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A newly identified polysaccharide from *Ganoderma atrum* attenuates hyperglycemia and hyperlipidemia

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ABSTRACT

A polysaccharide from *Ganoderma atrum* (PSG-1) was purified and characterized, and its hypoglycemic and hypolipidemic effects were investigated in high fat diet- and streptozotocin-induced type 2 diabetic rats. Oral administration of PSG-1 at 200 or 400 mg/kg body weight significantly reduced fasting blood glucose and serum insulin levels. PSG-1 significantly decreased the levels of serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, free fatty acid and insulin resistance, and increased high-density lipoprotein cholesterol level and insulin sensitivity. In addition, PSG-1 inhibited the expression of pro-apoptotic protein, Bax and increased the expression of anti-apoptotic protein, Bcl-2 in pancreatic cells, suggesting that PSG-1 exerted a protective role in the pancreas of diabetic rats. These results indicated that PSG-1 may have a potential for the treatment of hyperglycemia, hyperlipidemia, hyperinsulinemia and insulin resistance in type 2 diabetes.

1. Introduction

Ganoderma, a basidiomycete white rot fungus, has been used as a traditional Chinese medicine in many Asian countries for centuries [1], and more than 120 species of *Ganoderma* have been identified. There are several biologically active components in *Ganoderma*, including polysaccharides, triterpenes and amino acids. Polysaccharides obtained from *Ganoderma lucidum* were reported to exhibit anti-oxidant [2], anti-tumor, anti-inflammatory [3], and hypoglycemic effects [4,5].

Ganoderma atrum is one of the most widely used medicinal herbs, and has a long history in traditional Chinese medicine. Among various components of *G. atrum*, the polysaccharides have been found to exert diverse biological activities. Recently, a novel polysaccharide, named PSG-1, has been isolated from *G. atrum* in our laboratory. The primary structural features and molecular weight of the purified PSG-1 (purity 99.8%) were characterized, and PSG-1 was found to show a potent antioxidant activity [6]. We have also reported that PSG-1 possessed antitumor activity and cardiomyocytes protective effect, and attenuated oxidative stress [7].

Diabetes mellitus is the most common metabolic disorder caused by absolute or relative insulin deficiency, characterized by hyperglycemia as well as impaired metabolism of carbohydrate, fat and protein [8]. Type 1 diabetes and type 2 diabetes are the two main types of diabetes, while type 2 diabetes accounts for approximately 90% of all diagnosed cases of diabetes [9,10]. Over the past half-century, changes in diet and other aspects of lifestyle have resulted in the dramatic rise in the prevalence and incidence of type 2 diabetes in virtually every country in the world. Meanwhile, reduction in physical activity, increase in energy intake, and the aging of the population play pivotal roles in bringing about this rapid change [11].

To the best of our knowledge, there was little study about the hypoglycemic and hypolipidemic effects of polysaccharides from *G. atrum* in type 2 diabetes. Therefore, the aim of this study was to evaluate the hypoglycemic and hypolipidemic effects of PSG-1 in an established animal model of type 2 diabetes. Our results

Abbreviations: PSG-1, polysaccharide from *Ganoderma atrum*; STZ, streptozotocin; BW, body weight; HFD, high fat diet; SND, standard normal chow diet; OGTT, oral glucose tolerance test; HOMA-IR, the homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index.

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demonstrated that PSG-1 may have a potential for the treatment of hyperglycemia, hyperlipidemia, hyperinsulinemia and insulin resistance in type 2 diabetics.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin (STZ) and 1, 1-dimethylbiguanide hydrochloride were purchased from Sigma Chemical Co (St. Louis, MO, USA). All other chemicals were obtained from Nanjing Jiancheng Biochemistry Co. Ltd. (Nanjing, China). *G. atrum* was purchased from Ganzhou, Jiangxi Province, China. One touch glucometer (Accu-chek Performa) was obtained from Roche Diagnostics, Germany.

2.2. Preparation of PSG-1

The polysaccharide, PSG-1 was prepared from the fruiting bodies of *G. atrum* by boiling water according to the method by Chen et al. [6]. The chemical composition of PSG-1 was analyzed by the phenol-sulphuric acid, carbazole and sulphuric acid spectrophotometric method. The structure of PSG-1 was determined by methylation, GC-MS analysis and 1D/2D nuclear magnetic resonance (NMR) spectroscopy.

