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Dihydromyricetin delays the onset of hyperglycemia and ameliorates insulin resistance without excessive weight gain in Zucker diabetic fatty rats

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ABSTRACT

Many flavonoids are reported to be partial agonists of PPARγ and exert antidiabetic effects with fewer side effects compared with full agonists. Here, we assessed the effects of flavonoid dihydromyricetin (DHM) on glucose homeostasis in male Zucker diabetic fatty rats. Animals were treated with DHM (50 −200 mg kg^{-1}) or rosiglitazone (4 mg kg^{-1}) once a day for 8 weeks. We found that DHM reduced fasting blood glucose and delayed the onset of hyperglycemia by 4 weeks. Furthermore, DHM preserved pancreatic β-cell mass, elevated adiponectin and improved lipid profile more vigorously than rosiglitazone. Notably, DHM decreased body weight gain and fat accumulation in both liver and adipose tissue, while rosiglitazone caused a significant increase of body weight and fat accumulation. DHM inhibited phosphorylation of PPARγ at serine 273 more efficiently than rosiglitazone. These results suggest that DHM exerts antidiabetic effects without causing excessive body weight gain via inhibition of PPARγ phosphorylation.

1. Introduction

Diabetes is generally recognized as a cause of premature death and disability, and increases the risk of cardiovascular disease, kidney failure and blindness (Levitan et al., 2004). The global prevalence of diabetes has been increasing in recent decades. Type 2 diabetes is the most common type of diabetes and is characterized by progressive hyperglycemia and insulin resistance. There are different classes of antidiabetic drugs such as insulin sensitizers, secretagogues, alpha-glucosidase inhibitors, etc. At the beginning of type 2 diabetes, insulin secretion is increased rather than reduced due to the compensation of the organism. Therefore, medications to enhance insulin sensitivity exert excellent effects on blood glucose control.

Peroxisome proliferator-activated receptor (PPAR[1]) γ is a nuclear receptor family member that binds to DNA regulating target genes involved in lipid and glucose metabolism. Thus, PPARγ has been targeted to treat diabetes for decades. Previous researches have shown that phosphorylation of PPARγ at serine 273 (Ser273) increased expression of diabetogenic adipokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1 and resistin, and decreased expression of insulin-sensitizing hormone adiponectin (Choi et al., 2010). These adipokines play important roles in fat distribution, energy expenditure, insulin secretion, and sensitivity (Blüher and Mantzoros, 2015). Researches also found that both

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[1] Abbreviations: AUC (area under the curve); CDK5 (cyclin-dependent kinase 5); CT (computed tomography); DAB (3, 3′-diaminobenzidine); DHM (dihydromyricetin); ELISA (enzyme-linked immunosorbent assay); ERK (extracellular signal-regulated kinase); FGF (fibroblast growth factor); HDL (high-density lipoprotein); H&E (hematoxylin and eosin); HOMA-IR (homeostasis model assessment of insulin resistance); IL (interleukin); ITT (insulin tolerance test); LDL (low-density lipoprotein); NEFAs (nonesterified fatty acids); OGTT (oral glucose tolerance test); PBS (phosphate buffered saline); PPAR (peroxisome proliferator-activated receptor); RIA (radioimmunoassay); SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis); TC (total cholesterol); TG (triglyceride); TNF (tumor necrosis factor); TZDs (thiazolidinediones); ZDF (Zucker Diabetic Fatty); ZL (Zucker lean).

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cyclin-dependent kinase 5 (CDK5) and extracellular signal-regulated kinase (ERK) directly phosphorylated Ser273 of PPARγ (Banks et al., 2015). Thus, regulation of this ERK/CDK5 axis may contribute to antidiabetic treatment.

Full PPARγ agonists, such as thiazolidinediones (TZDs), improve insulin sensitivity, alleviate hyperglycemia and preserve pancreatic islet mass in diabetic patients (Atkinson et al., 2008; Smith et al., 2000). However, typical TZDs drug rosiglitazone potently increases lipid accumulation, promotes fat cell differentiation and stimulates fat cell gene expression (Choi et al., 2011). Therefore, clinical utility of rosiglitazone is limited due to its unwanted effects including weight gain, fluid retention, increasing risk of congestive heart failure and bone fracture (Aubert et al., 2010; Nesto et al., 2004; Staels, 2005). Partial agonists of PPARγ show weak transcriptional activity compared with classical full agonists. Nevertheless, they are more effective to block phosphorylation of PPARγ at Ser273 (Banks et al., 2015; Choi et al., 2010, 2011). Hence, partial agonists of PPARγ have gained attention for diabetic therapy because they exert potent antidiabetic effects with the inability to promote adipogenesis.

