Molecular & Cellular Toxicology (2020) 16:39–50
https://doi.org/10.1007/s13273-019-00056-7

ORIGINAL ARTICLE

Multi-tissue lipotoxicity caused by high-fat diet feeding is attenuated by the supplementation of Korean red ginseng in mice

Seon-A Jang[1] · Seung Namkoong[2] · Sung Ryul Lee[3] · Jin Woo Lee[2] · Yuna Park[4] · Gyeongseop So[2] · Sung Hyeok Kim[2] · Mi-Ja Kim[2] · Ki-Hyo Jang[2] · Alberto P. Avolio[5] · Sumudu V. S. Gangoda[5] · Hyun Jung Koo[6] · Myung Kyum Kim[4] · Se Chan Kang[1] · Eun-Hwa Sohn[2]

Accepted: 14 November 2019 / Published online: 3 December 2019

Abstract

Background Excessive intake of fat, one of the causes of obesity, is associated with low-grade inflammation in various susceptible organs and eventually causes tissue toxicity. This study examines the multifaceted suppressive effects of Korean red ginseng extract (KRG) on high-fat diet (HFD)-induced lipotoxicity and inflammatory responses in the aorta, liver, and brain.

Methods Male C57BL/6 mice were fed HFD with or without KRG for 12 weeks. The improvement effect in KRG on lipotoxicity and inflammatory potential was determined in the blood and the aorta, liver, and brain tissues.

Results KRG significantly inhibited 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity by > 20% in vitro. KRG supplementation suppressed HFD-associated body weight gain, lipid profile changes, and excessive fat deposition in the liver and increased leptin, insulin, and ALT levels in the blood. Inflammatory markers in the aorta, liver, and brain were also significantly reduced by KRG treatment. In microvascular endothelial cells, the 15% cyclic stretch-mediated upregulation of ICAM-1 and vascular cell adhesion protein-1 (VCAM-1) expression was significantly attenuated in the presence of KRG.

Conclusion KRG supplementation attenuates HFD-mediated body weight gain, lipid profile changes, and multi-tissue inflammatory responses.

Keywords Cell adhesion molecules · High-fat diet · Inflammation · Korean red ginseng · Lipotoxicity

Introduction

Obesity is a public health concern worldwide due to its increasing prevalence and compelling effects on the development of metabolic diseases (Haslam and James 2005; Gadde et al. 2018). Dietary fat is utilised as an energy source in the body and plays an important role in health (Lichtenstein et al. 1998). However, excessive consumption of fat and its aberrant deposition in the body is linked to the development of obesity (Lichtenstein et al. 1998) and lipotoxicity in non-adipose tissues such as the heart, skeletal muscle, pancreas, and liver (Schaffer 2003). Except in manifested metabolic

Seon-A Jang, Seung Namkoong and Sung Ryul Lee contributed equally to this work.

✉ Eun-Hwa Sohn
ehson@kangwon.ac.kr

[1] Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, Republic of Korea

[2] College of Health Sciences, Kangwon National University, Samcheok 25949, Republic of Korea

[3] Department of Convergence Biomedical Science, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan 47392, Republic of Korea

[4] Department of Bio and Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea

[5] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia

[6] Department of Medicinal and Industrial Crops, Korea National College of Agriculture and Fisheries, Jeonju 54874, Republic of Korea

disorders, the presence of high fatty acid due to an imbalance between fatty acid uptake and utilisation contributes to the accumulation of toxic lipids, which involves in organ toxicity via cell apoptosis and excessive oxidative, endoplasmic, and mitochondrial stress (Engin 2017). Inflammation associated lipotoxicity throughout the body contributes to multi-organ damage and increases susceptibility to insulin resistance, diabetes, hypertension, and neuropathological alterations (Wellen and Hotamisligil 2005). Although the underlying mechanisms by which a high-fat diet (HFD) exerts adverse effects on human health remains poorly understood, multi-organ inflammation, including in the brain, and increased oxidative stress are proposed as major players in morbidity associated with obesity (Musaad and Haynes 2007; Duan et al. 2018). In medical toxicology, obese patients display different physiological and pharmacokinetic characteristics; thus, special medical considerations are necessary to prevent the problems of drug overdose (Zuckerman et al. 2015)).

Due to the multi-causal aetiology and complex pathophysiology of obesity-associated complications, pharmacotherapy-based management is poor (Acosta et al. 2014; Hussain and Bloom 2011). Ginseng, the root of *Panax ginseng* Meyer, has been traditionally used as a medicine for treating and preventing diseases in East Asia and its health-promoting potentials attract worldwide attention (So et al. 2018). Korean red ginseng (KRG) is made via a specific manufacturing process that includes the steaming and drying of white ginseng. KRG contains Rg3, Rg5, and Rk1, which are the distinctive compounds in steamed *P. ginseng* (So et al. 2018; Kim et al. 2007). The pharmacological effects and mechanisms of KRG and its components have been studied extensively (So et al. 2018). Several studies reveal that ginseng possesses an anti-obesity potential (Li and Ji 2018; Choi et al. 2013). However, the multifaceted suppressive effect of KRG on HFD-mediated lipotoxicity and inflammatory responses occurring in the aorta, liver, and brain has not been well established.

In this study, the direct inhibitory effect of KRG on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity was determined in vitro. Next, male C57BL/6 mice were fed with HFD in the presence or absence of KRG supplementation (10 and 50 mg/kg body weight) via a gastric gavage for 12 weeks. Then, changes in body and organ weights, lipid profiles, and blood leptin, insulin, and alanine aminotransferase (ALT)/aspartate aminotransferase (AST) levels were investigated. In the liver tissue, fat accumulation was assessed via haematoxylin and eosin (H&E) and Oil Red O staining and mRNA expression levels of cyclooxygenase-2 (COX-2) were determined. To identify the suppressive effect of KRG on vascular inflammatory responses, the thickness of the aortic vascular wall was measured by H&E staining and the protein expression levels of intercellular cell adhesion molecule-1 (ICAM-1) in aortic tissues were evaluated. In addition, changes in the protein expression levels of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) caused by a 15% cyclic stretch, which mimics hypertensive conditions (Gangoda et al. 2018), were determined in the cerebrovascular cells. Finally, protein expression levels of amyloid-β precursor protein (APP), β-secretase1 (BACE1), and Tau, which are important factors involved in neurological inflammation, were assessed in the hippocampal tissue.

