Alleviation of diabetic complications by ginsenoside Rg3-enriched red ginseng extract in western diet-fed LDL−/− mice

Evelyn Saba 1,correo electrónico: rheemh@knu.ac.kr (M.H. Rhee).
Seung-Hyung Kim 2,*, Sung-Dae Kim 3, Sang-Joon Park 1, Dongmi Kwak 1, Jun-Hwan Oh 4, Chae-Kyu Park 4, Man Hee Rhee 1,∗

1 Department of Veterinary Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea
2 Institute of Traditional Medicine & Bioscience, Daejeon University, Daejeon, Republic of Korea
3 Research Center, Dongnam Institute of Radiological and Medical Sciences, Busan, Republic of Korea
4 R&D Headquarters, Korean Ginseng Corporation, Daejeon, Republic of Korea

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A B S T R A C T
In this study, we precisely showed how the Rg3-enriched red ginseng extract (Rg3-RGE) lowers glucose, triglyceride, and low-density lipoprotein [LDL] levels in LDL−/− mice. Aspartate aminotransferase/serum glutamic-oxaloacetic transaminase, alanine aminotransferase/serum glutamate-pyruvate transaminase, and steatohepatitis were found to be reduced, and atheroma formation was inhibited by Rg3-enriched red ginseng extract.

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Epidemiologically, cardiovascular diseases (CVDs) are the major causes of morbidity and mortality worldwide. The high prevalence of CVDs is primarily attributed to the increased use of nonorganic health products and sedentary lifestyle [1]. A large percentage of the population now suffers from diabetes, which is a risk factor for the occurrence of CVDs [2]. Patients who suffer from diabetes are more prone to myocardial infarction, congestive heart failure, and reinfarction [3]. Diabetes and CVDs show common manifestations, such as arterial dysfunction, dyslipidemia, hyperglycemia, and alteration of cell types in the endothelial layer of vessels, which lead to chronic heart disease [4–6]. Many allopathic therapies are being used successfully for the treatment of CVDs and diabetes; however, if prolonged, their side effects can become serious. Therefore, managing diabetes by inhibiting atherosclerosis or CVDs using natural herbal medications can drastically improve lifestyle without causing any complications.

Panax ginseng can be considered the “king of herbs” and is a multifunctional supplement that has been reported to possess anti-inflammatory, anticancer, antistress, and antiplatelet activities, as well as to increase longevity and enhance libido [7]. The major active constituents in this herb are divided into three groups: protopanaxadiols, protopanaxatriols, and oleic acids. These components are called ginsenosides. Korean Red Ginseng extract (RGE), which contains these three subcomponents, has been studied extensively for its effects on cardiovascular diseases and diabetes. Rg3-enriched RGE (Rg3-RGE) is the same extract that has been enriched with the ginsenoside Rg3. Briefly, Red ginseng (stem/root = 75:25) was extracted with distilled water proceeding with 55% ethanol extraction. With multiple extractions, a concentrated extract was prepared. Subsequently the extract was then subjected to high performance liquid chromatography (HPLC) and resultant profile of constituents is given in Table 1 with Rg3 in most major quantities. Many of the properties of ginsenoside Rg3 have been elucidated in the past, including its potent effects on vascular diseases [8–12].

In this study, we report for the first time that Rg3-RGE reduces the levels of low-density lipoprotein (LDL), serum glutamic-oxaloacetic transaminase, and serum glutamate-pyruvate transaminase, as well as steatohepatitis and atheroma formation in LDL−/− mice in a dose-dependent manner. The aim of this study was to characterize the complications associated with diabetes, e.g., atherosclerosis, and to minimize and mitigate the occurrence of diabetes and thereby CVDs.

Male LDL−/− and C57BL/6 mice aged 6 wk were purchased from the Jackson Laboratory (Sacramento, CA, USA). Mice were provided...
a normal chow diet, while LDL−/− mice were provided a western diet (WD) for 12 wk. Animals were maintained in a pathogen-free facility according to international guidelines for animal care. All in vivo experiments were carried out with the permission and according to the protocols provided by the Institutional Animal Care and Use Committee of Daejeon University. Mice were divided into five groups (n = 6). The first group was fed only the normal chow diet. The second group received a WD, and the third group was treated with 5 mg/kg atorvastatin in addition to the WD. The fourth and fifth groups were administered Rg3-RGE at a dose of 2.5 mg/kg and 5 mg/kg, respectively, over the last 4 wk of WD feeding. At the end of 12 wk, blood and tissue samples were collected and either stored at −70°C or processed to investigate various parameters.

