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Research Paper

Rehmannia glutinosa (Gaertn.) DC. polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice

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ABSTRACT

Ethnopharmacological relevance: Rehmannia glutinosa (Gaertn.) DC. (RG) has been widely used as traditional Chinese herbal medicine for treatment of diabetes and its complications. The polysaccharide fraction of RG has been proposed to possess hypoglycemic effect by intraperitoneal administration, however, the mechanisms responsible for the hypoglycemic effect of RG polysaccharide (RGP) remain poorly understood. Here we studied the anti-hyperglycemic and anti-hyperlipidemic effect of oral administration of a purified RGP and its underlying mechanisms in streptozotocin (STZ)-induced diabetic mice.

Materials and methods: The preliminary structure of RGP was determined by GC and FT-IR. Mice were injected with STZ to induce type 1 diabetes. RGP at doses of 20, 40 and 80 mg/kg/day was orally administered to mice for 4 weeks, and metformin was used as positive control. After 4 weeks, the blood biochemical parameters, the pancreatic insulin contents, in vitro insulin secretion, the hepatic glycogen contents and mRNA expression of phosphoenolpyruvate carboxyl kinase (PEPCK) were assayed.

Results: RGP was composed of rhamnose, arabinose, mannose, glucose and galactose in the molar ratio of 1.00:1.26:0.73:16.45:30.40 with the average molecular weight of 63.5 kDa. RGP administration significantly decreased the blood levels of glucose, total cholesterol, triglycerides, low density lipoprotein-cholesterol, and increased the blood levels of high density lipoprotein-cholesterol and insulin in diabetic mice, concurrent with increases in body weights and pancreatic insulin contents. The in vitro study revealed that RGP significantly enhanced both basal and glucose-stimulated insulin secretions, as well as islet insulin contents in the pancreatic islets of diabetic mice. Moreover, RGP reversed the increased mRNA expression of PEPCK and the reduced glycogen contents in the liver of diabetic mice. Furthermore, RGP exhibited potent anti-inflammatory and anti-oxidative activities, as evidenced by the decreased blood levels of TNF-α, IL-6, monocyte chemoattractant protein-1, MDA, and also the elevated blood levels of SOD and GPx activities in diabetic mice.

Conclusions: Taken together, RGP can effectively ameliorate hyperglycemia, hyperlipemia, vascular inflammation and oxidative stress in STZ-induced diabetic mice, and thus may be a potential therapeutic option for type 1 diabetes.

Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; BW, body weight; CVD, cardiovascular disease; FBG, fasting blood glucose; FT-IR, Fourier transform infrared; GC, gas chromatography; GLUT2, glucose transporter 2; GPC, gel permeation chromatography; GPx, glutathione peroxidase; HDL-C, high density lipoprotein-cholesterol; IL-6, interleukin-6; KRB, Krebs Ringer bicarbonate buffer; LDL-C, low density lipoprotein-cholesterol; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; M-MLV, molony murine leukemia virus; PBS, phosphate buffered saline; PEPCK, phosphoenolpyruvate carboxyl kinase; RGP, *Rehmannia glutinosa* polysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean; SOD, superoxide dismutase; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TFA, trifluoroacetic acid; TG, triglycerides; TNF-α, tumor necrosis factor-alpha.

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1. Introduction

Rehmannia glutinosa (Gaertn.) DC. (RG), also named as Di-Huang in China, has been widely used as traditional Chinese herbal medicine for thousands of years. *Rehmannia* refers to the root of RG, a herb belonging to the Scrophulariaceae family. In ancient China, it was recorded in Chinese medical classics "Shennong's Herba" and was thought to be a "top grade" herb (Zhang et al., 1993). It is also the main component herb of the most frequently prescribed herbal formula for treatment of type 2 diabetes, Rehmannia Six Formula or *Liu-Wei-Di-Huang-Wan*. In the past decades, RG has been widely studied for treatment of diabetes and its complications (Hsu et al., 2014; Poon et al., 2011; Zhang et al., 2008). With regard to its bioactive components, some extracts and several compounds extracted from RG have been shown to possess hypoglycemic effects in diabetic and/or normal animals, including RG oligosaccharide (Zhang et al., 2004, 2014), catalpol (Dong and Chen, 2013; Huang et al., 2010; Shieh et al., 2011), monomer rehmannioside D (Yu et al., 2001) and RG ethanolic extract (Waisundara et al., 2008b).

Diabetes mellitus is one of the most costly chronic diseases with an estimated worldwide prevalence of 366 million in 2011. Vascular inflammation and cardiovascular disease (CVD) have been shown to be the leading causes of morbidity and mortality in the diabetic population and remain major public health issues. The proinflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and oxidative stress are widely recognized markers of vascular inflammation (Andreozzi et al., 2006; Devaraj and Jialal, 2000; Kern et al., 2001; Rader, 2000; Singh et al., 2005; Wellen and Hotamisligil, 2005). The levels of the cytokines and oxidative stress are elevated in the blood of many diabetic patients (Cavallo et al., 1991; Hussain et al., 1996; Jain, 2002, 2003; Kern et al., 2001). Moreover, elevated blood levels of TNF-α and IL-6 are known to impair insulin sensitivity, and promote vascular inflammation and the development of CVD (Andreozzi et al., 2006; Devaraj and Jialal, 2000; Halse et al., 2001; Kern et al., 2001; Saghizadesh et al., 1996; Singh et al., 2005).

