Carbohydrate Polymers 121 (2015) 199–206

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Hypoglycemic activity and potential mechanism of a polysaccharide from the loach in streptozotocin-induced diabetic mice

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ARTICLE INFO

Article history:
Received 20 August 2014
Received in revised form
12 December 2014
Accepted 15 December 2014
Available online 2 January 2015

Keywords:
Misgurnus anguillicaudatus polysaccharide
Diabetes
Insulin
Dyslipidemia
Oxidative stress
Inflammation

Chemical compounds studied in this article:
Streptozotocin (PubChem CID: 29327)
Glucose (PubChem CID: 5793)
Metformin (PubChem CID: 4091)
Cholesterol (PubChem CID: 5997)
Malondialdehyde (PubChem CID: 10964)
Glutathione (PubChem CID: 124886)

ABSTRACT

The present study was designed to investigate the hypoglycemic activity and the potential mechanisms of *Misgurnus anguillicaudatus* polysaccharide (MAP) in streptozotocin-induced diabetic mice. MAP oral administration significantly decreased the blood levels of glucose, TC, TG, LDL-C, and increased the blood levels of HDL-C and insulin in diabetic mice, concurrent with increases in body weights and pancreatic insulin contents. Moreover, MAP reversed the increased mRNA expressions of PEPCK and the reduced glycogen contents in the liver of diabetic mice. Concurrently, MAP 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 SOD and GPx activities in the serum of diabetic mice. Furthermore, MAP also significantly improved the blood markers of the impaired liver function and renal function in diabetic mice. Altogether, these results suggest that MAP may be a potential therapeutic option for type 1 diabetes.

1. Introduction

The loach (*Misgurnus anguillicaudatus*) serves as a kind of delicious nutritive food and also has long been employed as Chinese traditional medicine in folk remedies for the treatment of hepatitises, carbuncles, osteomyelitis, inflammation and cancers, as well as for the restoration of health disorders caused by aging and various pathogeneses (Qin, Huang, & Xu, 2002d). Generally, the mucus coat of fish skin contains a variety of secretions from epithelial cells and epidermal goblet cells, which have been implicated in many important biological functions (Whitear, 1984). In line with this, some vertebrate lectins, purified from the skin mucus or egg of the loach, were found to induce release of cytotoxin from macrophages or fresh murine bone narrow cells and lyse tumor cells but not normal spleen cells (Goto-Nance & Watanabe, 1995; Okutumi, Nakajima, Sakakibara, Kawauchi, & Yamazaki, 1987). A novel antimicrobial peptide named misgurin, consisting of 21 amino acid residues, was isolated from the loach and identified (Park, Lee, Park, Kim, & Kim, 1997). Moreover, a deaminated neuraminic acid-containing glycoprotein from the skin mucus of the loach, was isolated and characterized (Kimura et al., 1994).

The biological activities of the polysaccharides and polysaccharide–protein complexes isolated from mushrooms, fungi, algae, yeasts, plants, and animals have attracted more and

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BSA, bovine serum albumin; BUN, blood urea nitrogen; BW, body weight; CHD, coronary heart disease; FBG, fasting blood glucose; FT-IR, Fourier transform infrared; GC, gas chromatography; GPx, glutathione peroxidase; HDL-C, high density lipoprotein-cholesterol; IL-6, interleukin-6; LDL-C, low density lipoprotein-cholesterol; MAP, *Misgurnus anguillicaudatus* polysaccharide; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; M-MLV, molony murine leukemia virus; PBS, phosphate buffered saline; PEPCK, phosphoenolpyruvate carboxyl kinase; 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; TG, triglycerides; TNF-α, tumor necrosis factor-alpha.

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http://dx.doi.org/10.1016/j.carbpol.2014.12.037
0144-8617/

more attention in the biochemical and medical areas (Hurtley, Service, & Szuromi, 2001). In recent years, our group isolated and identified a free neutral polysaccharide from the mucus of the loach *M. anguillicaudatus* (Qin, Huang, & Xu, 2002c). *M. anguillicaudatus* polysaccharide (MAP) was shown to possess a variety of pharmacological properties including anti-inflammatory, anti-oxidative, anti-cancer and hypoglycemic activities, and enhancement of the immune system in vivo and/or in vitro (Qin, Ding, Huang, & Xu, 2008; Qin, Huang, & Xu, 2002a, 2002b, 2002c, 2002d; Qin, Zhou, Zhao, Huang, & Xu, 2001; Zhang & Huang, 2005a, 2005b, 2006).