Materials and methods

Chemicals and reagents

ALT, AST, and HMG-CoA reductase activity assay kits, Oil Red O, and simvastatin (SV) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Normal chow diet (D12450B; 10% kcal% fat; 4.057 kcal/g) and HFD (D12492; 60% kcal% fat; 5.243 kcal/g) was obtained from Research Diet, Inc. (New Brunswick, NJ, USA). Assay kits for determining insulin, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and HMG-CoA were purchased from Biovision (Milpitas, CA, USA). Serum leptin levels were measured using the R&D system assay kit (Minneapolis, MN, USA). Antibodies against ICAM-1, VCAM-1, COX-2, and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against APP, BACE1, and Tau were obtained from EMD Millipore (Billerica, MA, USA). Antibodies against β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA).

Preparation of KRG

Standardised KRG powder was kindly provided by Korea Ginseng Corporation (Seoul, Korea, 2016, H1269-6041). KRG extract was dissolved in phosphate-buffered saline (PBS) at a final concentration of 30 mg/mL and frozen at − 80 °C.

HMG-CoA reductase (HMGR) activity

The potential for KRG extracts to inhibit the rate-limiting enzyme of cholesterol biosynthesis was measured using the HMGR assay kit (Sigma-Aldrich, catalogue number CS1090) according to the manufacturer's protocol. Briefly, 1 × assay buffer, NADPH, and HMG-CoA substrate were sequentially added to a 96-well plate. PBS (2 µL), SV (10 µg/mL) and different concentrations of KRG (10–200 µg/mL) were added and the total volume was adjusted to 198 µL with 1 × assay buffer. After 2 µL of the purified catalytic domain of HMGR was added, an enzymatic reaction was initiated

at 37 °C. Colorimetric changes to NAPDH in the reaction mixtures were determined by measuring the absorbance at 340 nm using a 96-well microplate reader (VICTOR X3; PerkinElmer, Waltham, MA, USA) after 30 min. The control value was set at 1.0 and expressed as a relative ratio to the control value.

Cell culture

Human hCMEC/D3 brain microvessel endothelial cells, immortalised by lentiviral transduction using large SV40 T antigen (Weksler et al. 2005), were purchased from Applied Biological Materials Inc. (Richmond, British Columbia, Canada). Cells were maintained in M199 media (Sigma-Aldrich) supplemented with 10% foetal bovine serum (Gibco; NY, USA) and 1% penicillin/streptomycin (Gibco) at 37 °C in fully humidified air with 5% CO_2. Passages 17–19 were used for all experiments.

Cyclic stretching of cells

hCMEC/D3 cells (8×10^5 cells/mL) were seeded on silicon chambers (200-µm-thick transparent bottoms with side-wall thickness of 400 µm) coated with fibronectin (375 µg/mL). Silicon chambers had uniform support stretching across the cell substrate. After 24 h, cells were exposed to uniaxial pulsatile stretching as previously described (Gangoda et al. 2018). Briefly, under the conditions in cell culture, the chambers were mounted in the ShellPa stretch system (B-Bridge International, Mountain View, CA), in which one end of the chamber is fixed and the other is connected to an actuator operated by compressed air. Cells were stretched by 15%, defined as relative elongation of the chamber with a rate of 1 Hz in the presence or absence of KRG (100 µg/mL) for 18 h. There was minimal cell detachment under these stretching conditions.

Animal experiments

The animal facility and protocols were approved by the Animal Care and Use Committee of Kangwon National University (KW-161129-1). All procedures were undertaken in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the National Animal Welfare Law of the Republic of Korea. Six-week-old male C57BL/6 mice were purchased from the Korea Laboratory Animal (Daejeon, Korea) and acclimated for 1 week. Mice were housed in controlled environments at 22 °C (±2 °C) and a humidity of 53% (±5%) under 12 h light/dark cycles. Mice were randomly assigned to five groups ($n = 10$ per group) and fed a normal chow diet (ND), HFD, HFD with KRG (10 or 50 mg/kg/day) or SV (10 mg/kg/day) for 12 weeks. Food and water were provided ad libitum. Intragastric delivery of KRG and SV 6 days per week was carefully performed by a well-trained researcher to minimise animal stress. During the experiment, clinical signs and general appearance were observed twice per week. Weight gain and food intake (calculated by collecting and weighing uneaten food) were recorded twice per week.

Blood and tissue collection

After 4 h of fasting, under anaesthesia with diethyl ether, whole blood samples were collected from the abdominal vein and sera were separated by centrifugation at 1500×*g* for 10 min. Tissues (epididymal fat pad, liver, thymus, spleen, aorta, and hippocampus) were carefully excised, cleaned with sterile 0.9% NaCl solution, blotted dry with filter paper, and weighed. The weight of each tissue was expressed relative to body weight. After measuring the weights of each tissue, parts of the liver and aorta tissues were subjected to histochemical analysis. Other tissues were immediately frozen in liquid nitrogen and stored at − 80 °C.

Hepatic HMGR activity

To determine hepatic HMGR activity, liver microsomal proteins were prepared as described in previous studies (Zhang et al. 2015). Briefly, liver tissue samples were thawed on ice and weighed. The samples were finely homogenised in buffer A containing 50 mM of Tris–HCl (pH 7.0), 1 mM of KCl, and 1 mM of EDTA (10 mL for 1 g liver) and ultracentrifuged at 100,000×*g* for 1 h at 4 °C. Microsomal pellets were suspended in buffer A containing 50 mM of Tris–HCl (pH 7.5) and 250 mM of sucrose and were stored at − 80 °C. Microsomal protein concentrations were determined according to the BCA (bicinchoninic acid) protein assay. Hepatic HMGR activity was measured using the HMGR Assay Kit (Sigma-Aldrich, CS1090) according to the manufacturer's protocol. Total liver microsomal proteins were mixed with NADPH and HMG-CoA and incubated for 30 min at 37 °C. Absorbance at 340 nm was measured. Results are expressed as µmol of HMGR activity/min/mg protein.

