METABOLIC SYNDROME AND RELATED DISORDERS
Volume 12, Number 2, 2014

Pp. 125–131
DOI: 10.1089/met.2013.0098

Improvement of Insulin Sensitivity by a Novel Drug Candidate, BGP-15, in Different Animal Studies

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Abstract

Background: Insulin resistance has been recognized as the most significant predictor of further development of type 2 diabetes mellitus (T2DM). Here we investigated the effect of a heat shock protein (HSP) co-inducer, BGP-15, on insulin sensitivity in different insulin-resistant animal models and compared its effect with insulin secretagogues and insulin sensitizers.
Methods: Insulin sensitivity was assessed by the hyperinsulinemic euglycemic glucose clamp technique in normal and cholesterol-fed rabbits and in healthy Wistar and Goto–Kakizaki (GK) rats in dose-ranging studies. We also examined the effect of BGP-15 on streptozotocin-induced changes in the vasorelaxation of the aorta in Sprague–Dawley rats.
Results: BGP-15 doses of 10 and 30 mg/kg increased insulin sensitivity by 50% and 70%, respectively, in cholesterol-fed but not in normal rabbits. After 5 days of treatment with BGP-15, the glucose infusion rate was increased in a dose-dependent manner in genetically insulin-resistant GK rats. The most effective dose was 20 mg/kg, which showed a 71% increase in insulin sensitivity compared to control group. Administration of BGP-15 protected against streptozotocin-induced changes in vasorelaxation, which was similar to the effect of rosiglitazone.
Conclusion: Our results indicate that the insulin-sensitizing effect of BGP-15 is comparable to conventional insulin sensitizers. This might be of clinical utility in the treatment of T2DM.

Introduction

Insulin resistance plays a major role in the development of prediabetes and overt type 2 diabetes (T2DM).[1] Furthermore, clinical and experimental evidence suggests that both diabetes and insulin resistance cause endothelial dysfunctions, which may diminish the antiatherogenic role of the vascular endothelium.[2–4] Diabetic endothelial dysfunction is synonymous with decreased endothelium-dependent vasorelaxation to acetylcholine.[5] A recent large randomized clinical study of patients with stable coronary artery disease and T2DM observed that addition of an insulin-sensitizing drug, a thiazolidinedione or metformin, reduced peripheral artery disease, revascularization surgery, and leg amputations—even when adjusting for improved glycemic control.[6]

The peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist the thiazolidinediones (TZDs), so-called insulin sensitizers, enhance insulin action in muscle and fat tissues. The major side effect, seen with troglitazone, the first TZD, is liver damage. Because of this dangerous side effect, the Food and Drug Administration (FDA) removed troglitazone from the market. Two other glitazones, pioglitazone and rosiglitazone, cause weight gain and fluid retention[7]; moreover, rosiglitazone is thought to increase the risk of cardiovascular events.[8] The side effects and the necessity for a safe and effective insulin sensitizer encourage development of compounds that improve insulin sensitivity through other mechanisms.

BGP-15 [O-(3-piperidino-2-hydroxy-1-propyl)-nicotinic acid], a hydroxylamine derivative, does not belong to the glitazone

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family. Receptor binding experiments with BGP-15 do not interfere with ligand-inducible PPAR-α, -γ, or -δ signaling.[9,10] The insulin-sensitizing effect of BGP-15 in *ob/ob* mice activates HSP72 in skeletal muscles and prevents c-jun amino-terminal kinase (JNK) phosphorylation and subsequent insulin resistance.[11] Furthermore, our previous results indicate that BGP-15 may have additional cardiovascular protective activity, *i.e.*, it prevented ischemia/reperfusion-induced heart injury and restored vascular responsiveness in insulin resistance.[12–14] Recent studies demonstrate that BGP-15 confers protection against rapid pacing-induced atrial fibrillation in a *Drosophila melanogaster* model of cardiac arrhythmias.[12,15]

