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Effect of sitagliptin treatment on metabolism and cardiac function in genetic diabetic mice

Bianca Hemmeryckx [a], Melissa Swinnen [b], David J Gallacher [c], Hua Rong Lu [c], H. Roger Lijnen [a,*]

[a] Center for Molecular and Vascular Biology, KU Leuven, Leuven, Belgium
[b] Division of Clinical Cardiology, KU Leuven, Leuven, Belgium
[c] Translational Sciences, Safety Pharmacology Research, Janssen Research & Development, Janssen Pharmaceutical NV, Beerse, Belgium

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ABSTRACT

To investigate the chronic effect of sitagliptin (7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-(3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate, SIT) on metabolism and cardiac function in genetic diabetic Akita mice, 10 weeks old Akita mice were either exposed for 4 months to a high fat and high cholesterol (HF–HC) diet, with or without 10 mg/kg/day SIT, or were fed for 3 months with the same diet with or without 50 mg/kg/day SIT. SIT treatment of Akita mice at either a low or high dose did not affect body or liver weight. A significant increase in subcutaneous and gonadal fat mass was only observed for the 50 mg/kg/day dose of SIT. Furthermore, only the 50 mg/kg/day SIT dose resulted in an improvement of glycemic control, as evidenced by a decrease in fasting blood HbA1c levels and an increase in plasma adiponectin levels. Echocardiographic analysis revealed that Akita mice kept on the HF–HC diet with 10 mg/kg/day of SIT for 4 months showed an increase in ejection fraction and fractional shortening, whereas the higher dose (50 mg/kg/day) had no effect on these parameters, but instead induced left ventricular (LV) hypertrophy as evidenced by an enlarged LV internal diameter, volume and mass. Thus, in the diabetic Akita mouse SIT is cardioprotective at a low dose (10 mg/kg/day), whereas improvement of glycemic control requires a higher dose (50 mg/kg/day) which, however, induces LV hypertrophy. This mouse model may thus be useful to study the safety of anti-diabetic drugs.

1. Introduction

Diabetes mellitus (DM) has reached epidemic proportions: globally, in 2010 an estimated 285 million people suffered from this metabolic disease (Shaw et al., 2010); 90% of DM patients is type 2 (T2DM) (Zimmet et al., 2001). The pathology of T2DM is characterized by defects in insulin sensitivity and secretion, resulting in hyperglycemia (Taylor, 1999). Current therapies for T2DM are associated with adverse drug effects such as hypoglycemia (Micheli et al., 2012), weight gain (Quinn et al., 2008), gastrointestinal problems (Stein et al., 2013), liver toxicity (Marcy et al., 2004) and increased risk for cardiovascular diseases (Nesto et al., 2003). To better understand the molecular mechanisms underlying these complications, suitable preclinical rodent models are needed. In a previous study, we have used the type 1 and type 2 diabetic Akita mouse model to establish side effects of the thiazolidinedione rosiglitazone (RGZ), including weight gain and increased risk for cardiovascular disease (Hemmeryckx et al., 2013). These mice are heterozygous for a spontaneous mutation (C96Y) in the insulin 2 gene, which prevents proper folding of the insulin protein and results in subsequent pancreatic β-cell endoplasmatic reticulum stress and apoptosis. Akita mice develop hyperglycemia, hypoinsulinemia, polydipsia and polyuria at an age of 3–4 weeks (Hong et al., 2007; Yoshioka et al., 1997). Consequently, insulin production/secretion is very low. In humans, type 1 diabetes is an auto-immune disorder. It is believed that the disease develops due to destruction of the pancreatic β-cells by auto-antibodies evoked by viruses or environmental toxins. As a result, insulin production/secretion is very low or nonexistent. Although the pathophysiology of the disease in humans and the Akita mouse model differs, the outcome is very similar: a very low level of insulin production/secretion. Insulitis however, induced by an auto-immune response in type 1 diabetic patients leading to β-cell death and hypoinsulinemia, does not occur in the Akita mice. In addition, they show symptoms of human type 2 diabetes such as insulin resistance in skeletal muscle (caused by reduced glucose transporter-4 levels), liver and brown adipose tissue that is

* Correspondence to: Center for Molecular and Vascular Biology, KU Leuven, Campus Gasthuisberg, CDG, Herestraat 49, Box 911, B-3000 Leuven, Belgium. Tel.: +32 16 345771; fax: +32 16 345990.
E-mail address: roger.lijnen@med.kuleuven.be (H. Roger Lijnen).