Diabetes mellitus is one of the most costly chronic diseases with an estimated worldwide prevalence of 366 million in 2011. In recent years, the anti-diabetic activity of polysaccharides have attracted considerable attention (Chen et al., 2013; Fu et al., 2012; Zhang, Zheng, Zhang, & Hai, 2012; Zhu et al., 2013). Our preliminary study showed that MAP exhibited hypoglycemic effect in streptozotocin (STZ)-induced diabetic mice (Qin et al., 2002a), however, the molecular mechanisms underlying the hypoglycemic effect of MAP remain unclear. Therefore, the present study aimed to investigate the hypoglycemic activity and potential mechanisms of MAP in a STZ-induced diabetic mouse model.

2. Materials and methods

2.1. Materials and reagents

The loach (*Misgurnus anguilliacaudatus*, weight 8 ± 1.5 g, length 8.5 ± 3 cm) was purchased from market in Wuhan City, China. 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). All other chemicals were of the highest commercial grade available.

2.2. Preparation of MAP

MAP was isolated and purified as described in our previous report (Qin et al., 2002c). It was further identified that the average molecular weight was 130 kDa by gel permeation chromatography; the major structure monomers of MAP were composed of galactose, fucose and mannose (5:4:1) by gas chromatography (GC) (Supplemental Fig. 1A and B); the monomers link each other by α-1,3 bonds through periodate oxidation test, Smith degradation test and Fourier transform infrared (FT-IR) spectrum (Supplemental Fig. 1C). In the periodate oxidation test, no periodate was consumed and no formic acid was produced. In the Smith degradation test, the hydrolyzed product contained monoses only, but no glycerol and erythritol. These data are same as our previous results (Qin et al., 2002c).

Supplemental Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.12.037.

2.3. Animals

Male Kunming mice (20 ± 2 g) were obtained from Hubei Research Center for Laboratory Animals (Wuhan, China) and allowed one week for environmental acclimation. The mice were housed in a temperature (22 ± 2 °C)-controlled room with a 12-h day/night cycle. Throughout the study period, the mice were allowed free access to food and water ad libitum unless otherwise noted. Animal procedures were approved by the Institutional Animal Care and Use Committee at Huazhong University of Science and Technology, and were performed according to the NIH guidelines (NIH publication #85-23, revised in 1996), as well as the guidelines of the Animal Welfare Act.

2.4. Induction of diabetes in mice

For diabetes induction, mice were fasted overnight and then intraperitoneally injected with 100 mg/kg STZ. STZ was dissolved in freshly prepared sodium citrate buffer (pH 4.5) and immediately injected within 20 min of preparation. Control mice were intraperitoneally injected with citrate buffer alone. Five days after STZ injection the diabetic state was assessed by measuring fasting blood glucose (FBG) concentrations after fasting overnight. Mice with FBG concentrations over 11 mmol/L in addition to polyuria and other diabetic features were considered as diabetic.

2.5. Experimental design

All the mice were randomly divided into six groups with 10 mice in each group. Group 1 served as normal controls and received vehicle only (normal saline). Group 2 served as diabetic control and received vehicle. Group 3 received the standard drug metformin (150 mg/kg body weight). Groups 4, 5 and 6 received MAP at 50, 100 and 200 mg/kg body weight, respectively. The vehicle or test drugs were administered via oral gavage (suspended in normal saline). The treatment with MAP was started 5 days after STZ injection and was lasted for 4 weeks. Body weights and FBG concentrations were monitored weekly. At the end of experimental period the mice were fasted overnight, and then sacrificed by exsanguination under diethyl ether anesthesia. Serum was obtained from the blood by centrifugation, and frozen at -80 °C until analysis. The pancreas and liver from each animal were immediately excised and rinsed in phosphate buffered saline (PBS, pH 7.4), and then stored at -80 °C until analysis.

2.6. Measurement of blood glucose and serum insulin levels

Blood samples for glucose assay were obtained from the tail veins of mice after fasting overnight. Blood glucose was measured with an Accu-check Advantage glucometer and blood glucose test strips (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were determined by using an insulin ELISA kit with mouse insulin as a standard (ALPCO Diagnostics, Windham, NH) according to the manufacturer's instructions.

2.7. Blood lipid profile, liver function and renal function tests

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, and blood lipid profiles including triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C) were determined using commercially available kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions.

2.8. Measurement of pancreatic insulin contents

Pancreatic insulin was extracted by an acid/ethanol method as previously described (Noh et al., 2013). The pancreatic insulin contents were determined using an ELISA kit (ALPCO Diagnostics, Windham, NH) and normalized by protein content.