The aim of this series of experiments was to assess the insulin-sensitizing effect of BGP-15 alone, and in combination as well, in comparison with oral antidiabetics such as metformin, rosiglitazone, and glibenclamide. Furthermore, we studied the therapeutic effect of BGP15 and rosiglitazone on vasorelaxation induced by acetylcholine in aortic preparations isolated from streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Experimental animals

Adult, male, white New Zealand rabbits, weighing 3–3.5 kg, adult male Wistar and Goto–Kakizaki (GK) rats weighing 220–320 grams and male Sprague–Dawley rats weighing 270–290 grams (aged 9–14 weeks), housed in an animal room (12-hr light/dark periods a day, temperature of 22–25°C, humidity of 50–70%) and fed commercial laboratory chow and tap water *ad libitum* were used after a 2-week adaptation period.

Food was withdrawn at least 12 hr prior to commencement of experiments to determine insulin sensitivity. A bolus injection of STZ 50 mg/kg intravenously (i.v.) was administered via tail vein 12 weeks before use.

Preparation of isolated thoracic aorta

Male Sprague–Dawley rats were anesthetized with sodium pentobarbital (Nembutal®, Sigma, 60 mg/kg). After thoracotomy, the thoracic aorta was removed and placed in ice-cold Krebs solution and continuously aerated with 95% O_2, 5% CO_2. The descending thoracic aorta was cut into rings approximately 5 mm in length. Ring preparations ($n = 4$) were set up in parallel for isometric tension measurement by maintaining the tissues under a resting preload tension of 2 grams, in 10-mL organ baths (thermostatically controlled for 37 ± 0.2°C) containing Krebs–Henseleit solution (pH 7.4) and maintained under oxygenation at 37°C. Precontraction of the aorta was induced by phenylephrine. This maximum constriction was previously determined by increasing dose of the α-adrenergic agonist. Maximum vasorelaxation was obtained by increasing the dose of acetylcholine to the precontracted vascular preparation. Tension measurement was carried out with Isosys System (EXP-D isolated organ amplifier, AIF-01 computer interface and IBM-compatible personal computer Experimetria Ltd., Budapest).

Hyperinsulinemic euglycemic glucose clamp

Whole-body insulin sensitivity was determined by hyperinsulinemic euglycemic glucose clamp (HEGC) method as described.[16] Human regular insulin was infused at a constant rate (13–15 mU/kg per min, Actrapid; Novo Nordisk, Copenhagen, Denmark) via one of the venous catheters over 120 min. This insulin infusion rate yielded plasma insulin immunoreactivity of 100 ± 5 μU/mL in the steady state. Blood samples (0.2 mL) were taken from the arterial cannula for blood glucose concentration measurement at 10-min intervals. Blood glucose concentration was maintained constant (5.5 ± 0.5 mmol/L) by a variable rate of glucose infusion via the second venous cannula. When blood glucose had stabilized for at least 20–30 min, this condition was defined as the steady state. In this state, additional blood samples (0.3 mL) were taken for plasma insulin determination three times at 10-min intervals. The glucose infusion rate (mg/kg per min) during the steady state was the measure of glucose disposal characterizing insulin sensitivity.

Effect of BGP-15 on insulin sensitivity in normal and cholesterol-fed rabbits

The rabbits were randomized into two groups. One group of the animals was continued to be fed normal rabbit chow, whereas the other group was fed chow enriched with 1.5% cholesterol over a period of 8 weeks. Each group was divided into six treatment groups ($n = 6$); one of them was untreated (without treatment) and the others were treated with BGP-15 at 5, 10, 20, 30, or 50 mg/kg *per os*, once a day for 5 days. BGP-15 was provided by N-Gene Research Laboratories Ltd. (Budapest, Hungary).