Although the polysaccharides have been suggested to be the main chemical components responsible for the bioactivities and pharmacological properties of RG (Li et al., 2004), little information is available concerning RG polysaccharides with antidiabetic effects. In this regard, Kiho et al. (1992) have shown that the ethanol precipitate fraction obtained from the hot water extract from rhizome of *R. glutinosa* Libosch. f. *hueichingensis* Hsiao, mainly composed of pectin-like polysaccharide, exhibits hypoglycemic activity in STZ-induced diabetic mice by intraperitoneal administration of this fraction. Furthermore, they also suggest that its hypoglycemic activity exists in the polysaccharide moiety. However, so far no purified polysaccharide from RG with potential hypoglycemic activity has been reported. In the study by Kiho et al. the route of intraperitoneal administration is different from that in traditional use of this herb, where the herb is used orally. It remains to be determined whether this hypoglycemic effect will remain when RG polysaccharides are orally administrated. Furthermore, the mechanisms for the effect of RG polysaccharides on glucose homeostasis in diabetic animals remain obscure. Also, the effects of RG polysaccharides on other conditions associated with diabetes have not been addressed previously, e.g., dyslipidemia, vascular inflammation and oxidative stress. Therefore, the present study aimed to investigate whether oral administration of purified RG polysaccharide (RGP), a novel polysaccharide which had not been studied for its antidiabetic effect, could improve hyperglycemia, hyperlipemia, vascular inflammation and oxidative stress in a STZ-induced diabetic mouse model, as well as the potential molecular mechanisms. Moreover, the preliminary structure of RGP was also characterized.

2. Materials and methods

2.1. Materials and reagents

R. glutinosa (Gaertn.) DC. was collected from their natural habitat in Shanxi Province of PR China. The plant was authenticated by a botanist from Shanxi Ci Yuan Biotechnology Co. Ltd. (Xi'an, China), where a voucher specimen (No. 20120028) was deposited. The purified RGP (with a purity of 98%) was obtained from Shanxi Ci Yuan Biotechnology Co. Ltd. STZ and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). Mouse TNF-α and IL-6 ELISA kits were purchased from eBioscience (San Diego, CA). Mouse MCP-1 ELISA kit was purchased from ALPCO Diagnostics (Windham, NH). TRIzol reagent was obtained from Invitrogen. Molony murine leukemia virus (M-MLV) reverse transcriptase (200 U) and oligo (dT) were purchased from Promega. 10 mM dNTP was from Roche. 2 × SYBR Green PCR Master Mix was obtained from Toyobo (Japan). Inositol, hydroxylamine hydrochloride, acetic anhydride, pyridine, trifluoroacetic acid (TFA), methanol, and acetic acid were from Shanghai Chemicals and Reagents Co. (Shanghai, China). The standard monosaccharides (rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose) were purchased from the National Institutes for Food and Drug Control (Beijing, China) and Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest commercial grade available.

2.2. Characterization of RGP

2.2.1. Determination of contents of carbohydrate, sulfuric radical, protein and uronic acid

The content of carbohydrate in RGP was determined by phenol–sulfuric acid method using glucose as the standard (Dubois et al., 1956). The content of sulfate radical was determined as described previously (Doigson and Price, 1962). The content of protein was determined by the Bradford method using bovine serum albumin as the standard (Bradford, 1976). The content of uronic acid was determined according to the method of Blumenkrantz and Asboe-Hansen (1973) by using D-glucuronic acid as the standard.

2.2.2. Purity and molecular weight determination

The purity and molecular weight of RGP was determined by size-exclusion HPLC chromatography instrument (Agilent 1100, USA) with a gel permeation chromatographic (GPC) column of PL aquagel-OH MIXED (8 μm, 300 × 7.5 mm^2) at 35 °C. Sample was dissolved in 0.05 M Na_2SO_4 and filtered through a 0.45-μm filter, applied to gel permeation column, eluted with 0.05 M Na_2SO_4 at a flow rate of 1.0 ml/min and then detected by a refractive index detector. Standard dextrans with different molecular weights (10,000, 40,000, 70,000, 500,000, 2,000,000 Da) passed through the column, and a standard curve was plotted according to the retention time and the logarithm of their respective molecular weights. The molecular weight of RGP was calculated by comparison to the standard curve.

2.2.3. Analysis of monosaccharide composition by gas chromatography (GC)

The monosaccharide composition of RGP was analyzed by GC according to the reported method with slight modifications (Chen et al., 2008). RGP was hydrolyzed with 2 M TFA in a sealed glass tube at 110 °C for 4 h, turning into monosaccharide compositions. The residual solution was concentrated, and the excess of acid was removed by repeated concentration with methanol. The residual were acetylated by the addition of a mixture of hydroxylamine hydrochloride and pyridine, followed by acetic anhydride. The monosaccharide standards (rhamnose, arabinose, fucose, xylose,

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