INFLUENCING AND INVESTIGATING INSULIN RESISTANCE BY A MOLECULE WITH A NEW MODE OF ACTION

PhD theses

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INTRODUCTION

The global spread of insulin resistance (IR) syndrome and its consequence: type 2 diabetes (2DM) along with their complications present an enormous health problem. The inestimable financial burden of this medical care may be decreased only by the prevention of the disease. According to several large international studies lifestyle changes and medical treatment are good enough to prevent or slow down the progression of IR and the early stage of diabetes (IFG, IGT).

The bone mineral density (BMD) is lower in 1DM, while higher in 2DM. This phenomenon was seen as the consequence of the higher BMI in 2DM. Decreased bone mass, higher fracture risk, longer fracture healing time and the decreased biomechanical integrity of bones are the consequences of insulin deficiency in patients with 1DM.

The incidence of obesity, metabolic syndrome, 2DM and osteoporosis increases worldwide, so the global spread of diabetes is driven by the same obesity which results in stronger bones in women. If the worsening of glucose metabolism is in relation with the state of bones, then the change of this relationship will be detectable in the prediabetic state and the worsening of energy homeostasis will be in proportion with the change of bone metabolism.

In the last decade understanding the pathomechanism of IR and 2DM meant new knowledge: a relationship was found between intracellular heat shock protein (HSP) levels and metabolic disturbance in insulin dependent tissues (muscle, fat). 2DM and IR are

characterized by decreased HSP72 expression in muscle, which is closely associated with the total body glucose utilization. Induction of HSP72 by heat shock, transgenic methods, and pharmacologic means augments insulin signaling, improves mitochondrial function.

BGP-15, an HSP inducer, hydroximic acid derivative was initially developed to alleviate the toxic side effects of cytostatic agents.

The secretion of inflammatory cytokine, TNF-α increases in obesity and 2DM which results in blocking insulin receptors by activating serine/threonine kinases.

HSP72 decreases the activity of the inhibitor of κB kinase (IKK) and c-jun amino terminal kinase (JNK), which has a very important role in inflammatory signaling. Inflammatory processes via JNK play a pivotal role in the development of IR. HSP72 deficiency increases IRS-1 phosphorylation at serine 307, while insulin signaling decreases. The activation of HSPs can markedly block the activation of JNK, so this may protect against the development of IR.

BGP-15 increases the expression of HSP72 *in vitro*, inhibits the phosphorylation of JNK - serine/threonine kinase - and the development of IR.

The object of our study was the clinical investigation of a drug candidate which acts as an insulin sensitizer via the induction of HSP confirmed by preclinical data.

AIMS

The early recognition, diagnosis and appropriate treatment of the metabolic disorder are crucial to prevent the manifestation of 2DM.

Our aim was to study the relationship between the carbohydrate/energy homeostasis and the bone state, furthermore to study the therapeutic benefits of this relationship. We searched for the relationship between insulin sensitivity (IS) and BMD and the effect of changing glucose tolerance on this relationship. If the improvement of IR results in the increase of bone formation, why does the insulin sensitizer rosiglitazone increase the risk of fractures? We looked for the answer whether the fracture risk is in relation with the change of IS or only with the characteristic side effect of the drug, in the prediabetic state.

Our aim was to study the insulin sensitizing effect of the HSP-inducer BGP-15 with a new mode of action in different insulin resistant animal models (rabbit, rat) reflecting the prediabetic stages and to compare it with antidiabetic agents used in clinical practice.

We wanted to study the effect of BGP-15 on endothelial dysfunction in diabetic rat model.

Furthermore, our aim was to test BGP-15 with a new mode of action, which improves IR in a clinical study on insulin resistant volunteers.

We wanted to study the effect of BGP-15 on the IR inducing side effects of an antipsychotic drug used in everyday practice, olanzapine. Based on preclinical data BGP-15 had a beneficial effect on olanzapine-induced metabolic dysfunction.

METHODS

Experimental animals

We studied the insulin sensitizer effect of BGP-15 in adult, male, white New Zealand rabbits, weighing 3-3.5 kg as well as in insulin resistant Goto–Kakizaki (GK) rats weighing 220–320 grams. The assessment of the effect of BGP-15 on ex vivo relaxation of aortae was performed in male Sprague–Dawley rats weighing 270–290 grams. The animals were housed in an animal room (12-hr light/dark periods a day, at a temperature of 22–25 C° and humidity of 50–70%) and fed commercial laboratory chow and tap water ad libitum. They were used after a 2-week adaptation period.

Intravenous glucose tolerance test (IvGTT)

IvGTT was used to measure glucose induced insulin secretion, given that the effects can be avoided caused by the uncertainty of glucose absorption and gut hormones (incretins) during the intravenous glucose load. Iv glucose administration was performed after a 12 hour fast. Volunteers were given 0.3 g x kg $^{-1}$ bodyweight of 40% glucose intravenously. The blood sampling points were 0, 3, 5, 10, 15, 20, 30, 40, 50 and 60 min.