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partly due to hyperglycemia (Hong et al., 2007; Yoshioka et al., 1997). However, in T2DM patients insulin-stimulated glucose uptake is reduced and the insulin resistance in skeletal muscle and liver is associated with an increased intracellular lipid accumulation, while in Akita mice the opposite pattern is observed (Hong et al., 2007). The reduced amount of lipids in muscle and liver may be explained by the increased whole body lipid oxidation seen in these mice. A species-associated difference in lipid metabolism therefore exists. To compensate for the altered lipid metabolism, Akita mice display an increase in insulin-stimulated glucose uptake, which allows for glucose to be directed to lipogenesis in white adipose tissues. To further validate this diabetic mouse model in testing the safety of anti-diabetic drugs, sitagliptin (7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-(3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate, SIT, Januvia®) was administered at two physiological doses (10 and 50 mg/kg/day). SIT is presumed to have a greater impact on type 2 diabetes than RGZ, as its capacity to improve overall glycemic control is higher, and it restores pancreatic islet cell mass (Mu et al., 2009). SIT selectively inhibits the activity of the enzyme dipeptidyl peptidase 4 (DPP-4), leading to increased circulating levels of the incretin hormone glucagon-like peptide 1 (GLP-1) and subsequent promotion of insulin secretion and normalization of blood glucose (Deacon, 2007).

2. Materials and methods

2.1. Animal model

Male Akita ($Ins2^{Akita}$; JAX003548) mice in a C57Bl/6J genetic background were purchased from Jackson Laboratories (Bar Harbor, MN, USA). At the age of 10 weeks, mice kept in individual microisolation cages on a 12 h day/night cycle at 20–22 °C, were started for 4 months on 5 g/day of a high fat-high cholesterol (HF–HC) diet (Ssniff EF Clinton/Cybulsky (II) mod. 19.5% fat and 1.25% cholesterol; E15751-30, Ssniff Spezialitäten GmbH, Soest, Germany), supplemented with ($n=8$) or without ($n=11$) 10 mg/kg/day SIT (MSD Ltd., Hertfordshire, UK). In a second study, 10 weeks old Akita mice were kept for 3 months on the HF–HC diet ad libitum with ($n=9$) or without ($n=10$) 50 mg/kg/day SIT. Water was always available ad libitum. Body weight and food intake were measured weekly and daily in the two studies, respectively. Before and at the end of the studies, after a fasting period of 6 h, blood was collected on 3.8% citrate via the retro-orbital sinus to measure fasting blood glucose and glycohemoglobin (HbA1c) levels. Plasma samples were prepared for determination of insulin, adiponectin, glucagon, and GLP-1 levels, and for active DPP4 levels. For comparison, some parameters (insulin and adiponectin) were determined for 10 weeks old wild-type (WT) C57BL/6J male mice (Janvier, Le Genest Saint Isle, France). In addition, transthoracic echocardiographic examinations were performed in anesthetized mice (2% isoflurane; Ecuphar, Oostkamp, Belgium) at the start and end of the treatment, using a MS 400 transducer (18–38 Mhz) (Visualsonics Inc., Toronto, Canada) on a Vevo 2100 equipment. Left ventricle (LV) diameter at end-diastole (LVIDd) and end-systole (LVIDs), muscle thickness in diastole (IVSd) and in systole (IVSs) and LV posterior wall thickness in end-diastole (LVPWd) and end-systole (LVPWs) were measured, and fractional shortening (FS), LV mass, end-diastolic volume (EDV) and end-systolic volume (ESV), ejection fraction (EF), stroke volume (SV) and cardiac output (CO) were calculated (Stypmann et al., 2009).

