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Dipeptidyl peptidase-4 inhibitor anagliptin ameliorates diabetes in mice with haploinsufficiency of glucokinase on a high-fat diet

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ABSTRACT

Objective. Type 2 diabetes is a chronic metabolic disorder characterized by hyperglycemia with insulin resistance and impaired insulin secretion. DPP-4 inhibitors have attracted attention as a new class of anti-diabetic agents for the treatment of type 2 diabetes. We investigated the effects of anagliptin, a highly selective DPP-4 inhibitor, on insulin secretion and insulin resistance in high-fat diet-fed mice with haploinsufficiency of glucokinase (GckKO) as animal models of type 2 diabetes.

Materials/Methods. Wild-type and GckKO mice were administered two doses of anagliptin by dietary admixture (0.05% and 0.3%) for 10 weeks.

Results. Both doses of anagliptin significantly inhibited the plasma DPP-4 activity and increased the plasma active GLP-1 levels in both the wild-type and GckKO mice to a similar degree. After 10 weeks of treatment with 0.3% anagliptin, body weight gain and food intake were significantly suppressed in both wild-type and GckKO mice. In addition, 0.3% anagliptin ameliorated insulin resistance and glucose intolerance in both genotypes of mice. On the other hand, treatment with 0.05% anagliptin was not associated with any significant change of the body weight, food intake or insulin sensitivity in either genotype of mice, but it did improve the glucose tolerance by enhancing insulin secretion and increasing the β-cell mass in both genotypes of mice.

Conclusions. High-dose anagliptin treatment improved glucose tolerance by suppression of body weight gain and amelioration of insulin resistance, whereas low-dose anagliptin treatment improved glucose tolerance by enhancing insulin secretion.

Abbreviations: DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; IRS-2, insulin receptor substrate-2; CREB, cAMP response element-binding protein; Gck, glucokinase; GIR, glucose infusion rate(s); EGP, endogenous glucose production; R_d, rate of glucose disappearance; ITT, insulin tolerance test; OGTT, oral glucose tolerance test.

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1. Introduction

Type 2 diabetes is a chronic metabolic disorder characterized by hyperglycemia with insulin resistance and impaired insulin secretion. Progression to type 2 diabetes is influenced by genetic and environmental or acquired factors, such as a sedentary lifestyle and dietary habits that promote obesity. Most patients with type 2 diabetes are obese, and obesity is associated with insulin resistance. β-cell mass in adults exhibits plasticity, and adjustments in β-cell growth and survival maintain the balance between insulin supply and the metabolic demand. For example, obese individuals who do not develop diabetes exhibit an increase of the β-cell mass that appears to compensate for the increased metabolic load and obesity-associated insulin resistance. However, this β-cell adaptation eventually fails in the subset of obese individuals who develop type 2 diabetes [1–3]. In fact, most individuals with type 2 diabetes show a net decrease of the β-cell mass [1,4,5]. Thus, type 2 diabetes is a disease of relative insulin deficiency.

Glucagon-like peptide-1 (GLP-1), which is a gut-derived incretin hormone, stimulates glucose-dependent insulin secretion via the cAMP/PKA pathway. In addition, GLP-1 exerts multiple actions, including decrease of the body weight through suppression of appetite, stimulation of β-cell proliferation, and inhibition of β-cell apoptosis [6]. However, GLP-1 is rapidly converted to a bioinactive form by dipeptidyl peptidase-4 (DPP-4), the key enzyme responsible for cleaving and inactivating at the penultimate alanine residue [7–9]. Thus, DPP-4 inhibitors to block the enzymatic inactivation of GLP-1 have emerged as a new class of anti-diabetic agents for the treatment of type 2 diabetes.

