Anti-diabetic Effect and Mechanism of Korean Red Ginseng in C57BL/KsJ db/db Mice

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Abstract: The present study was designed to investigate the anti-diabetic effect and mechanism of Korean red ginseng in C57BL/KsJ db/db mice. The db/db mice were divided into three groups: diabetic control group (DC), Korean red ginseng group (KRG, 100 mg/kg) and metformin group (MET, 300 mg/kg), and treated with drugs once per day for 10 weeks. Compared to the DC group, fasting blood glucose levels were decreased by 19.8% in KRG-, 67.7% in MET-treated group. With decreased plasma glucose and insulin levels, the insulin resistance index of the KRG-treated group was reduced by 27.6% compared to the DC group. The HbA1c levels in KRG and MET-treated groups were also decreased by 11.0% and 18.9% compared to that of DC group, respectively. Plasma triglyceride and non-esterified fatty acid levels were decreased by 18.8% and 16.8%, respectively, and plasma adiponectin and leptin levels were increased by 20.6% and 12.1%, respectively, in the KRG-treated group compared to those in DC group. Histological analyses of the liver and fat tissue of mice treated with KRG revealed significantly decreased number of lipid droplets and decreased size of adipocytes compared to the DC group. From the pancreatic islet double-immunofluorescence staining, we observed KRG has increased insulin contents, but decreased glucagon production. To elucidate action mechanism of KRG, effects on AMP-activated protein kinase (AMPK) and its downstream target proteins responsible for fatty acid oxidation and gluconeogenesis were explored in the liver. KRG activated AMPK and acetyl-coA carboxylase (ACC) phosphorylations, resulting in stimulation of fatty acid oxidation. KRG also caused to down regulation of SREBP1α and its target gene expressions such as FAS, SCD1 and GPAT. In summary, our results suggest that KRG exerted the anti-diabetic effect through AMPK activation in the liver of db/db mice.

Key words: Korean red ginseng, C57BL/KsJ db/db mice, diabetes, AMPK, fatty acid oxidation.

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eye, kidney, nerve and blood vessel.1, 2) This disorder affects nearly 10% of the world’s population.3) The management of diabetes is considered a global problem and a cure has yet to be discovered. Modern drugs, including insulin and other hyperglycemia agents such as biguanides, sulphonylureas etc. control the blood glucose level only when they are regularly administered, but these treatments are tedious and have several disadvantages.4-6)

Plants have long been used as therapeutic purposes, and many of the currently available drugs are directly or indirectly derived from plants. Following the recommendations of the World Health Organization Expert Committee on Diabetes Mellitus, it is important to investigate hypoglycemic agents of plant origin used in traditional medicine.7)

Ginseng is a well-known medical plant used in traditional oriental medicine. The root of ginseng has been used as health product or natural remedy for a long time.8) Korean red ginseng (KRG), steamed root of Panax ginseng C.A. Meyer, has been known to have potent biological activities such as radical scavenging, vasodilating, anti-tumor and anti-diabetic activities.9-13) However, the mechanism of the beneficial effects of KRG on diabetes is yet to be elucidated. Therefore, in the present study, we investigated the anti-diabetic effect and action mechanism of KRG extract in C57BL/KsJ db/db mice.
MATERIALS AND METHODS

Materials KRG water extract was donated from KT&G (Seoul, Korea). The extracts 10 g were diluted in the 2 L water, freeze dried and kept at deep freezer until use.

Animals Five-week old C57BL/KsJ db/db mice were purchased from ORIENT BIO (Sungnam, Korea). All animals were acclimatized to the laboratory environment for 2 weeks before the experiment. Mice were allowed to freely access to drinking water and food under constant room temperature (22 ± 2°C) and humidity (50 ± 10%) conditions with an automatic 12 hr light and 12 hr dark cycle and cared for and treated in accordance with guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. Mice were randomly divided into three groups: diabetic control group (DC), KRG group (100 mg/kg), metformin group (MET, 300 mg/kg), and treated with drugs by oral administration once per day for 10 weeks. For oral administration, the drugs were diluted in the distilled water. During the experiment, body weight and blood glucose levels were measured once every week.

Assay of plasma parameters After 10 weeks treatment, the blood sample were collected and then centrifuged at 3,000g for 15 min at 4°C, and serum glucose, insulin, hemoglobin A1c (HbA1c), triglyceride (TG), non-esterified fatty acid (NEFA), adiponectin and leptin level were measured. Serum glucose concentration was determined using the glucose oxidase method (Asan Pharmaceutical Co., Korea). Serum insulin concentrations were determined using a mouse insulin enzyme immunoassay kit. (Gunma, Japan). HbA1c was measured using a hemoglobin A1c kit (Biosystems S.A., Barcelona, Spain). Serum TG concentration was determined using a commercially available kits (Asan pharmaceutical Co., Seoul, Korea). Serum NEFA and adiponectin levels were determined using enzymatic colorimetric method (Eiken, Tokyo, Japan) and a mouse adiponectin ELISA kit (Adipogen, Korea), respectively. Leptin level was measured using a mouse leptin enzyme immunoassay kit (Linco Research, USA).

Histological analysis The pancreas, epidydimal fat tissue and liver were removed and fixed in 10% neutral buffered formalin. The tissues were subsequently embedded in paraffin and sectioned with 5 µm thickness (Leica,Wetzlar, Germany), and stained with hematoxylin-eosin for microscopic assessment (Olympus, Japan). To examine the insulin and glucagon contents in pancreas on the same sections, a double immunofluorescence technique was used. The sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed by 0.1% trypsin. To block nonspecific binding of immunoglobulin, the sections were incubated with normal serum blocking solution for 30 min at room temperature. Goat anti-insulin Ig G (1:75, Santa Cruz Biotechnology, Santa Cruz, USA) were applied overnight at 4°C, followed the tissue sections were incubated with normal serum blocking solution for 30 min at room temperature. Then rabbit anti-glucagon Ig G (1:75, Santa Cruz Biotechnology, Santa Cruz, USA) were applied overnight at 4°C, followed the tissue sections were incubated with goat anti-rabbit IgG-FITC (1:200, Santa Cruz Biotechnology, Santa Cruz, USA) for 30 min at room temperature. Double immunolabeling was detected with the aid of a fluorescence microscope (AX-70, Olympus, Tokyo, Japan).

Western blot analysis After sacrificed, liver was immediately removed and instantly soaked in liquid nitrogen and stored at -70°C. Protein extracts were prepared in protein extraction kit (Intron Biotechnology Inc., Seoul, Korea). Lysates (30 µg) were electroblotted onto a nitrocellulose membrane following separation on a 8% SDS-polyacrylamide gel electrophoresis. Blotted membranes were incubated for 1 hr with blocking solution (tris-bufferd saline/Tween 20, TBST) containing 5% skin milk (w/v) at room temperature, followed by incubation overnight at 4°C with 1:2000 dilution of AMP-activated protein kinase (AMPK), phosphoAMP-activated protein kinase (p-AMPK), acetyl-coA carboxylase (ACC), phospho- acetyl-coA carboxylase(p-ACC) primary antibody (Cellsignaling, USA). Membranes were washed four times with 0.1% TBST and incubated with 1:3000 dilution of horseradish peroxidase-conjugated goat anti-rabbit or donkey anti-rabbit IgG secondary antibody for 1 hr at room temperature. Membranes were washed four times in TBST and then developed by ECL (Amersham, Sweden).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Total mRNA was isolated from mice liver using an Easy-Blue kit (Intron Biotechnology Inc, Seoul, Korea) according to the manufacture’s instructions. From each sample, total RNA (10 µg) was reverse transcribed