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The novel dipeptidyl peptidase-4 inhibitor teneligliptin prevents high-fat diet-induced obesity accompanied with increased energy expenditure in mice

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ABSTRACT

Dipeptidyl peptidase-4 (DPP-4)-deficient mice exhibit prevention of obesity with increased energy expenditure, whereas currently available DPP-4 inhibitors do not induce similar changes. We investigated the impact of the novel DPP-4 inhibitor teneligliptin on body weight, energy expenditure, and obesity-related manifestations in diet-induced obese mice. Six-weeks-old C57BL/6N mice were fed a high-fat diet (60% kcal fat) *ad libitum* and administered teneligliptin (30 or 60 mg/kg) *via* drinking water for 10 weeks. Mice fed a high-fat diet showed accelerated body weight gain. In contrast, compared with the vehicle group, the administration of teneligliptin reduced body weight to 88% and 71% at dose of 30 mg/kg/day and 60 mg/kg/day, respectively. Although there was no change in locomotor activity, indirect calorimetry studies showed that teneligliptin (60 mg/kg) increased oxygen consumption by 22%. Adipocyte hypertrophy and hepatic steatosis induced by a high-fat diet were suppressed by teneligliptin. The mean adipocyte size in the 60-mg/kg treatment group was 44% and hepatic triglyceride levels were 34% of the levels in the vehicle group. Furthermore, treatment with teneligliptin (60 mg/kg) reduced plasma levels of insulin to 40% and increased the glucose infusion rate to 39%, as measured in the euglycemic clamp study, indicating its beneficial effect on insulin resistance. We showed for the first time that the DPP-4 inhibitor prevents obesity and obesity-related manifestations with increased energy expenditure. Our findings suggest the potential utility of teneligliptin for the treatment of a broad spectrum of metabolic disorders related to obesity beyond glycemic control.

1. Introduction

The World Health Organization estimates that >346 million people have diabetes. Diabetes is a metabolic disorder characterized by hyperglycemia resulting from decreased insulin secretion and increased insulin resistance. Long-term hyperglycemia can cause damage to various organs and thereby lead to the development of coronary artery disease, cerebrovascular disease, retinopathy, nephropathy, and neuropathy (Nathan, 1993). One of the most important risk factors for the pathogenesis of diabetes is obesity and insulin resistance associated with it. Moreover, some studies have provided empirical support for the assertion that modest weight losses of 5–10% of the initial weight are sufficient to produce significant, clinically relevant improvements in risk factors for cardiovascular disease in overweight and obese patients with type 2 diabetes (Van Gaal et al., 2005; Wing et al., 2011).

Dipeptidyl peptidase-4 (DPP-4) is a ubiquitously expressed transmembrane glycoprotein that cleaves N-terminal dipeptides from a variety of substrates, including growth factors and hormones, neuropeptides, and chemokines (Yazbeck et al., 2009). A substrate of DPP-4, glucagon-like peptide-1 (GLP-1), is released from L cells in the intestine after oral ingestion of nutrients and plays a key role in the regulation of insulin secretion and glucose homeostasis (Ahren et al., 1997; Drucker, 2007; Holst, 2002; Meier and Nauck, 2006). GLP-1 is rapidly degraded by DPP-4, resulting in a circulating half-life of only 1–2 min (Lambeir et al., 2003; Zander et al., 2002). Thus, inhibition of DPP-4 represents a therapeutic approach for the treatment of type 2 diabetes. Many DPP-4 inhibitors have emerged as agents to improve glycemic control in patients (Ahren, 2007; Chien et al., 2004; Mest and Mentlein, 2005).

Abbreviations: DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; RT-PCR, real-time polymerase chain reaction; O_2 consumption, oxygen consumption; UCP-3, uncoupling protein-3

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In humans, plasma levels of DPP-4 are correlated with obesity-related parameters such as body mass index, adipocyte cell size, and plasma levels of insulin (Lamers et al., 2011). Mice lacking the gene encoding DPP-4 exhibit not only improvements in glycemic control (Marguet et al., 2000) but also prevention of diet-induced obesity with increased energy expenditure (Conarello et al., 2003). Although most previously identified DPP-4 inhibitors reportedly have neutral effects on body weight both in animals and humans (Holst and Deacon, 2013; Kern et al., 2012; Liu et al., 2009; Omar et al., 2013), several DPP-4 inhibitors reportedly have anti-obesity effects in high-fat diet-induced obese mice. In contrast, the increase in energy expenditure caused by these inhibitors has not been shown (Dobrian et al., 2011; Kim et al., 2012; Lamont and Drucker, 2008; Shimasaki et al., 2013). We recently reported that the novel, potent, and long-lasting DPP-4 inhibitor teneligliptin improved postprandial hyperglycemia and dyslipidemia in Zucker fatty rats (Fukuda-Tsuru et al., 2012). In the present study, we investigated the effect of teneligliptin on body weight gain, energy expenditure, and obesity-related manifestations in diet-induced obese mice.