Hyperinsulinemic-euglycemic glucose clamp (HEGC)

Total body IS was determined by the HEGC method. Human insulin and 20% glucose were iv infused over a minimum of 120 min. Hyperinsulinemia (70-100mE/l) was maintained by a constant insulin infusion rate. The glucose infusion rate was adjusted to maintain blood glucose concentration at 5.5 ± 0.5 mmol l^{-1} (euglycemia). Glucose levels were checked every 5-10 min. When blood glucose was stabilized for 20 - 30 min, the steady state (equilibrium) condition appeared where the total body glucose utilization showed the degree of IS (M-value).

Homeostasis model assessment (HOMA)

HOMA-IR = [fasting insulin (mU l^{-1}) × fasting glucose (mmol l^{-1})] / 22.5. The higher the HOMA-IR value is, the worse the IR is.

The assessment of HOMA-BCF characterizes β-cell function: HOMA-BCF = [(20 × insulin (mU l^{-1}) / fasting glucose (mmol l^{-1}) –3.5].

The study of early abnormalities of glucose metabolism

The glucose utilization of peripheral tissues (total body, muscle and fat) were determined by HEGC technique in 291 subjects (aged between 18 and 60) and their relationship with the different metabolic parameters {area under the curve (AUC) calculated for glucose and insulin levels during oral glucose tolerance test (OGTT) and IvGTT, body mass index (BMI), triglyceride and total cholesterol}were studied. The study subjects were classified by OGTT based on their glucose tolerance. The healthy subjects with normal glucose tolerance (NGT)

were classified into NGT group, the subjects with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) were classified into glucose intolerant (GI) group and those with treatment naive 2DM were classified into 2DM group.

The human study of the relationship between IR and bone metabolism

We examined the effect of progressive insulin resistance on the relationship between glucose and bone metabolism in 20 healthy and 51 GI women. The parameters of carbohydrate and bone metabolism, bone mineral density (lumbar spine 1-4 and femoral neck) were measured, furthermore OGTT and HEGC were performed.

We also examined the relationship between glucose and bone metabolism in men. 22 NGT and 39 GI men participated in the study. OGTT, IvGTT and HEGC were done in order to determine glucose tolerance, insulin secretion and insulin sensitivity. Beyond blood sugar and lipid parameters adiponectin, leptin, osteocalcin (OCN), osteoprotegerin (OPG), sRANKL (soluble receptor activator of nuclear factor-κB ligand), sex hormon and dehydroepiandrosterone (DHEAS) were measured.

The body composition (muscle and fat content) was determined by DXA (dual-energy X-ray absorptiometry).

The study of the effect of BGP-15 on insulin sensitivity in insulin resistant subjects

IvGTT, HEGC and HOMA-IR were used to measure the insulin sensitizing effect of BGP-15. In order to meet the inclusion criteria of the study, insulin resistant (HOMA-IR > 2.5) males and females were supposed to be between 25 and 60 years of age; females were postmenopausal, or had undergone hysterectomy.

The volunteers were classified into IFG, IGT and drug naïve 2DM groups based on their OGTT results. The primary objective was the change in total body (M-1 value) and in muscle tissue (M-2 value) glucose utilization between days 0 and 28. The secondary objectives were HOMA-IR, a HOMA-BCF and insulin secretion during IVGTT ($AUC_{0\text{-}1h}$ of the insulin secretion). Exploratory analyses were performed on the following parameters: plasma FFA level, plasma glycerol and insulin level. The 28 day study consisted of 3 treatment groups (200 mg, 400 mg, placebo). IvGTT and HEGC tests were done on the 0 and 28 days.

The effect of BGP-15 on olanzapine-induced insulin resistance

We studied the effect of BGP-15 on the change in olanzapine-induced IS and on the change of body weight in healthy volunteers. Furthermore, the pharmacokinetic interactions between olanzapine and BGP-15 were assessed. The healthy volunteers were administered a 5mg beginning dose of olanzapine with either 400mg BGP-15 or placebo for 3 days, then they were administered a 10 mg dose of olanzapine with either 400mg BGP-15 or placebo for 14 days in this randomized

double-blind, placebo-controlled study. The primary efficacy variable was the area under the curve of steady state olanzapine plasma concentrations (AUC_{ss}). The secondary efficacy parameters were: the changes of body weight, M-values, HOMA-IR, HOMA-BCF between the first and the 18^{th} day.

In order to meet the inclusion criteria of the study, subjects (males and females were postmenopausal or had undergone hysterectomy) had to be between 18 and 55 years of age, with normal glucose metabolism and were supposed to be healthy based on physical examination.

RESULTS

Significant negative correlation was found between the total body (M-1), muscle tissue (M-2) and fat glucose utilization and glucose, insulin levels AUC (obtained from OGTT and IvGTT), BMI, triglyceride, total cholesterol levels in 291 patients.

Significant negative correlation was found between BMD and M-1 in healthy individuals, but this correlation ceased with the progression of glucose intolerance. Only the adiponectin showed correlation with BMD among the adipokines (leptin, adiponectin), while this relationship remained in lumbar spine, ceased in femoral neck with the worsening of glucose metabolism.

