

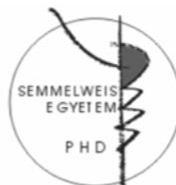
**EXAMINATION OF THE POTENTIAL ROLE OF
AN ANTIDIABETIC DRUG CANDIDATE -BGP-15-
IN THE TREATMENT OF SECOND
GENERATION ANTIPSYCHOTICS INDUCED
SIDE EFFECTS**

Doctoral (PhD) thesis

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Budapest
2013

INTRODUCTION

The incidence of metabolic disturbances is increasing worldwide due to lifestyle changes. Metabolic disorders significantly increase the risk of age related diseases, like cardio-vascular disorders, Alzheimer disease, and certain cancers.

According to WHO, nearly 54 million people suffer from some kind of severe mental illness. Severe psychiatric disorders, including schizophrenia, bipolar disorder principally treated with atypical antipsychotic drugs (AAPD), like clozapine (Clozaril), olanzapine (Zyprexa) and risperidone (Risperdal). However these drugs produce severe metabolic side effects, which lead to obesity, dyslipidemia, and glucose intolerance. These metabolic adverse effects result in poor compliance, manifestation of insulin resistance and type II diabetes, and an increased risk of cardiovascular complications. Currently these metabolic side effects cannot be effectively treated, which render a significantly more difficult application of second generation antipsychotic drugs.

AAPDs are supposed to be diabetogen molecules, thus the usage of antidiabetics have come to the front for the treatment of metabolic side effects. The conventional antidiabetics could not prevent these metabolic side effects. We have been examining the antidiabetic effects of hidroximic acid derivatives for a while. BGP-15 is a hidroximic acid, novel insulin sensitizer drug candidate, in human clinical phase II/b development stage. BGP-15 is the first drug candidate; its heat shock proteins (HSP)

inductive effect has been extensively examined and proved. BGP-15 improves insulin sensitivity through HSP induction. In the past two decades numerous effects of BGP-15 have been published. It seems that the drug candidate interferes with membrane hyper structures via their highly specific lipid interaction, sufficient to enhance stress signal.

Disturbances in lipid metabolism, lipid droplet accumulation, and endoplasmic retikulum play a role in promoting insulin resistance and diabetes. The activated c-jun amino terminal kinase (JNK) has great importance, as it increases serin phosphorylation of insulin receptor substrate (IRS), which inhibits insulin signaling. JNK activation can also be induced by chronic inflammation, mitochondrial dysfunction, and endoplasmic retikulum stress. It has been demonstrated that BGP-15 treatment diminishes the activation of JNK through increasing HSP72 protein expression, thus improves insulin sensitivity.

OBJECTIVES

Based on the well-known effects of the insulin sensitizer BGP-15 we supposed that BGP-15 can prevent the AAPD induced severe metabolic side effects. We aimed to confirm our hypothesis in different models, *in vitro*, *in vivo* studies. We proposed to prove the role of HSP72 induction with human *ex vivo* data as well.

1. The key role of adipocytes in the manifestation of type II diabetes is well known, thus we performed our experiments partly in *in vitro* differentiated 3T3-L1 adipocytes. The preadipocytes characteristic metabolic changes were shown by lipid droplet formation, which was forced with AAPD therapy. The role of lipid droplets in the development of diabetes is well known. Therefore we examined the effect of BGP-15 on an AAPD (clozapine) induced changes in lipid metabolism *in vitro* differentiated 3T3-L1 adipocytes.
2. For studying the possible protective effect of BGP-15 on a different system, we chose animal models. The AAPD therapy provoked metabolic disturbances in healthy, female Wistar rats, we administered risperidone for 21 days, clozapine for 2 months, clozapine for 35 days, and olanzapine for 5 days. We performed hyperinsulinemic euglycemic glucose clamp (HEGC) to measure the impairments and the effect of BGP-15. In these studies we also planned to compare the effect of BGP-15 and some currently

used antidiabetic drugs, metformin and rosiglitazone.

3. We examined the effect of the controversial anti-obesity drug, rimonabant and BGP-15 alone and also in combination in the genetic model of obesity and insulin resistance, in Zucker obese rats. We have done some comparative animal studies to evaluate the therapeutic indication and advantages of BGP-15 contrary to rimonabant.
4. The heat shock protein induction effect of BGP-15 in different models has been confirmed. It is a matter of question if this induction can be seen in humans, or it is rather related to its antidiabetic effect on AAPD induced insulin resistance. In phase I studies we planned to examine the correspondence between insulin resistance and HSP level changes in olanzapine + placebo or olanzapine + BGP-15 treated healthy volunteers' peripheral mononuclear blood cells.

METHODS

***In vitro* studies**

The adipogenesis of 3T3-L1 preadipocytes was induced as in (Giri et al 2006). The control cells were given only cultured medium, at the same, 13 μ M clozapine and 10 μ M BGP-15 were added to the cells. Three days later the flow cytometric analysis was made (FACS Calibur, Beckton Dickinson). A scatter plot of 3T3-L1 cells was gated into four regions based on their granularity distribution. The R1 region was gated to include the majority of control cells (where adipogenesis was not induced) on the SSC scale. Regions R2 to R4 were gated to include the remaining range of SSC, and each region contained an equal range of SSC.

