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Research article

Ginsenoside Rb1 ameliorates liver fat accumulation by upregulating perilipin expression in adipose tissue of db/db obese mice

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ABSTRACT

Background: Ginsenoside Rb1 (G-Rb1), the major active constituent of ginseng, improves insulin sensitivity and exerts antidiabetic effects. We tested whether the insulin-sensitizing and antidiabetic effects of G-Rb1 results from a reduction in ectopic fat accumulation, mediated by inhibition of lipolysis in adipocytes.

Methods: Obese and diabetic db/db mice were treated with daily doses of 20 mg/kg G-Rb1 for 14 days. Hepatic fat accumulation was evaluated by measuring liver weight and triglyceride content. Levels of blood glucose and serum insulin were used to evaluate insulin sensitivity in db/db mice. Lipolysis in adipocytes was evaluated by measuring plasma-free fatty acids and glycerol release from 3T3-L1 adipocytes treated with G-Rb1. The expression of relevant genes was analyzed by western blotting, quantitative real-time polymerase chain reaction, and enzyme-linked immunosorbent assay kit.

Results: G-Rb1 increased insulin sensitivity and alleviated hepatic fat accumulation in obese diabetic db/db mice, and these effects were accompanied by reduced liver weight and hepatic triglyceride content. Furthermore, G-Rb1 lowered the levels of free fatty acids in obese mice, which may contribute to a decline in hepatic lipid accumulation. Corresponding to these results, G-Rb1 significantly suppressed lipolysis in 3T3-L1 adipocytes and upregulated the perilipin expression in both 3T3-L1 adipocytes and mouse epididymal fat pads. Moreover, G-Rb1 increased the level of adiponectin and reduced that of tumor necrosis factor-α in obese mice, and these effects were confirmed in 3T3-L1 adipocytes.

Conclusion: G-Rb1 may improve insulin sensitivity in obese and diabetic db/db mice by reducing hepatic fat accumulation and suppressing adipocyte lipolysis; these effects may be mediated via the upregulation of perilipin expression in adipocytes.

1. Introduction

Panax ginseng has been used for the treatment and prevention of various diseases for several millennia in oriental medicine [1]. In previous clinical and pharmacological studies, ginseng and its active components have been reported to be effective in the treatment of diabetes [2]. Ginsenosides are the major active constituents responsible for the pharmacological properties of ginseng, and G-Rb1 is the most abundant among > 40 ginsenosides [3,4]. Our previous studies showed that G-Rb1 stimulates glucose uptake through the insulin-like signaling pathway in 3T3-L1 adipocytes [5], and facilitates adipogenesis of 3T3-L1 preadipocytes by enhancing the expression of peroxisome proliferator-activated receptor γ2 (PPARγ2) and CCAAT/enhancer-binding protein α [6]. In addition, we found that ginsenoside Rb1 can bind to PPARγ as a ligand and inhibited lipolysis in 3T3-L1 adipocytes [7]. Another study reported that ginsenoside Rb1 has antiobesity and antihyperglycemic effects in diet-induced obese rats [8]. Furthermore, recent studies demonstrate that G-Rb1 can reduce liver fat accumulation in high fat diet (HFD)-induced obese rats and mice [9,10].

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In the pathogenesis of insulin resistance and diabetes, ectopic fat deposition, which is defined as the storage of triglycerides within cells of nonadipose tissue, is postulated to play an important role in the development of obesity-mediated insulin resistance [11]. In the obese and diabetic state, the level of circulating free fatty acids (FFAs) is often elevated, and most FFAs have spilled over from adipose tissue [11]. Circulating FFAs contribute to the incidence of insulin resistance in two ways. One is that FFAs interfere with the insulin-signaling pathway, and another is that they lead to excessive accumulation of intracellular lipid products in the liver and muscle [11,12]. Intrahepatic lipid content, and not visceral fat mass, is primarily related to hepatic insulin resistance [13]. Several interventional studies have demonstrated that a reduction in liver fat in patients with type 2 diabetes mellitus (T2DM) can improve insulin sensitivity and glucose metabolism [14,15]. Thus, reducing the ectopic fat content represents an effective strategy for the treatment of insulin resistance and T2DM.

Several factors contribute to lipid accumulation in the liver, including increased FFA release from visceral fat depots, increased lipogenesis, and reduced fatty acid oxidation in the liver. Failure to suppress FFA release from adipose tissue is known to be more important for fat accumulation in the liver [16]. Lipolysis in adipocytes is regulated by a complex signaling cascade, among which perilipin plays a central role in the regulation of lipolysis [17]. Perilipin double-regulates triacylglycerol (TG) metabolism by blocking lipase from approaching droplets to reduce the rate of basal lipolysis and facilitate hormonally stimulated lipolysis [18,19]. In 3T3-L1 adipocytes, the stimulation of lipolysis by tumor necrosis factor-α (TNFα) is in part mediated by promoting the rapid degradation of perilipin. Moreover, overexpression of perilipin in adipocytes inhibited TNF-α induced lipolysis [20]. In perilipin-null mice, basal adipocyte lipolysis was increased, and the development of glucose intolerance and insulin resistance was also promoted, probably due to the elevated levels of FFA [21].