Natural plant extracts such as flavonoids are reported to have anti-inflammatory, anti-cancer and anti-oxidant bioactivities with fewer side effects (Breinholt and Larsen, 1998; Fotsis et al., 1997; Plaumann et al., 1996). Recently, flavonoids including quercetin, kaempferol, chrysin and luteolin have been identified as partial agonists of PPARγ that can improve insulin sensitivity without promoting adipogenesis (El-Bassossy et al., 2014; Liang et al., 2001; Ramachandran et al., 2012). Dihydromyricetin (DHM), the most abundant flavonoid in *Ampelopsis grossedentata*, possesses a broad spectrum of biological and pharmacological properties, including anti-cancer, anti-inflammatory and anti-oxidative effects (Hou et al., 2015; Nazemiyeh et al., 2008; Ye et al., 2015; Zhao et al., 2014; Zhu et al., 2015). So far as we know, the antidiabetic effects of DHM as a partial agonist of PPARγ have not been systematically explored. Here, we investigated effects of DHM on glucose homeostasis, insulin sensitivity and body weight gain compared with rosiglitazone in Zucker diabetic fatty (ZDF) rat, a commonly used animal model of obesity and type 2diabetes, which has initial signs of insulin resistance and obesity at 6–7 weeks of age and develops into hyperglycemia at 10–12 weeks of age.

2. Materials and methods

2.1. Chemicals and reagents

DHM (CAS No. mast-120131108, HPLC ≥98%) was obtained from MUST Bio-Technology Co., Ltd. (Chengdu, China). Rosiglitazone was purchased from Salvage Pharmaceutical Co., Ltd. (Guiyang, China). Rat fibroblast growth factor (FGF) 21 and rat adiponectin enzyme-linked immunosorbent assay (ELISA) kits were obtained from Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China). Rat insulin, rat glucagon and rat C-peptide iodine [^{125}I] radioimmunoassay (RIA) kits were bought from North Institute of Biological Technology (Beijing, China). Rat serum biochemical assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). ImmunoCruz™ goat ABC Staining System, antibodies against insulin, PPARγ, phosphor-CDK5 (Ser159), CDK5, phosphor-ERK1/2 (Thr202/Tyr204), ERK and β-actin were obtained from Santa Cruz Biotechnology (Dallas, TX). Antibody against phosphor-PPARγ (Ser273) was obtained from Biosynthesis Biotechnology Co., Ltd. (Beijing, China).

2.2. Animals and treatments

A total of 30 six-week-old male Zucker diabetic fatty (ZDF) rats and 5 male Zucker lean (ZL) rats weighting 183–214 g were purchased from Vital River Laboratories International Inc. (Beijing, China) and housed three per cage in a specific pathogen free (SPF) grade room under controlled conditions of temperature (22 ± 2 °C), humidity (60± 5%) and 12 h light/dark cycle in the Laboratory Animal Centre of the Third Military Medical University. Animals were fed Purina 5008 (Labdiet, St. Louis, MO) chow and sterile water *adlibitum*. All animal care and experimental procedures adhered to the Institutional Animal Care and Use Committee of the Third Military Medical University (Chongqing, China).

After two weeks of acclimatization, ZDF rats (8 weeks of age) were randomized into five groups (n = 6/group): vehicle (ZDF control); 50, 100, or 200 mg kg^{-1} DHM; or 4 mg kg^{-1} rosiglitazone. Vehicle-treated ZL rats were non-diabetic healthy controls (ZL controls, n = 5) and they were given a daily dose of 2 ml of water by gavage. DHM and rosiglitazone were administered as aqueous suspensions (2 ml, gavage) once a day for 8 weeks to create three DHM-treated and one rosiglitazone-treated groups.

Body weight was tracked every other day. Food intake and fasting plasma glucose were monitored weekly. Food efficiency was calculated as body weight gain per unit of energy intake. At the end of the experiment, overnight-fasted animals were euthanized and whole blood was collected from the abdominal aorta under 5% chloral hydrate (5 ml kg^{-1}) anesthesia. Serum was separated by centrifugation at 4000 rpm for 10 min at 4 °C and stored at −80 °C for further biochemical analysis. Pancreas, liver, subcutaneous and visceral adipose tissues were quickly removed and weighed. For histology, tissues were immediately fixed in 10% formaldehyde for 24 h. Remained tissues were snap frozen and stored at −80 °C.

2.3. Biochemical analysis

After 6 h fasting (with food deprived at 9 a.m.), blood was collected from tail vein. Fasting blood glucose was measured using an OneTouch Ultra Meter (LifeScan, Milpitas, CA). Fasting insulin, glucagon, and C-peptide were analyzed with RIA Kits. FGF-21 and adiponectin were quantified by ELISA kits according to the manufacturer's instructions. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and nonesterified fatty acids (NEFAs) were measured with commercial kits. All experiments were repeated at least three times. A homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula(Mather, 2009): HOMA-IR = glucose (mmol L^{-1}) ·insulin (mU L^{-1})/22.5.

2.4. Glucose and insulin tolerance

Oral glucose tolerance test (OGTT) was measured one week prior to animal sacrifice. Animals were fasted for 6 h. Tail blood glucose was quantified prior to oral glucose administration (2 g kg^{-1}) (Andrikopoulos et al., 2008), and after the glucose challenge at 15, 30, 60, and 120 min. Insulin tolerance test (ITT) was measured 6 days after OGTT. After 6 h fasting, animals were given recombinant human insulin (1 IU kg^{-1}, abdominal sc) and blood glucose was measured at 0, 15, 30, 60, 90, and 120 min with an OneTouch Ultra Meter. Overall changes in glucose during OGTT and ITT were calculated as areas under the curve (AUC).

2.5. Body compositions analysis

Body compositions were evaluated at week 7 by Quantum FX microCT Imaging System (Perkin Elmer Inc., Waltham, MA). Computed tomography (CT) images were obtained from the abdominal region (between vertebrae L1 and L6) of animals under