***In vivo* studies**

The atypical antipsychotic studies were carried out with adult female Wistar rats (Charles River) weighing 210-230 g. The rimonabant and BGP-15 combination studies were carried out with adult male Zucker obese rats weighing 400-420g.

The rats were treated orally with olanzapine, clozapine, BGP-15 and rimonabant and subcutaneously with risperidone and capsaicin.

Hyperinsulinemic euglycemic glucose clamp (HEGC)

Following one night fasting, human regular insulin was infused at a constant rate (5-12 mU/kg/min) via a catheter inserted into one of the jugular veins for over 120

minutes. This insulin infusion rate was adjusted to procedure 100 μ U/ml in steady state in each species. Blood samples were taken from an arterial cannula introduced into one of the external carotis arteries for blood glucose concentration at 10 minutes intervals. Blood glucose concentration was maintained constantly (5,5 mmol/l) by a variable rate of glucose infusion. When blood glucose stabilized for at least 20 minutes, we defined this condition as steady state. The glucose infusion rate during steady state was used to characterize insulin sensitivity.

***Ex vivo* studies in part of the human clinical pharmacological study**

The 17-day long pharmacokinetic interaction clinical phase I study of BGP-15 and olanzapine combination was randomized, double blind and placebo controlled. Blood drawn at the beginning and end of the human study was biochemically examined. The HSP72 protein level of the peripheral monocytes was determined by Western blot method.

Capsaicin pre-treatment

1. day - 10mg/kg 2% capsaicin solution subcutaneously
 2. day - 30mg/kg 2% capsaicin solution subcutaneously
 3. day - 50mg/kg 2% capsaicin solution subcutaneously
- The systemic capsaicin pre-treated animals were further treated after a 7-day period of recovery.

RESULTS

Thesis summary of our original observations

- BGP-15 administration reduced fat accumulation induced by clozapine in 3T3-L1 preadipocytes. Distribution of BGP-15 treated cells and control cells was nearly equal on bases of their lipid contain.
- BGP-15 prevented clozapine, olanzapine and risperidone induced weight gain and insulin resistance in healthy Wistar rats. BGP-15 treatment alone did not have any effect on insulin sensitivity and weight of healthy animals.
- BGP-15 – contrary to conventional antidiabetics - significantly decreased olanzapine provoked weight gain and insulin sensitivity impairment.
- BGP-15 in combination with rimonabant produced a degree of insulin sensitization at much lower BGP-15 or rimonabant doses in the genetic model of insulin resistance, in Zucker obese rats.
- Systemic capsaicin pre-treatment itself significantly increased insulin sensitivity in Zucker obese rats, which cannot be further amplified by either BGP-15, rimonabant, or their combination.

- In phase I human clinical trial BGP-15 administration reversed the olanzapine induced drop in HSP72 protein expression in healthy volunteers' peripheral mononuclear blood cells.

CONCLUSIONS

Atypical antipsychotic drugs (AAPD) are mainstays in the treatment of severe psychiatric disorders. Metabolic side effects, obesity, hypelipidemia and glucose intolerance have been attributed to AAPDs, and these metabolic side effects are still unsolved. In this dissertation we examined the possibility of the treatment of AAPD induced metabolic side effects of the hidroximic acid, antidiabetic drug candidate BGP-15, in human clinical phase II/b development stage.

BGP-15 prevented AAPD side effects induced metabolic syndrome in cell culture as well as in animal models; moreover, BGP-15 had greater effect compared with conventional antidiabetics. According to our results we believe that it may be suitable to reduce the metabolic side effects of AAPD.

Based on our human studies the well-known chaperone induction effect of BGP-15 plays a role in the mechanism of antidiabetic effects, as demonstrated in the clinical trials. It is very likely that in the BGP-15 insulin sensitizer effect HSP72 plays a crucial role. AAPD induced reduction in HSP72 protein expression in peripheral mononuclear blood cells was prevented by BGP-15.

The Zucker obese experiments results confirm our previous finding that BGP-15 is a potent insulin sensitizer, and in combination with rimonabant a much lower dose of both drugs is sufficient to produce an insulin sensitization effect.

In conclusion, the second generation antipsychotics and BGP-15 combination might be a suitable treatment for psychiatric patients without the severe metabolic side effects of AAPDs.

PUBLICATIONS

Publications in the topic of the dissertation:

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ACKNOWLEDGEMENTS

I am very much indebted to Prof. József Mandl, director of the Institute, coordinator of the Pathobiochemistry Doctoral Programme, and to Prof. László Vígh, director of the Institute of Biochemistry of the BRC of the HAS for accepting me as a student and for their continuous support and who contributed to the success of my project with their indispensable help.

I would like to express my sincere thanks to Dr. Kálmán Tory for his scientific advice, support and generosity upon which I could always rely. I am grateful to Dr. Attila Kolonics, Dr. Ibolya Horváth, Dr. Zsolt Török, Dr. Gábor Balogh, and Prof. Zoltán Szilvássy for their bright ideas, and constructive criticism and to Mrs Turbók for her advice and help with my experiments.

Last but not least, many thanks to my Family, especially to my Father for backing my plans and aims, and providing a firm emotional background I could always rely upon.