2.3. Experimental animals

Sixty male Wistar rats weighing approximately 180–200 g were obtained from Shanghai Slaccas Laboratory Animal Company Ltd. (Certificate Number SCXK (hu) 2007–0005, Shanghai, China). Before starting the experiments all the animals were acclimatized to the laboratory conditions for 1 week. They were housed at an ambient temperature of $25 \pm 2\,^{\circ}C$, 12/12 h of light–dark cycle with *ad libitum* food and water. All animals used in this study were cared for according to the Care and Use of Laboratory Animals Guidelines published by the United States National Institute of Health (NIH Publication 85-23, 1996). All experimental procedures involving the use of animals were approved by the Animal Care Review Committee, Nanchang University.

2.4. Induction of type 2 diabetes

After acclimation, ten rats fed a standard normal chow diet (SND) consisting of 12% fat, 60% carbohydrate and 28% protein (as a percentage of total kcal), the others were fed a high fat diet (HFD) consisting of 40% fat, 42% carbohydrate and 18% protein. After 8 weeks of dietary manipulation, all rats were fasted for 12 h (with free access to water). Rats fed on HFD were injected with a single low dose of STZ (30 mg/kg body weight (BW), in 154 mmol/L isotonic saline, pH 7.2, Sigma, St. Louis, MO, USA) into the tail vein to induce type 2 diabetes as described [12,13], while those fed on SND received an equivalent volume of saline [5]. Hyperglycemia was confirmed by the elevated glucose levels more than 11.1 mmol/L in whole-blood samples, determined at Day 4 and then at Day 7 after STZ injection.

2.5. Experimental design

Forty HFD/STZ-induced type 2 diabetic rats were randomly divided into four groups: un-treated diabetic group, positive control group (1,1-dimethylbiguanide hydrochloride, 70 mg/kg BW) [14] and the other two groups receiving different doses of PSG-1 (200 mg/kg BW and 400 mg/kg BW, respectively). For the three treatment groups, administration of PSG-1 by gastric gavage was conducted once daily over a 4 week period. Ten normal rats fed on SND served as a non-diabetic control group, received the same volume of vehicle instead of PSG-1 solution once a day during the same period. All animals continued on their original diets for the duration of the study.

The effects of PSG-1 on diabetic rats were determined by measuring fasting blood glucose (FBG) levels, food and fluid intake amount, changes in BW once a week.

2.6. Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight (12 h) fasted animals. Glucose solution (2 g/kg BW) was administered orally at 0, 30, 60, 120 and 180 min respectively, blood was drawn from a tail vein to measure the blood glucose level with one touch glucometer (Accu-chek Performa).

2.7. Measurement of serum insulin levels

At the end of the 4 weeks experimental period, rats were fasted for 12 h, blood samples were then collected under anaesthetized conditions and placed into prechilled tubes. The blood samples were then immediately centrifuged at $1000 \times g$ for 10 min and the serum was removed for further analyses. Serum insulin level was assayed with Insulin Radioimmnoassay Kit (China Diagnostic Medical Corporation, Beijing, China).

2.8. Assessment of insulin sensitivity

The homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) have been reported to be useful and easy estimates of insulin sensitivity. The HOMA-IR index uses the following formula described by Matthews et al. [15]: HOMA-IR = [fasting insulin (μIU/mL) $\times$ fasting glucose (mmol/L)]/22.5. QUICKI was derived from fasting glucose and fasting insulin levels according to the report by Katz et al. [16] with the formula: QUICKI = 1/{log [FASTING insulin (μIU/mL)] + log [fasting glucose (mg/dL)]}.

2.9. Measurements of serum lipids

Levels of serum lipids including total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by an enzymatic method, using the corresponding flex reagents on a multi-analyzer Dimension RxL Max (Dade Behring Diagnostics, Marburg, Germany). According to Nauck et al. [17], low-density lipoprotein cholesterol (LDL-C) levels were calculated by the following formula: LDL-C = TC – [HDL-C + TG (mmol/L)/2.2]. Free fatty acid (FFA) content in the serum was determined by an assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) as the manufacturer's instructions.

2.10. Histopathological analysis

The pancreatic samples were collected under anaesthetized conditions and a portion of pancreas was fixed in 10% formalin for a week at room temperature. After fixation, the specimens were dehydrated from 70% ethanol up to 100% ethanol in a graded series, then cleared in xylene and embedded with paraffin wax in molds. The specimens were then sectioned into 5 μm thick using a rotary microtome. Sections were stained with hematoxylin and eosin dye, and photomicrographs were obtained under light microscope.

2.11. Immunohistochemical analysis

The pancreas tissue samples collected for histology were subjected to immunohistochemical analysis to determine the