Mice were fasted overnight and blood was harvested the following day. Serum samples were obtained in order to investigate glucose, LDL, total cholesterol, triglyceride, glutamic-oxaloacetic transaminase, and glutamate-pyruvate transaminase levels using enzymatic methods (FUJI DRI-CHEM 4000i; FUJI, Shizuoka, Japan). For hematoxylin and eosin staining, liver and aortic tissues were embedded in paraffin, dehydrated, and Use Committee of Daejeon University. Mice were divided into four groups (C14/C6). The first group was fed only the normal chow diet, while LDL−/− mice were provided a western diet (WD) for 12 wk. Animals were maintained in a pathogen-free facility according to international guidelines for animal care. All in vivo experiments were carried out with the permission and according to the protocols provided by the Institutional Animal Care and Use Committee of Daejeon University. Mice were divided into five groups (n = 6). The first group was fed only the normal chow diet. The second group received a WD, and the third group was treated with 5 mg/kg atorvastatin in addition to the WD. The fourth and fifth groups were administered Rg3-RGE at a dose of 2.5 mg/kg and 5 mg/kg, respectively, over the last 4 wk of WD feeding. At the end of 12 wk, blood and tissue samples were collected and either stored at −70°C or processed to investigate various parameters. Mice were fasted overnight and blood was harvested the following day. Serum samples were obtained in order to investigate glucose, LDL, total cholesterol, triglyceride, glutamic-oxaloacetic transaminase, and glutamate-pyruvate transaminase levels using enzymatic methods (FUJI DRI-CHEM 4000i; FUJI, Shizuoka, Japan). For hematoxylin and eosin staining, liver and aortic tissues were embedded in paraffin, dehydrated, and cut into 4 μm-thick sections. These were later analyzed using a light microscope.

| Ginsenosides | Contents (mg/g) |
|-------------|----------------|
| Rb1         | 3.86           |
| 20(S)-Rg3   | 44.91          |
| Rg2         | 1.20           |
| Rb2         | 1.53           |
| Rd          | 1.60           |
| Rf          | 1.28           |
| Rh1         | 3.71           |
| 20(S)-Rg2   | 3.55           |
| 20(R)-Rg3   | 6.78           |
| Total       | 67.41          |

Table 1: Profile of Rg3-enriched ginseng extract constituents

Fig. 1. Rg3-RGE attenuated serum glucose and liver biomarker levels in LDL−/− mice. (A–C) Serum glucose, GOT, and GPT levels were determined using an ELISA kit after oral treatment of the mice with Rg3-RGE over 4 wk. Bar graphs represent means ± SEM (n = 6). A p value of <0.05 was considered significant compared with the atorvastatin group (positive control). (D) This figure part represents the following: (a) normal liver section of mice treated with regular chow diet; (b) LDL−/− mice fed with a western diet for 12 wk; (c) LDL−/− mice treated with atorvastatin at 5 mg/kg as positive control; and (d) LDL−/− mice given Rg3-RGE at (d) 2.5 mg/kg and (e) 5 mg/kg. GOT, glutamic-oxaloacetic transaminase; GPT, glutamate-pyruvate transaminase; LDL, low-density lipoprotein; Rg3-RGE, Rg3-enriched red ginseng extract; SEM, standard error of the mean.
examination of the aortas also showed that endothelial thickening (as indicated by black arrows), associated with a high-fat diet and leading to atheromatic plaque formation [20], potentially decreased by Rg3-RGE treatment [Fig. 2F(a–e)]. In summary, we can conclude that our study has demonstrated a comprehensive link between diabetes and atherosclerosis, and showed their alleviation using a natural herbal product, i.e., Rg3-RGE. With future studies, we will be able to further clarify the link between diabetes and CVDs.

Conflicts of interest

All authors declare no conflicts of interest.

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