Glucokinase (Gck) is the key rate-limiting enzyme in glucose metabolism in the β-cells. Gck catalyzes the conversion of glucose to glucose 6-phosphate, which is a critical process in glucose sensing for insulin secretion by the pancreatic β-cells. It has been shown that maturity-onset diabetes of the young type 2 (MODY2) can be caused by mutation in a single Gck gene allele [10,11]. Moreover, in type 2 diabetes, the mRNA expression and activity of Gck are significantly reduced, which is associated with impaired glucose-stimulated insulin release [12,13]. Mice with haploinsufficiency of Gck (GckKO mice) also exhibit glucose intolerance associated with a reduction in the insulin secretion in response to glucose [14]. In addition, GckKO mice show insufficient β-cell growth in response to high-fat diet-induced obesity-linked insulin resistance, leading to the development of diabetes [15]. Thus, GckKO mice fed a high-fat diet are considered as a useful animal model of diabetes, which show a time course of the disease similar to that seen in patients with type 2 diabetes.

In the present study, we investigated whether anagliptin, a highly selective DPP-4 inhibitor, might ameliorate glucose intolerance in high-fat diet-fed GckKO mice. Treatment with 0.3% anagliptin ameliorated the insulin resistance by suppression of body weight gain, which resulted in a decrease of the fasting plasma glucose and improvement of the glucose tolerance. On the other hand, treatment with 0.05% anagliptin improved glucose tolerance by enhancing insulin secretion, which was attributed to an increase of the β-cell mass, but did not suppress the body weight gain or ameliorate the insulin resistance. Taken together, both low and high doses of anagliptin improved glucose tolerance in the high-fat diet-fed GckKO diabetic mice. These findings suggest that anagliptin could be a potentially efficacious agent for the treatment of type 2 diabetic patients.

2. Materials and methods

2.1. Animals and genotyping

GckKO mice were generated as described previously [14]. Then, the original GckKO mice were back-crossed more than seven times with the C57BL/6 mice. The mice were housed under a 12-h light/dark cycle and fed standard chow (CE-2; CLEA) until 8 weeks of age and then allocated to either an HF diet alone or an HF diet containing a DPP-4 inhibitor. All of the experiments in this study were conducted on 8-week-old male littermates. The animal care and experimental procedures were approved by the Animal Care Committee of the University of Tokyo.

2.2. DPP-4 inhibitor treatment study

The composition of the HF diet (High Fat Diet 32; Clea Japan) was as described previously [15]. DPP-4 inhibitor was admixed with the HF diet at 0.05% or 0.3% (wt/wt). The DPP-4 inhibitor used in this study was anagliptin [16], prepared by Sanwa Kagaku Kenkyusho, Ltd.

2.3. Measurement of the plasma DPP-4 activity

Plasma DPP-4 activity was measured using a fluorometric assay with Gly-Pro-MCA (Peptide Institute, Osaka, Japan), modified from a previously published method [17]. In brief, 10 μL of a plasma sample was mixed with 90 μL of the reaction buffer (0.2 mmol/L Gly-Pro-MCA, 0.1 mg/mL BSA, 25 mol/L HEPES, 140 mol/L NaCl, pH 7.8). The mixture was incubated for 20 min at room temperature in the dark, and the reaction was stopped by the addition of 100 μL of 25% acetic acid. The fluorescence intensity of the liberated 7-amino-4-methylcoumarin (AMC) was measured with a 96-well plate fluorometer (1420 ARVOsx, PerkinElmer) at an excitation wavelength of 390 nm and emission wavelength of 460 nm. Plasma DPP-4 activity was calculated as nmol AMC/min/mL plasma, and the result in the treated samples was expressed as a percentage of that in the control.

2.4. Measurement of the plasma parameters

Plasma adiponectin levels were determined with a mouse adiponectin enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical). Plasma leptin levels were determined with a mouse leptin ELISA kit (Morinaga Institute of Biological Science). Plasma levels of active GLP-1 were assayed with a Glucagon-Like Peptide-1 (Active) ELISA kit (Millipore).

2.5. Insulin tolerance test

Mice were given free access to food and then fasted during the study. They were intraperitoneally challenged with human

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