Effect of BGP-15, rosiglitazone and metformin on insulin sensitivity in healthy Wistar and in GK rats

The experiments were carried out with male Wistar and GK rats. Oral daily doses of BGP-15 (5–30 mg/kg), rosiglitazone (2 mg/kg Avandia®, SmithKline Beecham plc, Brentford Middlesex, UK), metformin (100 mg/kg, Adimet®, Ratiopharm, Hungary), and distilled water (control group) were applied for 5 days. Insulin sensitivity was assessed by HEGC prior to and at the end of the treatment period 4–6 hr after the last BGP-15 dose. Four groups were used to test the effect of BGP-15 (5, 10, 20, and 30 mg/kg) by the HEGC procedure, whereas two separate groups were used to test the effect of rosiglitazone (2 mg/kg) and metformin (100 mg/kg) by this method. The doses of these latter drugs were selected as described.[17]

Effect of BGP-15 on insulin sensitivity in combination with a sulfonylurea antidiabetic drug in GK rats

The animals were divided into six treatment groups of seven animals each. Animals were treated once a day orally with distilled water (control group), BGP-15 (3, 10, and 30 mg/kg) alone, glibenclamide (1 mg/kg Gilemal®, PannonPharma, Hungary) alone, and BGP-15 (10 mg/kg) in combination with glibenclamide (1 mg/kg) for 5 days. Control rats received 1.0 mL of distilled water. Insulin sensitivity was assessed by HEGC prior to and at the end of treatment period 4–6 hr after the last BGP-15/glibenclamide dose.

The effects of BGP-15 on ex vivo relaxation of aorta obtained from normal and STZ-diabetic rats

STZ-treated Sprague–Dawley rats were considered diabetic and retained for experiments if their blood glucose

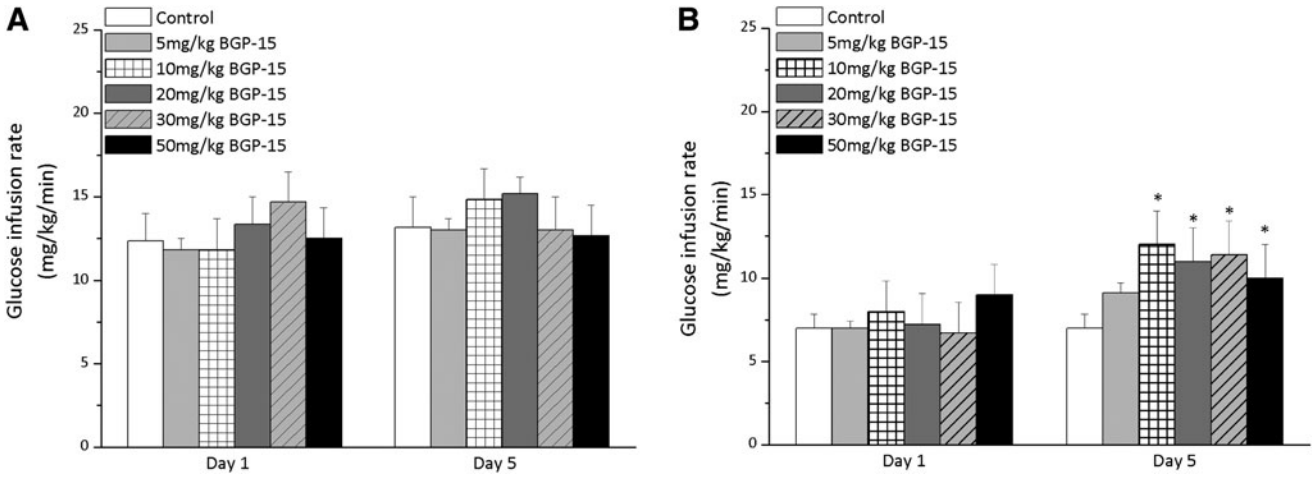

FIG. 1. Effect of BGP-15 on insulin sensitivity in normal **(A)** and cholesterol-fed rabbits **(B)**. The results are expressed as means ± standard deviation (SD) ($n = 6$ animals per group). (*) Significantly different from the control group, $P < 0.05$.

concentration was higher than 20 mmol/L. BGP-15 (in doses of 20 and 50 mg/kg), and rosiglitazone (4 mg/kg) were given to STZ diabetic rats orally over 5 days. Age-matched, saline-treated, male Sprague–Dawley rats served as controls.