Analysis of lipid profiles, liver functions, leptin, and insulin levels

Serum TG, TC, HDL-C, LDL-C, ALT, AST, leptin, and insulin levels were measured according to the manufacturer's instructions.

Histopathological analysis

Livers were perfused with 0.9% normal saline to remove blood and the largest lobule was sectioned and fixed in 4% formaldehyde. Paraffin-embedded liver and aorta tissue

sections (3 μm thick) were stained with H&E. Liver sections were stained with Oil Red O. Blinded histological analysis was performed by a trained histologist. For quantitative analysis, images were photographed at 200 × (100 × in the case of aorta tissue) magnification randomly and analysed using the Image J software program (National Institute of Health, Rockville, MD, USA).

Quantitative RT-PCR analysis of COX-2

mRNA expression level of COX-2 was determined using reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from livers using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and processed using a cDNA Synthesis kit (TAKARA, Tokyo, Minato-ku, Japan). SYBR master mix kit (TAKARA) was used for PCR and cDNA was amplified using specific COX-2 (forward, 5′-CGT AGCAGATGACTGCCCAA-3′; reverse, 5′-CTTGGGGGT CAGGGATGAAC-3′) and β-actin (forward, 5′-TGTCCA CCTTCCAGCAGATGT-3′; reverse, 5′-AGCTCAGTAACA GTCCGCCTAGA-3′) primers. Quantitative real-time RT-PCR reactions were performed on a LightCycler 96 Instrument (Roche, Basel, Switzerland). Relative quantitative evaluation of each gene was performed using the comparative cycle threshold method (Livak and Schmittgen 2001).

Immunoblot analysis

Aorta, hippocampus, and hCMEC/D3 cells were harvested and washed with PBS. Tissues and cells were lysed with a homogeniser while immersed in PRO-PREP™ Protein Extraction Buffer (Intron Biotechnology, Seongnam, Korea) for 1 h on ice. After centrifugation at 13,000×*g* for 30 min at 4 °C, lysates were collected and protein concentration was determined using a BCA protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein were subjected to 10% SDS–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane electrophoretically. The membrane was blocked with 5% skim milk and incubated with primary antibodies against ICAM-1, VCAM-1, APP, BACE1, and Tau. Blots were developed using enhanced horseradish peroxidase (HRP)-conjugated secondary antibodies and re-probed with anti-β-actin or anti-GAPDH antibodies to verify equal protein loading. Bands were visualised with EZ-Western Lumi Pico reagent according to the manufacturer's instructions.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's test (Graph pad prism 5 Software; San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

Results

Ginsenoside content in KRG

Korean red ginseng, provided by Korean Red Ginseng Cooperation (Lot No. H1269-6041), contained (as mg/g) ginsenoside Rg1 (G-Rg1): 1.92, G-Re: 2.83, G-Rf: 1.58, G-Rh1: 1.23, G-Rg2s: 2.69, G-Rb1: 9.38, G-Rc: 2.83, G-Rb2: 3.35, G-Rd: 1.38, G-Rg3s: 2.20, G-Rg3r: 0.89, arginine–fructose–glucose (AFG): 40.28, and ginseng polysaccharides: 98.02. AFG is a new product created during the heating of raw ginseng to red ginseng. The water content was 4.12% of the total weight.

Inhibitory effect of KRG on HMGR activity in vitro

To determine the direct inhibitory potential of KRG on HMGR, in vitro experiments using the purified HMGR were performed (Fig. 1). SV at 10 μg/mL significantly inhibited HMGR activity (52.3% inhibition) compared to the untreated control. KRG had an inhibitory effect on HMGR activity in a dose-dependent manner and statistical significance was shown at doses of 100 (28.6% inhibition) and 200 μg/mL (57.1% inhibition). To be used as an adjuvant therapy with statins and to minimise the adverse effects caused by strong inhibition of HMGR (Carris et al. 2017), the treating doses of KRG on HFD mice were set at 10 and 50 mg/kg to exclude the direct anti-obese effects of KRG possibly mediated by the inhibition of HMGR.

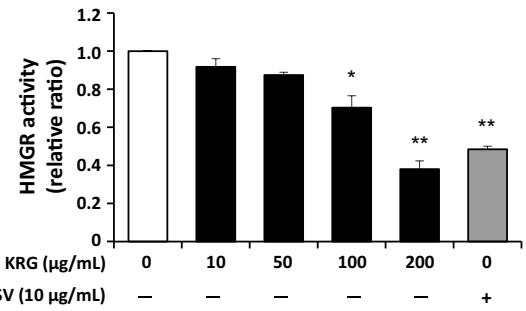

Fig. 1 Inhibitory effect of KRG on HMGR in vitro. HMGR activity in vitro was determined using a HMGR assay kit (Sigma) in the presence or absence of KRG. SV was used as a positive control for HMGR inhibition. Data are the mean ± SD ($n = 5$) and are expressed as a relative ratio of the untreated control (CON). * and ** indicate $p < 0.05$ and $p < 0.01$ compared to CON group. *HMGR* 3-hydroxy-3-methylglutaryl-coenzyme A reductase, *KRG* Korean red ginseng extract, *SV* simvastatin

Effect of KRG on changes in body weight and organ weight

During the 12-week experiment, there were no treatment-related mortality or behavioural changes such as salivation, lethargy, and tremors at any given dose. The effects of KRG on body weight and organ weight changes are shown in Table 1. There were no significant differences in baseline body weight among the groups ($p > 0.05$). After 12 weeks, the weight gain of the HFD group was 2.9-fold higher than that of the ND group ($p < 0.05$). The SV group had lower body weight gains (by 4.8%) than the HFD group, but there was no statistical significance ($p > 0.05$). Mice supplemented with KRG at 10 (31.1% reduction, $p < 0.05$) and 50 mg/kg (58.7% reduction, $p < 0.01$) showed significant suppression of body weight gain compared to HFD mice (Table 1). KRG treatment at doses of 10 and 50 mg/kg suppressed weight gain more effectively than the SV treatment.