Based on these observations, we hypothesized that G-Rb1 exerts insulin-sensitizing and antidiabetic effects in part by inhibiting lipolysis in adipocytes to reduce ectopic lipid accumulation in the liver. To test this hypothesis, we investigated the effect of G-Rb1 on hepatic lipid accumulation in obese diabetic db/db mice as well as the effect of G-Rb1 on the expression of perilipin in adipocytes.

2. Materials and methods

2.1. Materials

G-Rb1, rosiglitazone, and dimethyl sulfoxide were obtained from Sigma–Aldrich (St Louis, MO, USA). Recombinant human insulin was purchased from Lily (Fegersheim, France) and recombinant mouse TNFα from Gibco (Grand Island, NY, USA). Perilipin A goat polyclonal antibody, adipose triglyceride lipase (ATGL) rabbit monoclonal antibody, and abhydrolase domain-containing 5 (ABHD5) goat polyclonal antibody from Abcam (Cambridge, MA, USA). Hormone sensitive lipase (HSL) rabbit polyclonal antibody and phospho-HSL (Ser563) rabbit polyclonal antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). β-actin mouse monoclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The BCA protein assay kit and West Pico chemiluminescent substrate were purchased from Pierce (Rockford, IL, USA). Unless otherwise specified, all other reagents were of analytic grade.

2.2. Animals and treatment

Male obese and diabetic db/db mice and their nondiabetic counterparts, male C57BLKS/J mice, were obtained from MTE Ltd. (Jiangsu, China). Mice were maintained on a 12/12 h light/dark cycle at 25°C, and provided free access to standard rodent chow and tap water. Diabetic db/db mice were randomly divided into two groups. In the G-Rb1 treated group, G-Rb1 was administered by intraperitoneal injection at a dose of 20 mg/kg body weight to nondiabetic mice, and control animals received vehicle alone. Body weight and food intake were recorded daily. After 14 d of treatment, the mice were fasted for 10 h, and blood was collected from the orbital vein of mice after they were deeply anesthetized using sodium pentobarbital (30 mg/kg, intraperitoneal). Epididymal fat and liver were surgically removed and stored at −80°C until the assays were performed. All experiments were approved by the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine, Nanjing, China.

2.3. Measurement of plasma samples

Plasma glucose levels were measured using a glucometer. Insulin was measured using a commercially available enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). The homeostasis model assessment of basal insulin resistance was used to calculate an index from the product of the fasting concentrations of plasma glucose (mM) and plasma insulin (μU/L) divided by 22.5 [22]. FFA content was measured using the LabAssay NEFA kit (Wako, Osaka, Japan). Plasma concentrations of cholesterol, triglycerides (TG), and high- and low-density lipoprotein cholesterol were assessed with an autobiochemistry instrument using biochemical methods. Serum TNFα and adiponectin were measured using commercially available enzyme-linked immunosorbent assay kits (Millipore, St Charles, MO, USA).

2.4. Liver TG assay

The liver TG assay was performed as previously described [23]. In brief, approximately 100 mg of liver tissue was digested overnight with ethanolic KOH at 55°C, and the digest was extracted twice with 50% ethanol. After neutralization with $MgCl_2$, the supernatant was used for triglyceride measurement using a TG kit (Sigma–Aldrich).

2.5. Histological examination of the liver

Tissue sections were cut from frozen liver samples using a microtome (Leica, Wetzlar, Germany) and mounted on gelatin-coated slides. Oil red O staining was performed as previously described [24]. In brief, the sections were fixed in 4% formaldehyde in phosphate-buffered saline for 1 h and then stained with 0.6% (w/v) Oil Red O solution (60% isopropanol, 40% water) for 1 h at room temperature. Sections were then washed three times with 60% isopropanol to remove unbound dye and photographed at 100× magnification.

2.6. Measurement of lipolysis in 3T3-L1 adipocytes

Mouse 3T3-L1 fibroblasts were obtained from the American Type Culture Collection (Manassas, VA, USA). 3T3-L1 fibroblasts were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and differentiated into adipocytes as previously described [25]. On Day 10 of differentiation, 3T3-L1 adipocytes were incubated with or without TNFα (10 ng/mL) in Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin for 12 h. Subsequently, the medium was changed. Adipocytes were treated with G-Rb1 or rosiglitazone at a concentration of 10μM in the presence of 10 ng/mL TNFα for 24 h. After treatment, the medium was removed and cells were incubated with phosphate-buffered saline for 1 h. The glycerol content in the supernatant was measured using free glycerol reagent (Sigma–Aldrich).