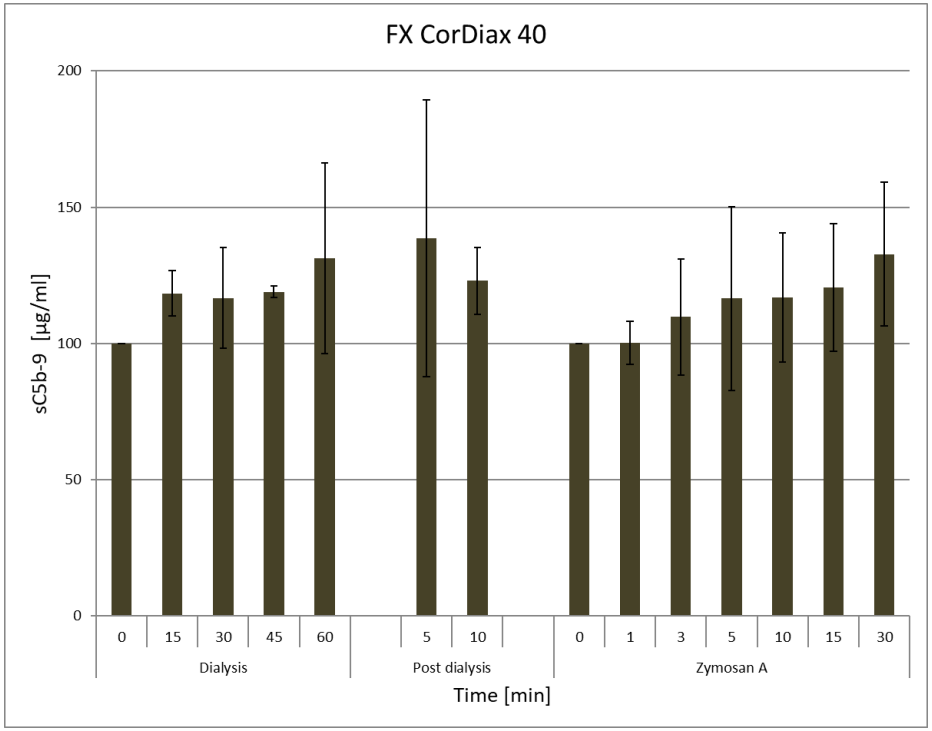


Complement changes during hemodialysis, reinfusion of extracorporeal blood and zymosan – no dialyser

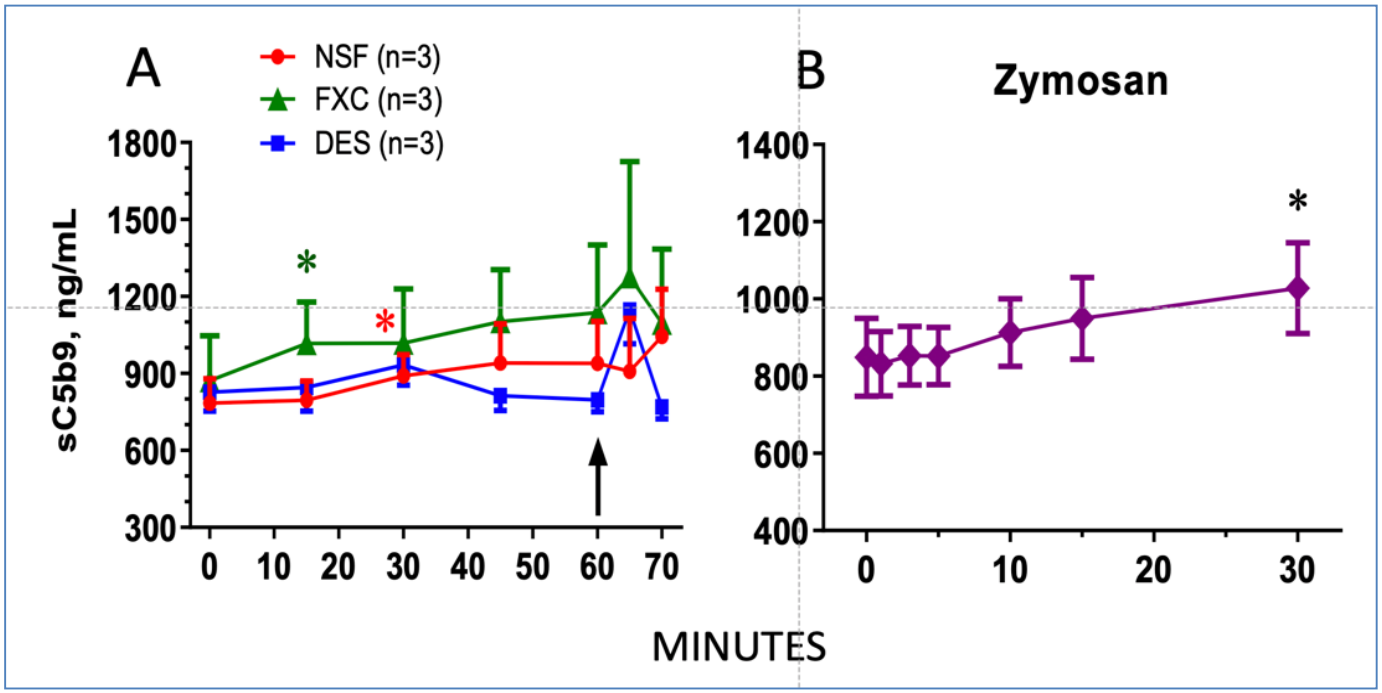


Complement changes during hemodialysis, reinfusion of extracorporeal blood and zymosan – with dialyser





Complement changes during hemodialysis, reinfusion of extracorporeal blood and zymosan



Conclusions

- **Pigs may provide a model for dialyzer reactions using PAP and TXB2 endpoints**
- **The high-speed return of ECB to the circulation seems critical: it induces pathophysiological changes triggering thromboxane release and a consequent pulmonary hypertension**
- **Measurement of sC5b-9 revealed the role of complement during and following hemodialysis**
- **Complement activation is likely to occur in the extracorporeal circulation**
- **The model may be useful to clarify further mechanisms of hypersensitivity reactions during hemodialysis**

Acknowledgement

- Gábor Szénási
- Rudolf Urbanics
- Ákos Pethő
- Maria Velkei
- Tamás Mészáros
- Kozma Gergely
- Tamás Fülöp
- Tamás Bakos
- Erik Őrfi
- János Szebeni

Thank you for your attention!