In the HFD group, the weights of the liver, thymus, and spleen (Table 1) were slightly decreased compared to the ND group but there was no statistical significance ($p > 0.05$). Supplementation with KRG enhanced the decreases in organ weight in HFD mice, but these effects were not statistically significant ($p > 0.05$). The accumulation of the epididymal fat pad was increased by 2.67-fold in HFD mice, but KRG and SV treatments were not effective in its reduction (Table 1).

Effect of KRG on the food intake rate

Gain and loss of body weight may be associated with food intake rate and energy content of the food. When analysing the food intake rate during the 12-week experimental period, there were no significant differences in food intake rates among groups (Fig. 2). This result indicated that the body weight gain shown in HFD mice was the result of the high-energy diet intake compared to the normal diet (Table 1). The suppression of body weight gain caused by KRG intake in HFD mice was associated not with a reduction in food intake rate but with the potential anti-obesity effects of KRG because there were no significant differences in food intake rate among groups.

Effect of KRG on serum lipid levels

Along with body weight gain (Table 1), HFD mice had significantly altered lipid profiles (Fig. 3a–d): TG (30% increase, $p < 0.05$), TC (92% increase, $p < 0.05$), HDL-C (50% increase, $p < 0.05$), and LDL-C levels (108.3% increase, $p < 0.05$). The SV treatment significantly

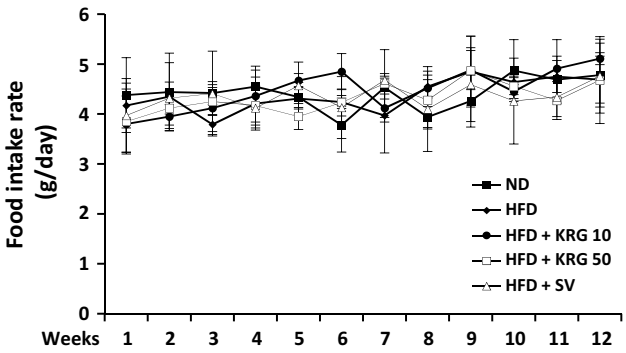

Fig. 2 Food intake rate. Mice were fed with normal chow diet (ND), high-fat diet (HFD), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via gastric gavage. Data are mean ± SD (each group, $n = 10$). There were no significant differences among groups. *KRG* Korean red ginseng extract, *SV* simvastatin

Table 1 Changes in body and tissue weights

Parameter ($n = 10$ per group)	ND	HFD for 12 weeks			
		–	10 mg/kg KRG	50 mg/kg KRG	10 mg/kg SV
Body weight (g)					
Initial body weight	20.4 ± 1.11	19.9 ± 0.98	19.6 ± 0.57	20.6 ± 0.44	20.0 ± 0.76
Final body weight	27.9 ± 2.51	41.8 ± 3.93#	36.3 ± 2.62*	34.4 ± 1.80**	40.9 ± 5.30
Weight gain (g)	7.5 ± 1.11	21.9 ± 2.73#	16.7 ± 1.47*	13.8 ± 1.34**	20.9 ± 4.12
Tissue weight (g/kg body weight)					
Epididymal fat pad	11.56 ± 2.8	42.47 ± 1.78##	41.21 ± 3.18	40.75 ± 4.58	43.16 ± 3.48
Liver	49.17 ± 1.03	39.70 ± 2.04	40.96 ± 3.62	41.24 ± 1.19	44.88 ± 2.56
Thymus	1.59 ± 0.05	1.16 ± 0.09	1.58 ± 0.21	1.42 ± 0.06	1.64 ± 0.31
Spleen	2.67 ± 0.05	2.31 ± 0.10	2.13 ± 0.47	2.92 ± 0.26	2.54 ± 0.21

All values are mean ± SD ($n = 10$ per group)

ND normal chow diet, *HFD* high fat diet (Research Diet D12492; 60% kcal% fat), *KRG* Korea red ginseng extract, *SV* simvastatin (10 mg/kg body weight) treatment on HFD mice

*, **Indicate $p < 0.05$ and $p < 0.01$ compared with the HFD group, respectively

