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Hypoglycemic effect of protopanaxadiol-type ginsenosides and compound K on Type 2 Diabetes mice induced by High-Fat Diet combining with Streptozotocin via suppression of hepatic gluconeogenesis

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ABSTRACT

Compound K (CK) is a final intestinal metabolite of protopanaxadiol-type ginsenosides (PDG) from *Panax ginseng*. Although anti-diabetic activity of CK have been reported with genetic mouse models (*db*/*db* mice) in recent years, the therapeutic usefulness of CK and PDG in type 2 diabetes, a more prevalent form of diabetes, remains unclear. In the present investigation, we developed a mouse of non-insulin-dependent diabetes mellitus that closely simulated the metabolic abnormalities of the human disease. For this purpose, type 2 diabetes was induced in male ICR mice by combining of streptozotocin. The male ICR mice fed with HFD for 4 weeks received 100 mg/kg of STZ injected intraperitoneally. After 4 weeks, mice with fasting (12 h) blood glucose levels (FBG) above 7.8 mmol/L were divided into 3 groups ($n = 12$) and treated with vehicle (diabetes model, DM), 300 mg/kg/day of PDG and 30 mg/kg/day of CK for 4 weeks while continuing on the high-fat diet. Hypoglycemic effects of CK and PDG were consistently demonstrated by FBG levels, and insulin-sensitizing effects were seen during oral glucose tolerance testing (OGTT). Moreover, the mechanism of hypoglycemic effect in type 2 diabetic mice was examined. Gluconeogenic genes, Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase), were decreased in two treatment groups with CK showing greater effects. These findings demonstrated the hypoglycemic and insulin-sensitizing capabilities of CK on type 2 diabetes induced by HFD/STZ via down-regulation of PEPCK and G6Pase expression in liver.

1. Introduction

Type 2 diabetes is a complex, heterogenous, polygenic disease characterized mainly by insulin resistance and pancreatic β-cell dysfunction [1]. Impaired postprandial insulin secretion because of functional defects and the loss of surviving pancreatic β-cells leads to hyperglycemia and a subsequent decline in insulin sensitivity [2,3]. Recently, more and more studies found that gluconeogenesis is a main cause of the elevated hepatic glucose production, contributing 50–60% of the released glucose [4]. Excessive hepatic glucose production via the gluconeogenesis pathway is partially responsible for the elevated glucose levels observed in patients with T2DM [5,6]. The rate

Abbreviations: PDG, protopanaxadiol-type ginsenosides; CK, compound K; T2DM, type 2 diabetes mellitus; HFD, high-fat diet; OGTT, oral glucose tolerance testing; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; ISI, insulin sensitivity index.

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of gluconeogenesis is regulated by the activity of two rate-limiting gluconeogenic enzyme, Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase) [7]. Inhibition of hepatic gluconeogenesis (suppression of PEPCK and G6Pase expression) contributes to glycemic control in the diabetic patients by insulin sensitizers [8]. Therefore, inhibitors of hepatic gluconeogenesis are potentially excellent targets in the treatment of T2DM.

Although several drugs are available for the treatment of diabetes, side effects and adverse reactions are of great concern. Recently, many researchers are seeking natural products or dietary interventions to prevent or treat T2DM. For thousands of years with medical practice, a great deal of valuable experience has been accumulated in the traditional Chinese medical system for diabetes therapy. To date, ginseng (*Panax ginseng* C.A Meyer) was considered as one of the most powerful complementary and alternative medicine for diabetes treatment [9,10]. Generally, the pharmacological properties of ginseng are mainly attributed to ginsenosides which mainly classified into protopanaxadiol-type ginsenosides (PDG, e.g. ginsenosides Rb1, Rb2, Rc, Rd, Rg3 and Rh2) and protopanaxatriol-type ginsenosides (PTG, e.g., ginsenosides Reand Rg1) based on sapogenins with a dammarane skeleton [11]. During our previous investigation on anti-obesity activity of ginsenosides, PDG was found to significantly inhibit pancreatic lipase activity *in vitro* and decrease the plasma lipids in mice fed with a high-fat diet [12]. It is well known that PDG are metabolized by intestinal bacteria after oral administration to their final derivative 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol, also named compound K (the major form of PDG absorbed in the intestine) [13,14]. Recently, CK have received increasing attention in view of various biological activities including anticancer [15,16], anti-inflammation [17] and hepatoprotective effect [18]. The structure of CK is shown in Fig. 1.