Mice were euthanized by *ip* injection of 60 mg/kg Nembutal (Abbott Laboratories, North Chicago, IL, USA). Inguinal subcutaneous (SC) and gonadal (GON) fat pads, liver, and heart were removed, weighed and stored for further analysis. The tibia length was also recorded.

All animal experiments were approved by the local ethical committee (KU Leuven P091/2010) and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996).

2.2. Assays

Glycosylated hemoglobin (HbA1c) in plasma was measured by ion exchange HPLC (Selvin et al., 2005). SIT levels in plasma and extracts of heart tissue were determined by LC–MS/MS (courtesy of Janssen Pharmaceutica N.V., Beerse, Belgium). Blood glucose concentrations were measured using Glucocard strips (Menarini Diagnostics, Firenze, Italy). Plasma levels of insulin (Mercodia, Uppsala, Sweden), adiponectin and glucagon (R&D Systems Europe, Lille, France), GLP-1 (Cusabio, Wuhan, China) and active DPP4 enzyme levels (Enzo Life Sciences, Antwerpen, Belgium) were measured using commercial ELISA's. Hematocrit levels in blood samples were determined using a Cell-Dyn 3500R (Abbott Diagnostics, Abbott Park, IL, USA). Plasma volume was estimated as 1 minus the hematocrit value measured at the end of the study multiplied by the estimated blood volume (8% of total body weight) (Ortiz et al., 2002; Reynolds, 1953).

2.3. Statistical analysis

Data are shown as means ± S.E.M. for the number of animals studied. Statistical significance between groups is evaluated by non-parametric Mann–Whitney *U*-test. Values of $P<0.05$ are considered statistically significant.

3. Results

3.1. Effect of SIT on body composition and metabolic parameters

The body weight of 10 weeks old Akita mice was slightly lower than that of age-matched WT mice (20 g ± 0.17 g versus 21 ± 0.28 g, $P=0.04$). Diabetic status was confirmed by higher fasting blood glucose (369 ± 14 mg/dL versus 104 ± 8.3 mg/dL, $P<0.0001$) and glycohemoglobin levels (6.8 ± 0.09% versus 2.6 ± 0.052%, $P<0.0001$), as well as hypoinsulinemia (0.078 ± 0.010 ng/μl versus 0.22 ± 0.088 ng/μl, $P>0.05$) in Akita as compared to WT mice.

Upon HF–HC diet feeding, the body weight of the Akita mice increased to 24 ± 0.50 g ($P<0.0001$) after 3 months and to 24 ± 0.40 g ($P<0.0001$) after 4 months. Co-administration of SIT at a dose of 10 mg/kg/day during 4 months or of 50 mg/kg/day during 3 months did not affect food intake, total body weight, liver weight, heart weight or tibia length (Table 1). Whereas the lower dose of SIT did not affect SC or GON fat mass, administration of the higher dose resulted in a significantly increased SC ($P=0.04$) and GON ($P=0.01$) adipose tissue mass (Table 1).

Fasting blood glucose and plasma insulin and glucagon levels were not affected by either dose of SIT, whereas at the higher dose blood HbA1c and plasma GLP-1 levels were decreased and plasma adiponectin concentrations increased as compared to HF–HC diet feeding without addition of SIT (Table 2).

3.2. Effect of SIT on cardiac function

Echocardiographic analysis revealed that the hearts of Akita mice upon HF–HC loading showed a significant reduction in FS and EF, due to an increase in diastolic and systolic LVID and LV volume (EDV/ESV), resulting in a higher LV mass. In addition, SV and CO were also enhanced by the diet. These diet-induced changes in cardiac parameters were more pronounced after 4 as compared to 3 months (Table 3).