Statistical analysis

The data were analyzed with one-way analysis of variance (one-way ANOVA) followed by the Student *t*-test modified according to the Bonferroni method for repeated measures.[18] The comparisons between treatment groups were calculated with one-way ANOVA pairwise analysis. Differences were considered statistically significant at $P < 0.05$.

Results

Insulin-sensitizing effects of BGP-15 in normal and cholesterol-fed rabbits

Repetitive oral doses of BGP-15 (once a day) induced a significant increase in insulin sensitivity during 5-day treatments with 10, 20, 30, and 50 mg/kg of BGP-15, respectively, in cholesterol-fed but not in normal rabbits. BGP-15 doses of 10 and 30 mg/kg were the most effective doses because these showed a 50% and a 70% increase in insulin sensitivity, respectively. The lowest dose studied, 5 mg/kg per day, had a moderate effect, but it did not reach the level of statistical significance (Fig. 1).

Effect of BGP-15, rosiglitazone, and metformin on insulin sensitivity in GK rats

All of the applied doses of BGP-15 showed a significant improvement of insulin sensitivity, which was dose-dependent up to the level of 20 mg/kg in genetically insulin-resistant GK rats. The most effective dose observed at 20 mg/kg of BGP-15 showed a 71% increase in glucose infusion rate after 5 days of treatment. While BGP-15 improved insulin sensitivity more than each of the other drugs on the market (rosiglitazone and metformin), BGP-15 proved statically superior to metformin and trended better than rosiglitazone (Fig. 2).

Effect of BGP-15 on insulin sensitivity in combination with glibenclamide in GK rats

The doses of 10 and 30 mg/kg BGP-15 caused a significant increase in peripheral glucose uptake after 5 days of treatment. BGP-15 (10 mg/kg) in combination with glibenclamide (1.0 mg/kg) also increased insulin sensitivity. This combined effect of BGP-15 and glibenclamide yielded a 97% increase in glucose infusion rate compared to the control group (Fig. 3).

The effects of BGP-15 on ex vivo *relaxation of aortae obtained from normal and STZ diabetic rats*

The decreased maximum vasorelaxation effect of acetylcholine in the 9- to 14-week-old STZ diabetic rats was normalized by 5 days of BGP-15 (20 and 50 mg/kg) and rosiglitazone (4 mg/kg) treatment. BGP-15 (20 mg/kg) produced a rosiglitazone-like effect both in shape and strength. Oral administration of BGP-15 in 20- or 50-mg/kg doses given for 5 days protected against STZ-induced diabetic changes in the vasorelaxation of the aorta. The effect of BGP-15 is comparable to that of rosiglitazone (Fig. 4).

Discussion

We evaluated the effect of BGP-15 on insulin sensitivity in New Zealand white rabbits, Wistar, and GK rats, and Sprague–Dawley rats in comparison with metformin, rosiglitazone, and glibenclamide. As an insulin sensitizer, BGP-15 outperformed metformin and equaled or superseded the rosiglitazone drug effect. Additionally, our compound proved to be effective in increasing insulin sensitivity when combined with a sulfonylurea agent. Five days of treatment with multiple different doses clearly showed the insulin-sensitizing effect of BGP-15 in cholesterol-fed rabbits but not in normal rabbits, which served as controls. This series of experiments confirmed that BGP-15 had a beneficial effect only in the insulin-resistant state.

BGP-15 treatment restored cholinergic vasorelaxation in aorta obtained from STZ diabetic rats. Vascular smooth muscle has been recently regarded as a nonclassical insulin-sensitive tissue. Diabetes impairs endothelium-dependent