Prior to this investigation, some researcher have also demonstrated the reduction of blood glucose and increase in insulin sensitivity in *db*/*db* mice [19], as well as reducing of HepG2 cellular triglyceride accumulation with CK treatment [20]. Up to now, however, little attention has been focus on the role of CK for treating type 2 diabetes, a more prevalent form of diabetes. Furthermore, given that CK inhibits hepatic gluconeogenesis, this possibility may represent a new mechanism for CK in the control of blood glucose. In summary, the primary objective of this paper was to carry out the hypoglycemic effect of CK and PDG on the HFD/STZ mice and to look into the mechanism of anti-diabetic activity. The data presented here suggest that CK exerts anti-diabetic effects partly via down-regulation of expression of PEPCK and G6Pase. To the best of our knowledge, this is the first report on CK ameliorates hyperglycemia via suppress hepatic gluconeogenesis.

2. Materials and methods

2.1. Materials

STZ was purchased from Sigma Chemicals, insulin was purchased from Eli Lilly, Changchun, China; Glucose, total cholesterol (TC), triglyceride (TG) test kit were obtained from BHKT Clinical Reagent Co., Ltd, Beijing, China; Iodine [^{125}I] Insulin Radioimmunoassay Kit was purchased from Tianjin Nine Tripods Medical & Bioengineering Co., Ltd, Tianjin, China; Other reagents were purchased from Beijing Chemical Factory, Beijing, China. Compound K used in this study was isolated and purified from *P. ginseng* roots by a series of chromatography procedures in our laboratory, and their structures were elucidated by comparison of spectral data. Its purity was determined to be more than 98.5% by HPLC-UV analysis.

2.2. Biotransformation of PDG to CK

PDG was isolated from ginseng roots by the previous method [12]. The snailase was used to convert PDG to CK under optimized conditions. In brief, the snailase were incubated with PDG in a pH 4.5 sodium acetate buffer with agitation at temperature of 50 °C and enzyme load of 20% for reaction time of 24 h. The mixture was subsequently placed in a water bath at 90 °C to terminate the enzymatic reaction. The reaction mixtures were individually evaporated, dissolved in methanol, and separated through repeated silica column chromatography to obtain CK. Fig. 2 showed the chromatograms of PDG before and after enzymatic preparation.

2.3. Diet and animal model

Regular chow consisting of 5% fat, 53% carbohydrate, 23% protein, with total calorific value 25.0 kJ/kg and high fat diet consisting of 22% fat, 48% carbohydrate, and 20% protein with total calorific value 44.3 kJ/kg were ordered from the Experimental Animal Holding of Jilin University.

Ninety male ICR mice (Experimental Animal Holding of Jilin University), 18 to 22 g, were housed individually in cages in a temperature-controlled room with a 12-hour light: dark cycle. After 1 week of acclimation with free access to regular rodent chow and water, the mice were randomly divided into 2 groups. Group 1 ($n = 12$, normal control [CON]) was fed regular chow. Groups 2 ($n = 78$, high-fat diet [HFD]) were fed the high fat diet. After 4 weeks of high-fat diet feeding, group 2 was injected (intraperitoneally) with STZ dissolved in citrate buffer (pH 4.5) at a dose of 100 mg/kg body weight and tested for fasting blood glucose (FBG) levels 4 weeks post-injection. The group 1 was injected with the citrate buffer vehicle. Mice in group 2 with FBS value above 7.8 mmol/L were randomly divided into 3 groups ($n = 12$ each) and continued on the high-fat diet. One group was used as a high-fat diabetic control (DM), and the other 2 were orally gavaged with PDG and CK dissolved in 0.5% CMC-Na at doses of 300 and 30 mg/kg body weight per day, respectively. Mice in the CON and DM groups were gavaged with 0.5% CMC-Na. Body weights and food intake was recorded weekly and daily, respectively.

At the end of the study, animals were fasted overnight and blood samples obtained from tails were collected into EDTA tubes and placed on ice. After centrifugation, plasma was collected and stored at −80 °C. Liver were immediately separated, collected and stored in liquid nitrogen till further analysis.

2.4. Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT)

Throughout the 4-week treatment period, fast blood glucose was measured weekly on lateral tail vein blood samples. Oral glucose tolerance testing was also performed during the last week of treatment. After a 12-hour fasting, the animals