#$p < 0.05$ compared to ND group

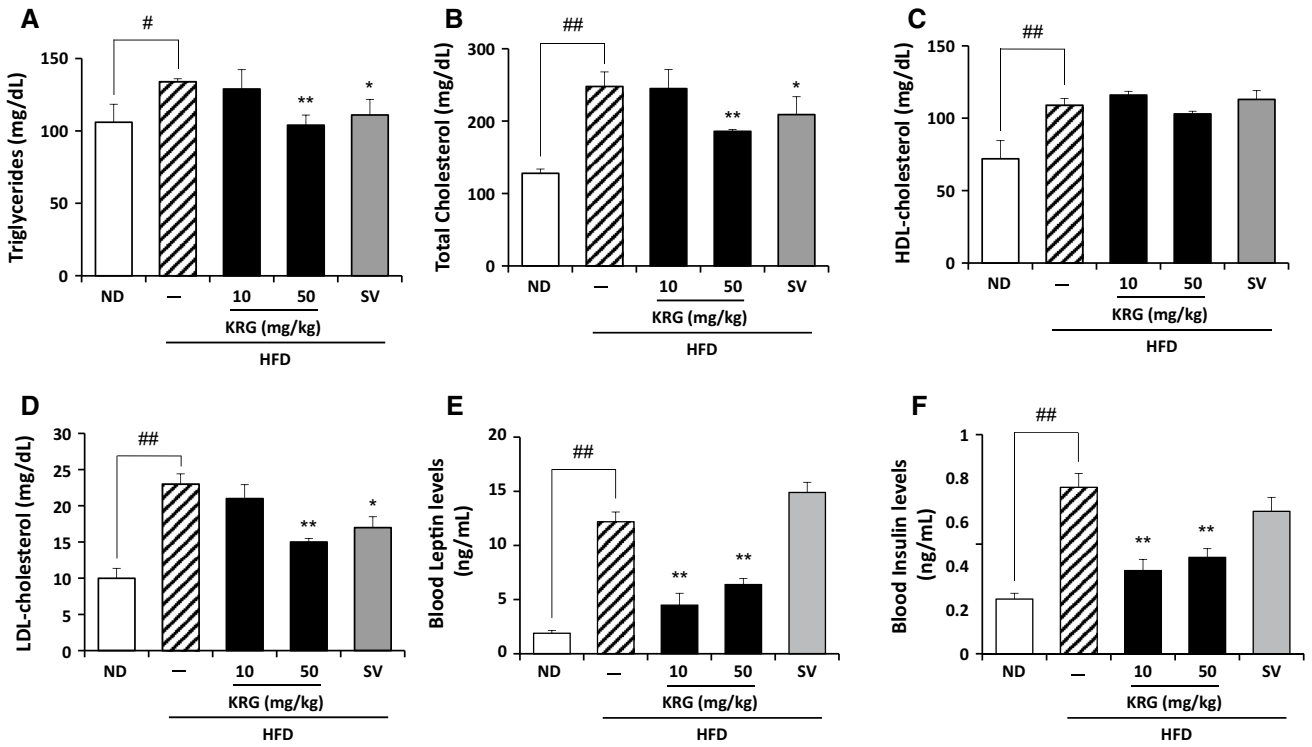

Fig. 3 Effects of KRG supplementation on the changes in lipid profiles, leptin, and insulin levels. Mice were fed with normal chow diet (ND), high-fat diet (HFD; Research Diet D12492; 60% kcal% fat), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via gastric gavage. At the end of the experiment, lipid profiles were analysed in the blood. **a** Triglycerides. **b** Total cholesterol. **c** HDL-cholesterol. **d** LDL-cholesterol. **e** Leptin. **f** Insulin. Data are mean ± SD (each group, $n = 10$). $^{\#}$ and $^{\#\#}$ indicate $p < 0.05$ and $p < 0.01$ compared to ND group. * and ** indicate $p < 0.05$ and $p < 0.01$ compared to HFD alone group. *KRG* Korean red ginseng extract, *SV* simvastatin

suppressed the increases in TG, TC, and LDL-C ($p < 0.05$). KRG at 10 mg/kg did not significantly reduce these parameters, but KRG at 50 mg/kg significantly reduced these parameters with more potency than the SV treatment (Fig. 3a–d). These results suggested that KRG intake was beneficial in correcting the undesired changes in serum lipid profiles caused by HFD.

Effect of KRG on blood leptin and insulin levels

When the effects of KRG supplementation on the circulating levels of leptin and insulin were determined, HFD significantly increased leptin levels by 5.1-fold compared to the ND group ($p < 0.01$), but KRG supplementation at 10 and 50 mg/kg reduced the increase in leptin level significantly in HFD mice (Fig. 3e). Because serum leptin levels were determined at the end of the experiment, it is unclear whether KRG supplementation led to the suppression of blood leptin during the experimental period. However, leptin levels are closely related to body weight; thus, decreased leptin levels increased under an HFD state indirectly reflected the lower body weight than the HFD control group. As shown in Fig. 3f, the HFD group showed a significant increase in insulin level by threefold ($p < 0.01$) compared to the ND group. KRG at a given dose significantly suppressed the increase in insulin levels which would eventually cause insulin resistance, whereas the SV treatment did not show significant reductions in insulin levels ($p > 0.05$). These results indicated that supplementation with KRG prevented hyperleptinemia and hyperinsulinemia caused by HFD intake.

Effect of KRG on hepatic inflammatory response

The liver is susceptible to damage from excessive accumulation of fat. HFD mice showed aberrant lipid profiles (Fig. 3); thus, the effects of KRG supplementation on fat accumulation in the liver were evaluated by H&E (Fig. 4a) and Oil Red O staining (Fig. 4b). In contrast to the ND group, the HFD alone group had abundant lipid droplets in the liver. However, increased lipid accumulation in the liver was significantly attenuated by KRG supplementation (Fig. 4c, d). Increases in ALT (Fig. 5a) and AST (Fig. 5b) levels in the HFD alone group were suppressed significantly by both KRG and SV treatments.

HFD mice showed a 1.9-fold significant increase in COX-2 mRNA expression level in the liver compared to