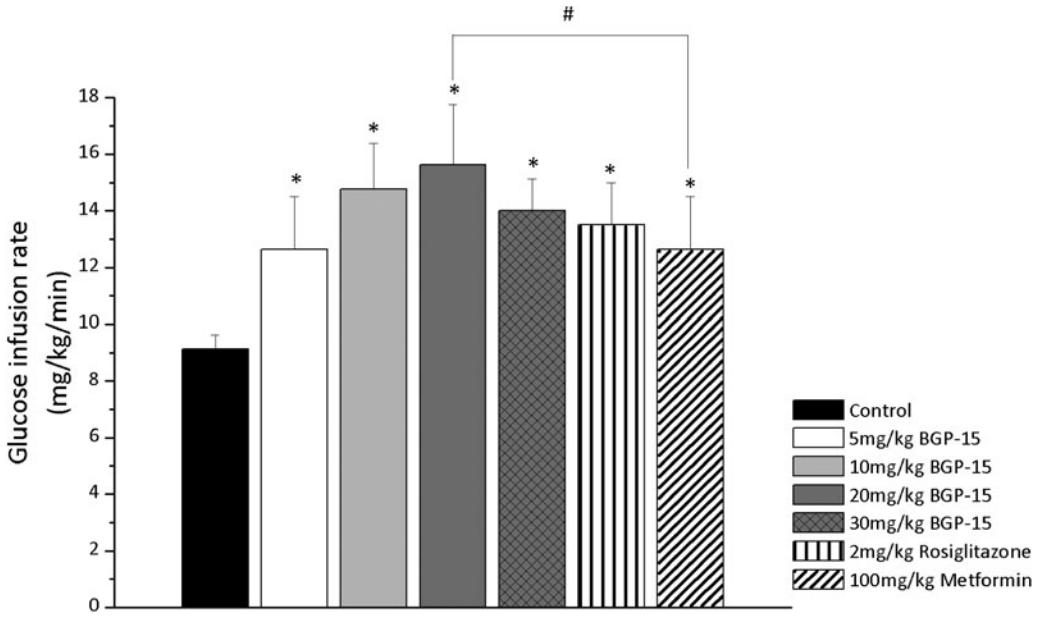

FIG. 2. Effect of BGP-15 on insulin sensitivity in Goto–Kakizaki (GK) rats with the hyperinsulinemic euglycemic glucose clamp (HEGC) method. Data are shown as means ± standard deviation obtained with six animals per group. (*) Significantly different from the control group, $P < 0.05$. (#) Significantly different from the metformin group, $P = 0.025$. Insulin sensitivity improvement with BGP-15 (20 mg/kg) compared to rosiglitazone showed a trend to a greater effect with BGP-15 ($p = 0.07$), but the improvement was not statistically significant. Data were analyzed with one-way analysis of variance (ANOVA) followed by the Student *t*-test modified according to the Bonferroni method for repeated measures. The comparisons between treatment groups were calculated with one-way ANOVA pairwise analysis.

vasorelaxation,[5] which can be prevented by rosiglitazone administration.[19] Rosiglitazone may act through increased nitric oxide (NO) bioavailability.[19] It has been demonstrated that insulin may restore neuronal nitric oxide synthase (NOS) expression in STZ-induced diabetic rats.[20] Moreover, restoration of acetylcholine vasorelaxation by BGP-15 could be extinguished by 7-nitro-indazole, an inhibitor of neuronal NOS.[21] These data are in line with our observation that BGP-15 can induce the expression of neuronal NOS in endothelial cells and that the BGP-15–induced increase in insulin sensitivity can be abolished by 7-nitro-indazole (unpublished). It is suggested that BGP-15 may ameliorate diabetes-induced impairment in vasorelaxation through a neuronal NOS-dependent mechanism.