Strong positive correlation was found between OCN and testosterone in men, which was independent from age, BMI and body fat content. No correlation was observed between OCN and FSH, estradiol or DHEAS. Correlations observed between OCN and OGTT glucose

AUC, ivGTT glucose AUC, fasting FFA, HDL-cholesterol were unaffected if data were adjusted with serum testosterone, but disappeared between OCN and muscle glucose utilization.

Serum testosterone is ranked as 24th out of 67 variables influencing OCN, meaning that its influence is stronger than that of BFP, BMI, M-3, HbA_{1c}%, glucose levels obtained from OGTT standing backwards in the queue. Leptin, adiponectin, OPG and sRANKL proved to have stronger impact on OCN than testosterone in variable ranking.

Each BGP-15 doses induced a significant increase in insulin sensitivity in cholesterol-fed but not in normal rabbits with maximum values appearing on the 3rd and 5th days of the treatment, except for the lowest dose (5mg/kg). BGP-15 doses of 10 and 20 mg/kg were the most effective.

BGP-15 improved IS in a dose-dependent manner to the same extent as rosiglitazone and better than metformin in insulin resistant Goto-Kakizaki rats.

Both BGP-15 doses of 20 and 50 mg/kg improved endothelial dysfunction, prevented the impairment of vasorelaxation of aortae obtained from streptozotocin (STZ)-induced diabetic rats. The effect of BGP-15 is similar to that of rosiglitazone.

A 28-day treatment with BGP-15 significantly improved total body glucose utilization and muscle tissue glucose utilization compared to baseline in insulin resistant individuals. Both total body glucose utilization and muscle tissue glucose utilization increased significantly in both daily 200 mg and 400 mg BGP-15 dose groups compared to placebo.

The geometric means were similar in both BGP-15 plus olanzapine and placebo plus olanzapine groups, but due to the high variance of the data the 90% confidence interval of the geometric mean: [0.732, 1.327] did not entirely fit in the previously determined interval [0.8, 1.25]. Therefore, the absence of a pharmacokinetic interaction between olanzapine and BGP-15 could not be concluded.However, based on the data it is likely that the interaction, if exists is minimal.

There was a statistically significant increase in body weight from baseline in both active and placebo groups on day 18 with no significant differences between treatment arms. The means of M-1 and M-2 values significantly decreased in both groups by the end of the treatment period (Day 18). The difference between the two treatment groups was statistically significant in both total body glucose utilization (M-1: p=0.006) and muscle tissue glucose utilization (M-2: p=0,002).

Bone formation markers and indexes showed a significant increase, while the characteristic resorption marker cathepsin K and ß-crosslaps decreased after 28 days of treatment with BGP-15.

CONCLUSION

Although clamp method is labor-intensive and burdensome for the volunteers, it is the most accurate, the "gold standard" method for measuring IS. However, it is true that several techniques have been revealed to quantify IS that can be used in every day practice, today the clamp is considered to be precise and fits for scientific purpose, so primarily it is still not replaceable in early human clinical studies. The relationship between BMD and tissue glucose utilization (M-value) characterizing insulin sensitivity ceases with the worsening of glucose metabolism.

Based on our studies the role of OCN in the relationship between bone metabolism and energy homeostasis observed in animal models is confirmed in humans as well. The role of OCN in bone-pancreas axis is gender specific in humans because its effect on IS is mediated through adiponectin in women and testosterone in men. Based on our preclinical and clinical data BGP-15 has been proved as an insulin sensitizer with a new mode of action. BGP-15 doses of 200 and 400 mg significantly improved insulin dependent glucose uptake characterizing IS in insulin-resistant individuals in a 28-day study.

A short-term treatment with olanzapine applied globally in psychiatric disorders can cause IR. The BGP-15 treatment might be suitable for the protection of this complication because the administration of olanzapine plus 400mg BGP-15 significantly decreased the development of IR compared to placebo plus olanzapine group. However, BGP-15 did not prevent from weight

gain, which supposed to be due to the short treatment time.

The BGP-15-induced improvement of IR was accompanied by the beneficial changes in bone remodeling, while the increase of M-value is associated with bone formation. According to our data the increased risk of fractures related to the glitazones is rather characteristic for this drug family and cannot be related to the change of IS, strengthening the need for development of insulin sensitizers with a different mode of action.

LIST OF MY PUBLICATIONS

The publications, which are associated with the dissertation:

1. **Literáti-Nagy B**, Tory K, Peitl B, Bajza A, Korányi L, Literáti-Nagy Z, Hooper PL, Vígh L, Szilvássy Z. (2014) Improvement of insulin sensitivity by a novel drug candidate, BGP-15, in different animal studies. Metab Syndr Relat Disord. 12(2): 125-131 (IF: 1.916)

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1. Buday B, Pach FP, **Literati-Nagy B**, Vitai M, Vecsei Z, Koranyi L. (2013) Serum osteocalcin is associated with improved metabolic state via adiponectin in females versus testosterone in males. Gender specific nature of the bone-energy homeostasis axis. Bone 57(1): 98-104. (IF: 4.461)

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