2. Materials and methods

2.1. Chemicals

Teneligliptin hydrogenbromide hydrate (3-[(2*S*,4*S*)-4-[4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-yl-carbonyl] thiazolidine hemipentahydrogenbromide hydrate) was synthesized in Mitsubishi Tanabe Pharma Corporation to $>95\%$ purity as confirmed by high-performance liquid chromatography (HPLC) analyses. Dosages are expressed in terms of the free base.

2.2. Animals and diets

All the procedures for animal experiments were approved by the Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation (Osaka, Japan).

Five-weeks-old male C57BL/6N mice were purchased from Charles River Laboratories, Inc. Mice were individually housed and maintained in a room with a 12-h light/12-h dark cycle. The room temperature and humidity were controlled at $20-25\ ^{\circ}C$ and 45–65%, respectively. The animals were provided with food and water *ad libitum* (as described below) in a specific pathogen-free environment. The mice were allowed to acclimatize for 5 days. A purified-ingredient high-fat diet with 60% kcal fat (D12492, 5.24 kcal/g; Research Diets, New Brunswick, NJ, USA) was used to exacerbate obesity and insulin resistance. A 10% kcal fat diet (D12450B, 3.85 kcal/g) was used as a normal diet.

2.3. Experimental protocol

Ten mice were fed a normal diet and 30 mice were fed a high-fat diet from 5 to 16 weeks of age. Five days after the initiation of feeding of a high-fat diet, 30 mice were randomly divided into the vehicle-treated group and teneligliptin (30 mg/kg or 60 mg/kg)-treated group on the basis of plasma levels of glucose, insulin and triglyceride as well as body weights. We confirmed that the dose of teneligliptin used in the present study increased plasma levels of active GLP-1 after a 10-weeks treatment in the preliminary study (1.00 ± 0.00 *vs.* 2.67 ± 0.49 pmol/l and 4.10 ± 1.29 pmol/l for vehicle *vs.* 30 and 60 mg/kg), and we did not observe drug-induced toxicity up to 300 mg/kg in the toxicity study. The mice were allowed to freely access drinking water free from teneligliptin (vehicle) or water containing teneligliptin for 10 weeks from 6 weeks of age. The initial day of treatment with teneligliptin was defined as day 1. The concentrations of teneligliptin in drinking water were calculated from the daily water consumption and mean body weight (which were measured 1 week before the preparation of the drinking water for each group). Drinking water was changed once a week because teneligliptin was found to be stable in water for a period of 1 week. Body weight and gross weight of the feeders and water bottles were measured once a week in the morning. Blood was collected on week 9 from the tail vein under non-fasting conditions to measure plasma levels of insulin, glucose, and triglyceride and on weeks 0 (pre), 3, 6, and 9 to measure DPP-4 activity. On week 10, energy expenditure was measured as described below. On the final day, the animals were weighed, and blood was then collected from the posterior vena cava using a heparin-containing syringe under ether anesthesia to measure the other plasma parameters described above. After blood collection, the liver, mesenteric white adipose tissues, epididymal white adipose tissues, and the soleus muscle were removed and their weight was measured. These organs were processed for the determination of lipid parameters, histopathological examination, and real-time polymerase chain reaction (RT-PCR).

2.4. Plasma parameters

Plasma levels of glucose, triglyceride, insulin, leptin, resistin, and high-molecular-weight adiponectin were determined using the Glucose CII-Test Wako Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Triglyceride E-Test Wako Kit (Wako Pure Chemical Industries, Ltd.), Mouse Insulin Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), Mouse Leptin ELISA Kit (Morinaga Institute of Biological Science, Inc.), Resistin Mouse ELISA Kit (Millipore, MA, USA), and Mouse/Rat High Molecular Weight Adiponectin ELISA Kit (Shibayagi, Gunma, Japan).

2.5. DPP-4 inhibition assay

In the DPP-4 inhibition assay, 20 μl plasma (10-fold diluted with assay buffer; phosphate-buffered saline containing 0.003% Brij-35 solution) and 20 μl glycyl-L-proline-4-methyl-coumaryl-7-amide (final concentration, 25 μmol/l; Peptide Institute, Osaka, Japan) were mixed with 160 μl of assay buffer to initiate the enzyme reaction, as described previously (Fukuda-Tsuru et al., 2012). After 1 h at 37 °C, the fluorescence intensity of 7-amino-4-methylcoumarin generated from glycyl-L-proline-4-methyl-coumaryl-7-amide was measured using an automated microplate reader at an excitation wavelength of 360 nm and emission wavelength of 465 nm. The fluorescence intensity of 7-amino-4-methylcoumarin corresponded to DPP-4 activity.

2.6. Energy expenditure

Oxygen consumption (O_2 consumption; VO_2) was measured for 4 h under fasting conditions and then for approximately 19 h under feeding conditions using the Oxymax System (Columbus Instruments, Columbus, OH, USA) during the last week of the treatment. O_2 consumption was measured by placing the animals and cages in chambers (1 animal/chamber). Data were normalized to body weight, as described previously (Conarello et al., 2003). Simultaneously, diurnal locomotor activity was measured using the ACTIMO Infrared Beam Sensor System (Bio Research Center, Nagoya, Japan).