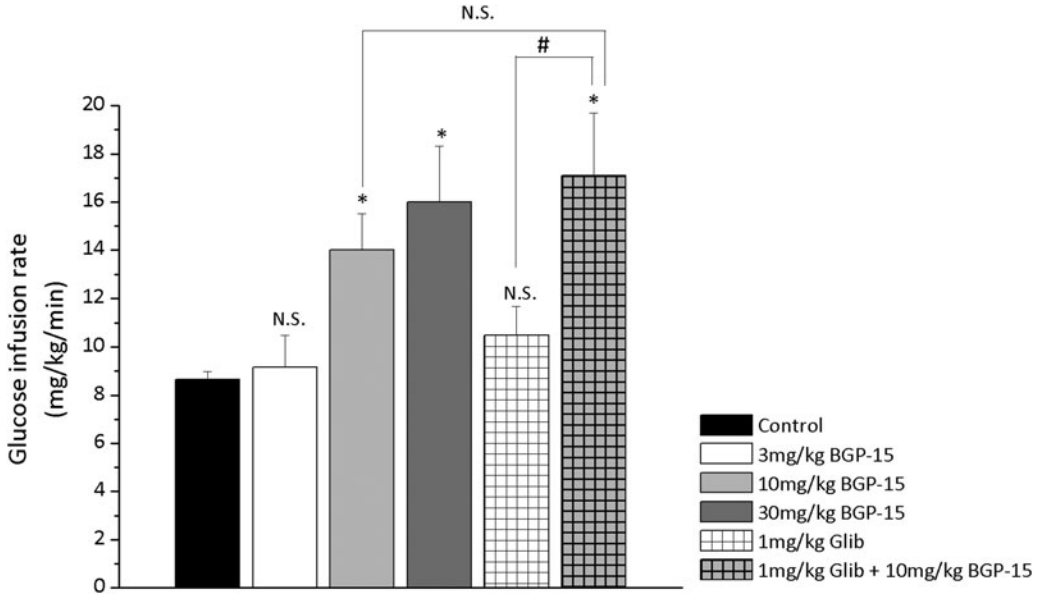

FIG. 3. The combined effect of BGP-15 and glibenclamide (Glib) on insulin sensitivity was measured by hyperinsulinemic euglycemic glucose clamp in Goto–Kakizaki insulin-resistant rats. Data are mean ± standard deviation (SD) ($n = 7$ animals per group). (*) Significantly different from the control group. (#) Shows a significant difference BGP + glibenclamide versus glibenclamide alone, $P < 0.05$. N.S., not significant.

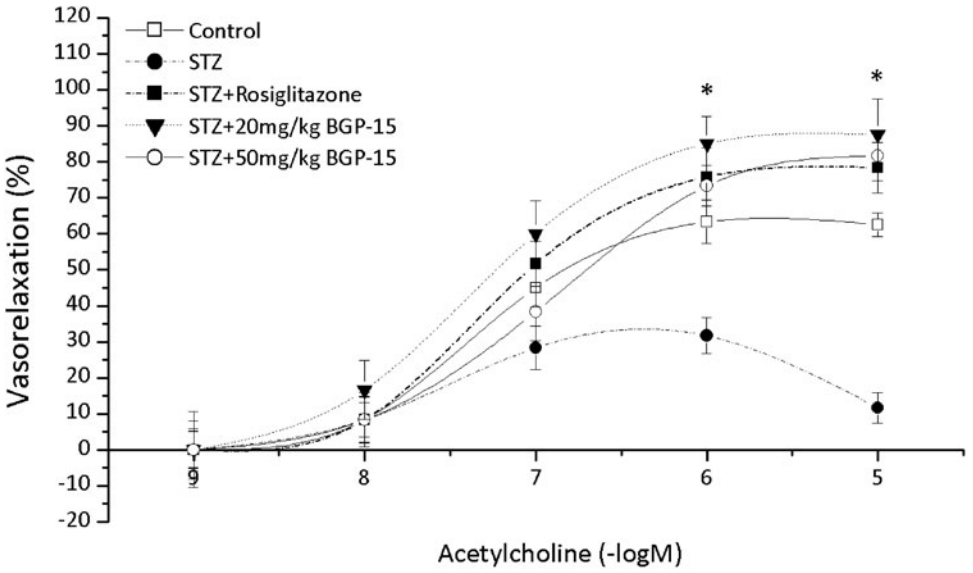

FIG. 4. The percentage of vasorelaxation by acetylcholine was calculated as 100% of the maximal contraction at steady state induced by phenylephrine EC_{50} (phenylephrine concentration producing 50% of maximal tension). Data were expressed as means ± standard error of the mean (SEM), and analyzed with one-way analysis of variance (ANOVA) followed by multiple comparison *post hoc* tests. (*) Significantly different from the streptozotocin (STZ) group, $P < 0.05$.

