Journal of Ethnopharmacology 210 (2018) 80–87

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Korean red ginseng (*Panax ginseng*) inhibits obesity and improves lipid metabolism in high fat diet-fed castrated mice

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ARTICLE INFO

Keywords:
3T3-L1 cell
Adipogenesis, dyslipidemia
Lipogenesis
Heaptic steatosis
Testosterone

ABSTRACT

Ethnopharmacological relevance: Korean red ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) has been historically used as a traditional drug for the prevention and treatment of most ageing-related diseases, such as obesity, dyslipidemia, diabetes, and cardiovascular disease. Elderly men with testosterone deficiency are strongly associated with many of the aforementioned metabolic diseases. The aim of this study was to determine the effects of ginseng on obesity and lipid metabolism in a mouse model of testosterone deficiency (castrated C57BL/6J mice).

Materials and methods: The effects of ginseng extract (GE) on obesity and lipid metabolism in high-fat diet (HFD)-fed castrated C57BL/6J mice were examined using hematoxylin and eosin staining, serum lipid analysis, and quantitative real-time polymerase chain reaction (PCR). The effects of GE, ginsenosides, and testosterone on adipogenesis were measured using Oil Red O staining, XTT assay, and real-time PCR.

Results: Compared with HFD mice, mice receiving HFD supplemented with GE (HFD-GE) for 8 weeks showed decreased body weight, adipose tissue mass, and adipocyte size without affecting food intake. Serum levels of triglycerides and total cholesterol were lowered in HFD-GE mice than in HFD mice. GE also markedly reduced HFD-induced hepatic lipid accumulation. Concomitantly, HFD-GE decreased mRNA expression of adipogenesis-related genes (SREBP-1C, PPARγ, FAS, SCD1, and ACC1) in visceral adipose tissues compared with HFD alone. Consistent with the in vivo data, GE and major active ginsenosides (Rb1 and Rg1) decreased lipid accumulation and mRNA expression of PPARγ, C/EBPα, and SCD1 in 3T3-L1 adipocytes compared with control. Similarly, testosterone also decreased lipid accumulation and mRNA levels of PPARγ, C/EBPα, and SCD1. These inhibitory effects were further increased by co-treatment of GE or ginsenosides with testosterone.

Conclusions: Our results demonstrate that ginseng can inhibit obesity and dyslipidemia in HFD-fed castrated mice, possibly by inhibiting adipogenic gene expression. In addition, our results indicate that ginseng may act like testosterone to inhibit adipogenesis, suggesting that ginseng may be able to prevent obesity, hyperlipidemia, and hepatic steatosis in men with testosterone deficiency.

1. Introduction

Obesity is the result of energy imbalance caused by an increased ratio of caloric intake to energy expenditure. The prevalence of obesity has rapidly increased in adult male men, and the related metabolic disorders of dyslipidemia, atherosclerosis, and type 2 diabetes have become global health problems.

Herbs have long been used to treat illness and improve health. In fact, herbs still account for about 80% of medical treatments in the developing countries, and approximately one-third of pharmaceutical drugs are derived from plant sources (Winslow and Kroll, 1998; Bent and Ko, 2004; Kang et al., 2010). Ginseng, in particular, has widely been used as a traditional herbal medicine in Korea, China, and Japan for more than 2,000 years (Yun, 2001; Park et al., 2012). Numerous studies have described the pharmacological effects of ginseng on the immune, central nervous, endocrine, and cardiovascular systems (Gillis, 1997; Attele et al., 1999; Lu et al., 2009). Ginseng has also been traditionally used in the prevention and treatment of most ageing-related metabolic syndrome, such as obesity, diabetes, and hyperlipidemia (School, 1986; Yin et al., 2008). Recently, ginseng is reported to induce weight loss in animal models of obesity and can effectively regulate signaling pathways and genes that play a role in obesity (Attele et al., 2002; Kim et al., 2005; Karu et al., 2007; Mollah et al., 2009; Lee et al., 2009, 2012). In addition, ginseng significantly inhibits visceral

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http://dx.doi.org/10.1016/j.jep.2017.08.032
Received 25 May 2017; Received in revised form 7 August 2017; Accepted 24 August 2017
Available online 24 August 2017

adipocyte hypertrophy (Lee et al., 2013, 2014, 2016), which is closely associated with metabolic syndromes including insulin resistance and hepatic steatosis (Jeong and Yoon, 2009; Lee et al., 2014). Our previous study demonstrated that ginseng decreases adipose tissue mass and prevents obesity in diet-induced and ovariectomized obese mice (Lee et al., 2013, 2014, 2016).

Obesity in the ageing men is associated with lowered testosterone levels (Michalakis et al., 2013; Fui et al., 2014; Traish, 2014; Kelly and Jones, 2015). Recent studies have shown that obese men have lower testosterone levels compared with lean men, and testosterone therapy in both ageing and hypogonadal men with testosterone deficiency results in weight loss and a lower risk of metabolic syndrome (Yassin and Doros, 2013; Francomano et al., 2014). Thus, we hypothesized that Korean red ginseng is able to inhibit weight gain and regulate obesity in castrated male mice, an animal model of men with testosterone deficiency, by regulating the expression of adipogenesis-associated genes.

