Journal of Ethnopharmacology 190 (2016) 231–240

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Black ginseng extract exerts anti-hyperglycemic effect via modulation of glucose metabolism in liver and muscle

Yun-Soo Seo [a,1], Mi-Yae Shon [b,1], Ryong Kong [a], Ok-Hwa Kang [a], Tian Zhou [a], Do-Yeon Kim [b], Dong-Yeul Kwon [a,*]

[a] Department of Oriental Pharmacy, College of Pharmacy and Wonkwang-Oriental Medicines Research Institute, Wonkwang University, Iksan, Jeonbuk 570-749, Republic of Korea
[b] International Ginseng and Herb Research Institute, Geumsan, 312-804, Republic of Korea

ARTICLE INFO

Article history:
Received 25 December 2015
Received in revised form
29 April 2016
Accepted 29 May 2016
Available online 31 May 2016

Keywords:
Black ginseng
Diabetes mellitus
Insulin signaling
Glucose tolerance
Skeletal muscle

ABSTRACT

Ethnopharmacological relevance: Ginseng (Panax ginseng C. A. Meyer, Araliaceae) has been used as a traditional medicine for thousands of years for the treatment of a wide variety of diseases, including diabetes. Processed ginseng named Black ginseng exhibits more potent biological activities than white and red ginseng. The aim of this study was to investigate the effects of black ginseng extract (GBG05-FF) on hyperglycemia and glucose tolerance in streptozotocin (STZ)-induced diabetic mice.

Materials and methods: Black ginseng was produced by a repeated steaming and drying process, subsequent extraction with 70% ethanol, filtration, and lyophilization. The effect of GBG05-FF on glucose uptake and related protein expression and phosphorylation were determined in C2C12 cells. Furthermore, we evaluated the anti-diabetic effects of GBG05-FF in STZ-induced diabetic mice.

Results: GBG05-FF significantly ($p < 0.05$) increased glucose uptake in C2C12 myotubes *via* AMPK, Sirt1 and PI3-K pathway. In addition, GBG05-FF improved the fasting blood glucose levels and glucose tolerance in STZ-induced diabetic mice. GBG05-FF decreased blood parameters such as glycated hemoglobin, triglyceride and total cholesterol. Quantitative RT-PCR assay revealed that in the STZ-induced diabetic mice treated with GBG05-FF, the expression of hepatic genes involved in gluconeogenesis (phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase)), glycogenolysis (liver glycogen phosphorylase (LGP)) and glycogenesis (glycogen synthase (GS)) was suppressed, while the expression of the genes involved in glucose uptake (glucose transporter (GLUT) 1, GLUT4) and β-oxidation (acyl-CoA oxidase (ACO), carnitine palmitoyl transferase 1a (CPT1a), mitochondrial medium chain acyl-CoA dehydrogenase (MCAD)) in muscle were increased. GBG05-FF delayed diabetes-associated muscle atrophy by activating mTOR. The major bioactive compounds including ginsenoside Rg1, Rg3(S), Rg3(R), Rg5, Rk1 and Rh4 were evaluated for glucose uptake effect in C2C12 myotubes; the data indicated that Rh4 significantly ($p < 0.05$) increased glucose uptake.

Conclusion: Collectively, the results suggested that GBG05-FF is a potentially useful agent for treatment of diabetes by increasing glucose uptake.

1. Introduction

Diabetes mellitus (DM) is a complex and progressive metabolic disease that is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Herder et al., 2007). Recent estimates project that the total worldwide population with diabetes will increase to 552 million by 2030 (Whiting et al., 2011). Despite the rapidly increasing prevalence of diabetes, effective treatment is still lacking. In addition, diabetes increases the risk of life-threatening diseases, making it as one of the 5 leading cause of death in the world (Chen et al., 2013) Furthermore, diabetes requires long-term medical care for glycemic control, which decreases the quality of life because of complications such as retinopathy, neuropathy, and nephropathy (Abu-Farha et al., 2015). Hence, controlling blood glucose to within normal levels is very important.

Hyperglycemia due to poor control of blood glucose level is considered a key factor of numerous mechanisms involved in development and progression of diabetic complications including increased oxidative stress, decreased nitric oxide bioavailability, glucose autoxidation and non-enzymatic protein glycation (Rahimi

* Corresponding author.
E-mail address: sssimi@wku.ac.kr (D.-Y. Kwon).
[1] These two authors shared co-first authorship.

http://dx.doi.org/10.1016/j.jep.2016.05.060
0378-8741/

et al., 2005). Thus, therapeutic agents that ameliorate circulating glucose levels mediated by glucose uptake and disposal in peripheral tissue such as liver, adipose tissue and skeletal muscle have received considerable attention. Especially, skeletal muscle is the major site for glucose utilization, which is closely associated with improvement of hyperglycemia (Shimoda et al., 2015). Firstly, insulin binding to cell surface receptors stimulates a complex cascade of downstream insulin signaling leading to glucose uptake. Stimulated insulin receptors phosphorylate insulin receptor substrate-1, which then activate liver kinase B1 leading to translocation of glucose transporter from the cytoplasm to cell surface (Park et al., 2014). Additionally, Liver Kinase B1 (LKB1; also known as Serine/Threonine Kinase 11 - STK11) is a major upstream kinase for AMP-activated protein kinase (AMPK) that leads to insulin-independent glucose uptake and modulation of mammalian target of rapamycin (mTOR) activation, which acts as a major molecule to coordinate the balance between cell growth and autophagy (Kapahi et al., 2010).