Hydroximic acid derivatives represent a new class of insulin sensitizers and have entered phase II clinical studies.[22] BGP-15 is the first of a new class of insulin sensitizers of the hydroximic acid derivative family, also known as "chaperone inducers" or "coinducers."[23–25] Mode of action studies showed that BGP-15 increases the expression of heat shock proteins (HSP-90α, HSP-72) and constitutive (c) NOSs (endothelial NOS, neuronal NOS) in cell cultures.[26] BGP-15 inhibited JNK and increased the level of HSP activity of cNOS *in vitro* and *in* vivo.[26,27] Decreased HSP expression is one of the cellular changes characteristic of diabetes.[28–30] Not only are the levels of HSP72 decreased in T2DM,[29–31] but whole-body glucose utilization is significantly correlated with HSP72 levels.[29] HSP72 acts on insulin sensitivity by inhibiting the inflammatory cascades, which activate the IκB kinase β (IKK-β) and the inflammatory signaling protein JNK, which can phosphorylate on tyrosine residues of insulin receptor substrates and thus inhibit insulin signaling.[25,32] Inflammation via the JNK pathway plays a pivotal role in the development of insulin resistance.[33] Furthermore, BGP-15 enhances mitochondrial biogenesis and improves mitochondrial dysfunction in pathological metabolic states.[34]

The HSP chaperone induction effect of hydroximic acid derivatives is based upon a direct interaction with heat shock factor-1 (HSF-1).[35] This key transcription factor induces expression of heat shock proteins; they bind to HSF-1 and stabilize the binding of HSF-1 to its DNA response element.[35] The importance of HSF-1, HSPs, and inflammation as they relate to insulin signaling and T2DM has been recognized recently.[36] The role of HSP-72 in the development of insulin resistance was demonstrated by Chung et al. in knockout *ob/ob* mice. A 15-day-long BGP-15 treatment significantly reduced fasting blood glucose and insulin levels and increased insulin sensitivity as assessed by glucose clamps.[11]

Where does BGP-15 fit into diabetic therapeutic armamentarium? Insulin resistance appears before the development of T2DM, a disease that often is accompanied by serious long-term complications and eventually heavy health economic consequences due to the tremendous cost of treatment. Numerous large studies have shown that the best way to reduce the expenditure is through prevention during the "prediabetic" stage or in the early stage of diabetes through change in lifestyle or pharmaceutical intervention.[37] Although changes in lifestyle have a greater positive effect on the restoration of insulin sensitivity, the more readily accepted insulin sensitizer drugs have had a big impact not only on the treatment but on disease prevention as well. Among insulin sensitizers, metformin[38] and the TZDs have been tested in these fields. The adverse effects of PPAR agonists such as TZDs have become an increasing problem in diabetes patients with cardiovascular disease (CVD). Rosiglitazone therapy was associated with a significantly increased risk of heart attack [odds ratio (OR) = 1.43, 95% confidence interval (CI), 1.03–1.98; $P = 0.03$), and an even higher risk of death from all cardiovascular diseases (OR = 1.64)[8].

Pioglitazone treatment, in contrast, showed significant protection from all-cause mortality.[39] However data regarding the increased risk in cardiovascular events with TZDs are still inconsistent. Recently pioglitazone was significantly associated with an increased risk of bladder cancer.[40]

Glucagon-like peptide-1–based therapy is gaining widespread use for T2DM, although there are concerns about risks for pancreatitis and pancreatic and thyroid cancers. There are also concerns that dipeptidyl peptidase-4 inhibitors might be oncogenic, given their effects on immune function,[41] but they need further elucidation.

Our results suggest that BGP-15 could be a promising drug candidate for improving insulin resistance and thus T2DM. Moreover it could be therapeutic in the prevention of T2DM and in the treatment of diabetic vascular complications. Our findings support the need for future investigations of BGP-15 therapy of T2DM patients in phase II–III studies, administered alone or combined with other agents.

Author Disclosure Statement

No competing financial interests exist.

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