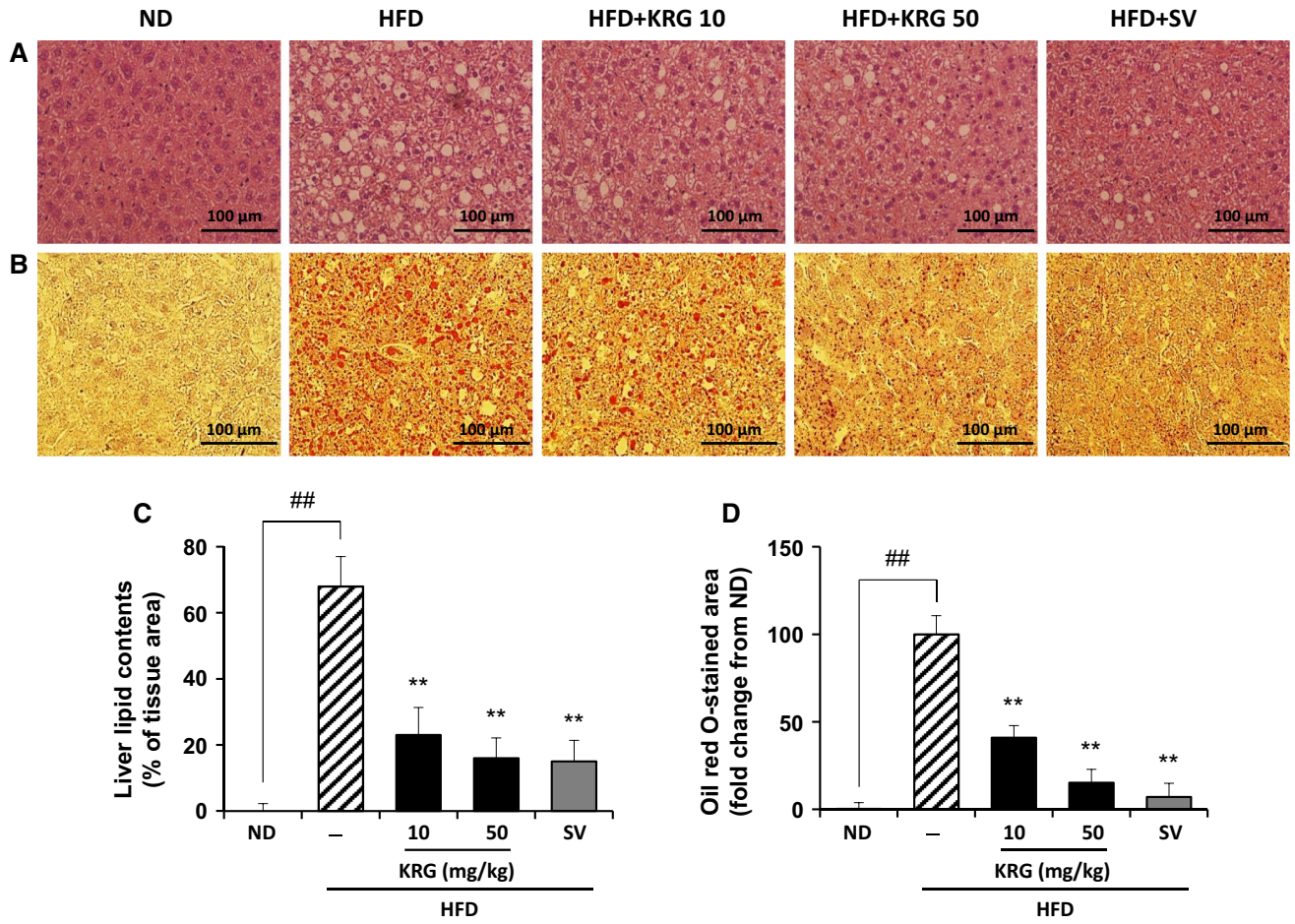

Fig. 4 Fat droplet and lipid contents in the liver tissue. Mice were fed with normal chow diet (ND), high-fat diet (HFD; Research Diet D12492; 60% kcal% fat), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via intragastric gavage. At the end of the experiment, liver tissues were isolated and fixed with 4% formaldehyde. Cross-sections of liver tissues were stained with haematoxylin and eosin (H&E) and Oil Red O staining. **a** Representative images of H&E staining. **b** Representative images of Oil Red O staining. Original magnification is ×200 and the bar represents 100 μm. Quantities of fat droplets (**c**) and lipids (**d**) in the liver were determined using Image J software. Data are mean ± SD (each group, $n = 10$). ## and ** indicate $p < 0.01$ compared to ND and HFD alone group, respectively. *KRG* Korean red ginseng extract, *SV* simvastatin

the ND group (Fig. 5c, $p < 0.01$). However, KRG supplementation (10 and 50 mg/kg) prevented this increase in COX-2 mRNA and the effect was more potent than that of SV ($p < 0.05$, Fig. 5c). These results suggested that KRG supplementation was beneficial in reducing hepatic fat accumulation, impairment of liver function, and inflammatory COX-2 expression.

Effect of KRG on hepatic HMGR activity in vivo

As shown in Fig. 5d, the enzyme activities of hepatic HMGR were highly augmented in HFD mice but with no statistical significance. Supplementation with KRG led to a significant reduction in HMGR activities in the liver and its potency was comparable to the SV treatment. This result suggested that the HMGR inhibitory activity of KRG shown in Fig. 1 was also in effect in HFD mice and that reduction in lipid profiles achieved by KRG treatment was, at least in part, associated with the HMGR inhibitory activity of KRG.

Effect of KRG on vascular inflammatory response

HFD mice showed a significant twofold thickening of the aorta compared to the ND group (Fig. 6a, b). Similar to the lipid-lowering drug SV, KRG supplementation suppressed HFD-associated aortic wall thickening. As shown in Fig. 6c and d, the protein expression levels of ICAM-1 were significantly increased by 1.7-fold ($p < 0.01$) and this increased level of ICAM-1 might be associated with the aberrant aortic wall thickening shown in Fig. 6b. Like SV treatment, KRG (10 and 50 mg/kg) supplementation caused significant suppression of ICAM-1 expression in the aorta ($p < 0.01$,