Ginseng (Panax ginseng C.A. Meyer, Araliaceae) is a traditional medicinal herb that is used for improvement of physiological function (Gillis, 1997). There are 3 types of ginseng in Korea i.e., white, red, and black. These types are classified according to changes in surface color by repeated steaming and drying process. Black ginseng contains newly discovered ginsenosides and exhibits more potent biological activities than white and red ginseng (Sun et al., 2009). Hence, the effects of Black ginseng have been addressed in many studies. Existing researches were focused on verifying effectiveness of ginseng on improvement of insulin resistibility or restoration of β-cell function, but this research decided that ginseng is also related with effective utilization of in-body glucose based on traditional use of ginseng. Therefore, this research is going to prove effectiveness of black ginseng on glucose metabolism intensively.

In this study, we focused on the effects of black ginseng extract (GBG05-FF) on skeletal muscle using C2C12 myotube and STZ-induced diabetic mice. First, we investigated the effects of GBG05-FF on glucose uptake in C2C12 myotubes and the mechanisms involved. Furthermore, we demonstrated the anti-diabetic effects of GBG05-FF and possible mechanisms involved in STZ-induced diabetic mice. Gensenosides were evaluated as the major active compound of GBG05-FF.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's medium (DMEM), penicillin, and streptomycin were obtained from Hyclone (Logan, UT, USA). Bovine serum albumin, LY294002, compound C, nicotinamide, streptozotocin and D-glucose were purchased from Sigma (St. Louis, MO, USA). Insulin Rα, P-mTOR, GLUT2, GLUT4 and peroxidase-conjugated secondary antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). P-IRS-1, P-LKB1 and P-AMPK were purchased from Cell Signaling Technology (Danvers, MA, USA). In addition, the RNeasy Mini kit and QuantiTect Reverse Transcription kit were purchased from Qiagen (Hilden, Germany). Finally, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA).

2.1.1. The preparation of black ginseng extract

The 5-year old Korean white ginseng (Panax ginseng C.A. Meyer) was purchased from a local ginseng center (Geumsan, Korea). The black ginseng was produced by repeatedly steaming white ginseng at 95 °C for 6 h and drying at 60 °C in an oven. The dried black ginseng samples were twice extracted in 10 volumes of 70% ethanol at 80 °C for 8 h by using a Soxhlet extractor with a heating mantle. The extracts were subsequently filtered and lyophilized. The extraction yields of black ginseng were minutely described on Table 1. Black ginseng extract was designated as GBG05-FF and used for research.

Table 1.
Yield and moisture content of GBF05-FF.

Sample	Moisture content (%)	Weight (kg)	Yield (%)
Fresh ginseng	70.64	10	100
Black ginseng	14.18	2.65	26.5
GBG05-FF	2.36	0.85	8.48

2.1.2. HPLC analytical methods

1 g dried black ginseng powder was extracted three times with 20 mL of 80% methanol at 80 °C for 2 h. Three replicate extracts were combined, the solvent was a rotary vacuum-evaporator at 40 °C, and the residue was dissolved in 10 mL of distilled water. The reaction mixture was extracted three times with water saturated n-butanol and evaporated in a vacuum. The residue was dissolved in 1 mL of 50% methanol and filtered through a 0.45 μm. Ginsenosides were assayed by HPLC using an Agilent 1260 system (Santa Clara, CA, USA) and a detection wavelength of 203 nm with a Poroshell 120 EC-C18 column (3.0 mm × 50 mm, 2.7 μm, Agilent). The column was eluted at 35 °C with a linear gradient of acetonitrile/water from 5:95 to 95:5 (v/v) at a flow rate of 0.8 mL/min. Standard ginsenosides Rg3, Rg5, Rk1, Rh4 were purchased from Ambo institute(Daejeon, Republic of Korea).

2.2. Animal and induction of diabetes

All animal experiments were conducted in accordance with the guidelines established by the Animal Ethics Committee of Wonkwang University, which also approved the protocols (Approval No. WKU15–101). ICR mice were purchased from SAMTAKO (Osan, Korea). Four-week-old male mice were housed with a 12-h light–dark cycle with free access to food and water., Intraperitoneal injection of streptozotocin (STZ) (60 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) was performed twice after 12 h fasting for 2 d to induce hyperglycemia. Mice with hyperglycemia (Fasting blood glucose > 200 mg/dL(11 mmol/L)) were chosen for subsequent experiments. Normal group mice were injected with an equal volume of 0.1 M citrate buffer. STZ-induced diabetic mice were divided into 3 groups with similar fasting blood glucose levels and weight. Two groups of diabetic mice were orally administrated GBG05-FF 300 mg/kg/day and 900 mg/kg/day for 5 weeks. Doses were chosen following guideline from KFDA which required more than 3 ford difference between experimental doses without toxicity. At the end of the study, the mice were sacrificed by CO_2 Anesthesia and Exsanguination. Blood is collected from the aorta. Liver and muscles were carefully removed and used for biochemical, histological and molecular assays including preliminary processing.

Experimental Design was as follows.

Group I (Normal): Normal control rat group were fed basal diet and treated with water throughout the experiment; Group II (STZ): STZ-induced diabetic rats were treated with water; Group III (GBG05-FF300): Diabetic rats treated with an oral dose of GBG05-FF 300 mg/kg b.w.; and Group IV (GBG05-FF900): Diabetic rats treated with an oral dose of GBG05-FF 900 mg/kg b.w.