In this study, we fed castrated mice a high-fat diet (HFD) supplemented with Korean red ginseng extract (GE). We found that treatment with GE decreases body weight gain, adipose tissue mass, adipocyte size, and inhibits dyslipidemia and hepatic steatosis. Concomitantly, adipogenesis-related gene expression was lowered by GE in HFD mice. Similarly, treatment with GE and ginsenosides inhibits lipid accumulation and adipogenic gene expression in 3T3-L1 adipocytes. In addition, testosterone also decreased lipid accumulation and mRNA levels of adipogenesis-ssociated genes. These inhibitory effects were more increased by co-treatment of GE or ginsenosides with testosterone. Our findings suggest that ginseng can regulate obesity and lipid metabolism in testosterone deficiency.

2. Materials and methods

2.1. GE preparation

The GE was prepared from 6-year-old *Panax ginseng* C. A. Meyer (Korea Ginseng Corporation, Seoul, Korea). A voucher specimen was deposited at the laboratory of Korea Ginseng Corporation and the batch number of ginseng used in our study is 6510100112048. Briefly, red ginseng was boiled in distilled water for 24 h at 95 °C. The aqueous extracts were filtered and freeze-dried under vacuum to produce GE powder.

For analysis of the quality of GE, GE powder (100 g) was placed into a 1-L flask with a refluxing condenser and extracted twice with 500 ml of water-saturated 1-butanol for 1 h at 80 °C. The extracted solution was passed through Whatman filter paper (No. 41) after cooling. The process was repeated twice. The residue and filter paper were washed with 100 ml of water-saturated 1-butanol, and then the filtrate was washed twice with 100 ml of water in a 2-L separating funnel. The butanol layer was then evaporated to dryness. The concentrate was extracted to remove any traces of fat with 100 ml of diethyl ether for 30 min at 36 °C in a flask with a refluxing condenser, after which the ether solution was decanted. The quality control of GE was analyzed by the HPLC/ELSD system and the HPLC profile of GE was described previously (Lee et al., 2014). The contents of ginsenosides in GE were reported previously and GE contained ginsenosides Rb1 and Rg1 (5 mg/g) as major active compounds (Lee et al., 2016).

2.2. Animal treatments

For all experiments, 8-week-old male wild-type C57BL/6 J mice were housed and bred at Mokwon University with a standard 12-h light/dark cycle. Prior to the administration of the special diets, the mice were given standard rodent chow and water ad libitum. The mice were castrated and then divided into three groups (n = 8/group). The first group received a low-fat diet (LFD, 10% kcal fat, Research Diets, Brunswick, NJ, USA) for 8 weeks. The second group received an HFD (45% kcal fat, Research Diets). The third group received an HFD supplemented with 5% GE (HFD-GE). For the HFD-GE preparation, 50 g GE powder was mixed with 1 kg HFD (Lee et al., 2013). Body weight was measured three times a week by a person blinded to each treatment group. Food intake was determined by estimating the amount of food consumed by the mice throughout the treatment period. Cages were inspected for food spillage, but only a little spillage was noticed and collected to measure food intake. After an 8-h fast on the last day of the study, the animals were sacrificed by cervical dislocation. Blood was collected from the retro-orbital sinus into tubes, and serum was separated and stored at −80 °C until analysis. Visceral (VSC) fat pads were removed, weighed, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Portions of the VSC fat pads and liver tissues were prepared for histology. Serum triglyceride levels were measured using an automatic blood chemical analyzer (CIBA Corning, Oberlin, OH). Levels of total cholesterol were measured using SICDIA NEFAZYME (Shinyang Chemical, Seoul, Korea). All animal experiments were approved by the Institutional Animal Care and Use Committees of Mokwon University and were carried out in accordance with the National Research Council Guidelines.

2.3. Histological analysis

The liver and VSC adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed for paraffin sections. Tissue sections (5 μm) were cut and stained with hematoxylin and eosin for examination by microscopy. To quantify adipocyte size, the stained sections were analyzed using the Image-Pro Plus analysis system (Media Cybernetics, Bethesda, MD, USA).

2.4. In vitro cytotoxicity test

Murine 3T3-L1 cells (ATCC, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (Invitrogen, Carlsbad, CA, USA) in a 37 °C incubator with a humidified atmosphere containing 5% CO_2. Cells were plated on a 96 well plate at a density of 1×10^4 cells/well and incubated for 24 h at 37 °C with culture medium in the presence of dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), and testosterone (100 nM). Cell viability was detected by 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl] − 2H-tetrazolium-5-carboxanilide disodium salt (XTT) assays using a Cell Proliferation Kit II (Roche, Basel, Switzerland).

2.5. 3T3-L1 differentiation and analysis of triglyceride content

3T3-L1 cells grown in DMEM containing 10% bovine calf serum were maintained at confluence for 2 days, after which the medium was replaced with DMEM containing 0.5 mM 1-methyl-3-isobutyl-xanthine, 1 μM dexamethasone, and 1 μg/ml insulin, and 10% fetal bovine serum (Invitrogen) (day 0). The cultures were incubated for 2 days to induce adipocyte differentiation, and then the medium was replaced with DMEM containing 10% fetal bovine serum for the remainder of the differentiation process. The cells were treated with dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), and testosterone (100 nM) on days 0–2 only, and the medium was changed every other day. Dimethyl sulfoxide was used as a solvent vehicle. On day 8, the cells were fixed in 10% formalin for 1 h and stained with Oil Red O for 2 h. For quantitative analysis, the Oil Red O stain was eluted by adding isopropanol and quantified by measuring absorbance at 520 nm.

2.6. Quantitative real-time polymerase chain reaction (PCR)

Total cellular RNA from VSC adipose tissues and 3T3-L1 cells was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY, USA) according to the manufacturer's instructions. Total cellular RNA (2 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase to generate an antisense cDNA template. The genes of