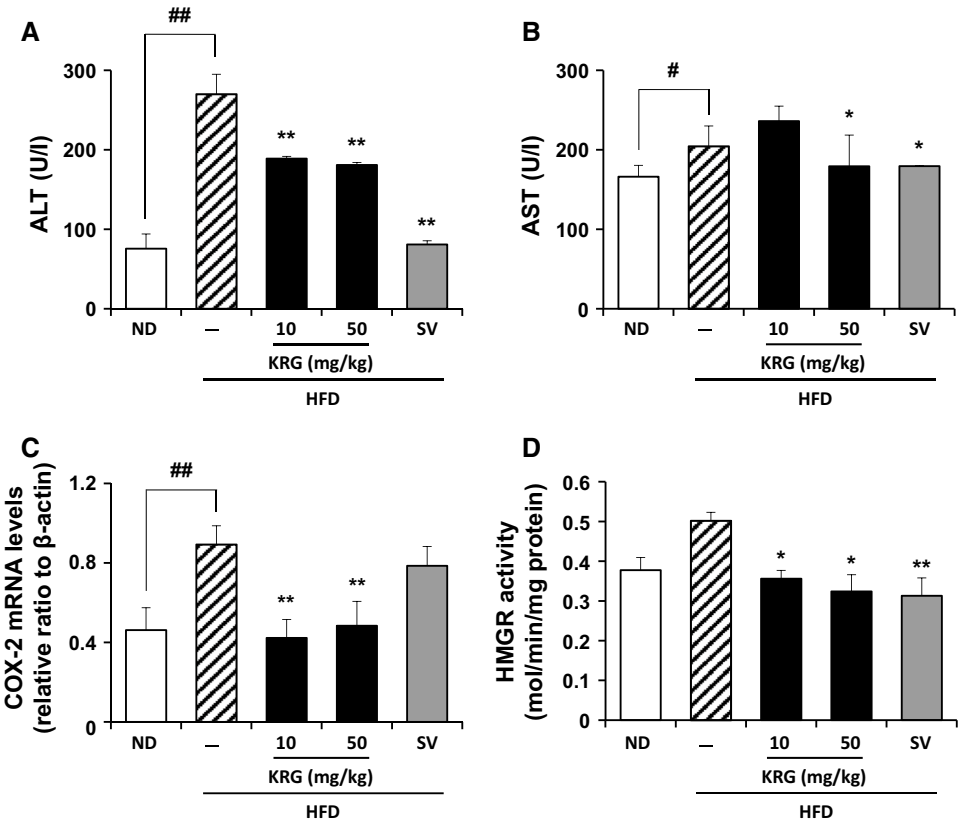

Fig. 5 Serum ALT and AST levels, mRNA expression level of COX-2, and HMGR activities in the liver tissue. Mice were fed with normal chow diet (ND), high-fat diet (HFD; Research Diet D12492; 60% kcal% fat), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via gastric gavage. At the end of the experiment, blood samples and liver tissues were collected. Serum levels of ALT (**a**) and AST (**b**) were determined using commercially available assay kits. The relative mRNA expression level of COX-2 (**c**) and HMGR activities (**d**) in the liver were determined using quantitative RT-PCR and commercially available assay kits, respectively. Data are mean ± SD (each group, $n = 10$). $^{\#}$ and $^{\#\#}$ indicate $p < 0.05$ and $p < 0.01$ compared to ND group. * and ** indicate $p < 0.05$ and $p < 0.01$ compared to HFD alone group. *HMGR* 3-hydroxy-3-methylglutaryl-coenzyme A reductase, *KRG* Korean red ginseng extract, *SV* simvastatin

Fig. 6d). This suppressive effect of KRG both on aortic wall thickening and ICAM-1 expression might have ameliorated obesity-associated vascular impairment. Additionally, suppression of hypertension-associated vascular inflammatory events will be another merit in preventing obesity-associated complications. Here, the pathological 15% cyclic stretch was used, which mimicked hypertension that occurs in the vascular system and the effect of KRG treatment on the changes of ICAM-1 and VCAM-1 proteins was determined in cerebral endothelial cells. As shown in fibroblasts, there is a possibility that mechanical stretch may redistribute or remodel the components of the cytoskeleton such as α- and β-actin (Langevin et al. 2006). Thus, we determined the protein level of GAPDH as a protein loading control instead of β-actin. As shown in Fig. 7, the protein expression levels of ICAM-1 and VCAM-1 were significantly increased by 0.6- and 1.3-fold, respectively, under the continuous cyclic stretch of 15% amplitude. However, the upregulated ICAM-1 and VCAM-1 expression were suppressed in the presence of KRG by 21.9% and 10.7%, respectively ($p < 0.05$). This result suggested that KRG supplementation was also effective in the suppression of vascular inflammatory responses caused by hypertension associated with obesity.

Effect of KRG on cerebral inflammatory response

Along with the correction of lipid profiles, it would be favourable if KRG mitigated aberrant changes occurring in the brain following an obese body condition. To test the suppressive effect of KRG against obesity-associated cerebral inflammatory responses, protein expression levels of APP, BACE1, and Tau were evaluated via immunoblot analysis. As shown in Fig. 8, HFD alone mice showed significantly increased expression levels of BACE1 (2.4-fold increase, $p < 0.01$) and Tau (0.15-fold, $p < 0.05$), whereas protein expression levels of APP were not significantly different ($p > 0.05$). KRG at the dose of 50 mg/kg suppressed HFD-associated upregulation of BACE1, cleaved APP to generate

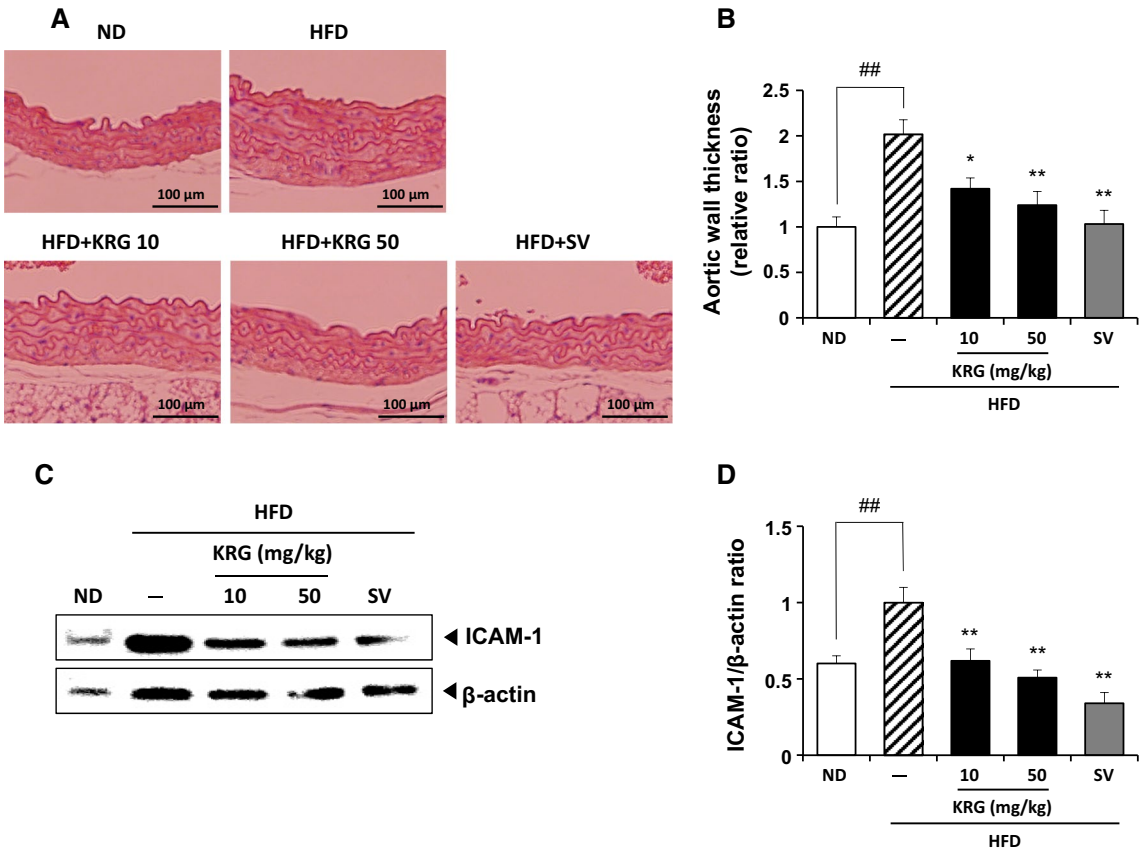

Fig. 6 Wall thickness and ICAM-1 protein expression levels in the aorta tissue. Mice were fed with normal chow diet (ND), high-fat diet (HFD; Research Diet D12492; 60% kcal% fat), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via gastric gavage. At the end of the experiment, isolated aorta tissues were stained with H&E staining. **a** representative H&E staining. Original magnification, × 100. The bar indicates 100 μm. The length of the aortic wall was measured using Image J software and is expressed as a relative ratio of the ND group (**b**). The protein expression levels of ICAM-1 in the aorta were determined via immunoblotting (**c**) and are expressed as a relative ratio of proteins levels of β-actin (**d**). Data are mean ± SD (each group, $n = 10$). ## and ** indicate $p < 0.01$ compared to ND and HFD alone group, respectively. *KRG* Korean red ginseng extract, *SV* simvastatin

amyloid-β peptides, and Tau expression (Fig. 8). Although HFD did not lead to the upregulation of APP expression, KRG at the dose of 50 mg/kg suppressed the basal level expression of APP. Collectively, KRG supplementation may have mitigated obesity-associated neurological alteration by inhibiting the expression of BACE1 and Tau.

Discussion

In this study, we demonstrated that 12-week HFD supplementation caused lipotoxicity and significant liver, vascular, and brain inflammatory responses. KRG had a direct inhibitory effect on HMGR activity and also suppressed the HFD feeding-caused increase in HMGR activity. KRG supplementation (10 and 50 mg/kg) was beneficial in alleviating lipid profiles and resulted in the suppression of body weight gain. In addition, KRG supplementation suppressed HFD-induced hepatic steatosis and inflammatory events in the liver, aorta, and brain.

Excessive lipid accumulation in non-adipose tissues causes various organ inflammation and eventually results in organ failure due to cell death and impairment in cellular functions, if not properly corrected (Schaffer 2003). A HMGR inhibitor (or statin), such as lovastatin, simvastatin, and atorvastatin are widely used for the treatment of hyperlipidaemia and cardiovascular disease. In this study, KRG displayed HMGR, the key enzyme of hepatic cholesterol biosynthesis, inhibitory potential in vitro (Fig. 1) and in vivo (Fig. 5d). This finding indicates that the lipid-lowering effect of KRG in HFD mice is, at least in part, associated with HMGR inhibition.

Although KRG supplementation was effective in reducing body weight gain (Table 1), improving lipid profiles (Fig. 3), and reducing fat and lipid accumulation in the liver (Fig. 4) in HFD conditions, the suppressive potential of KRG on the accumulation of the epididymal fat pad was not statistically

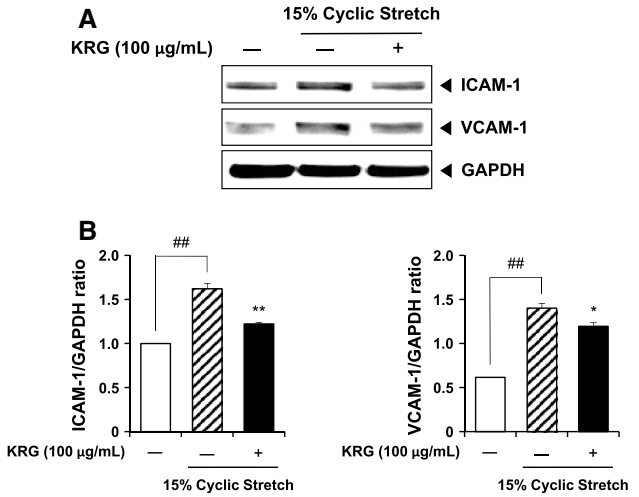

Fig. 7 ICAM-1 and VCAM-1 protein expression levels in hCMEC/D3 cells. hCMEC/D3 cells were seeded onto fibronectin-coated silicone chambers. Cells were 15% actuated arm stretched in the presence or absence of KRG (100 μg/mL) at a rate of 1 Hz for 18 h. Protein extracts were subjected to immunoblot analysis. **a** Representative immunoblot image. **b** Quantitative values of ICAM-1 and VCAM-1 protein expression levels normalised to GAPDH levels. Data are mean ± SD ($n = 4$). ## and ** indicate $p < 0.01$ compared to untreated control and 15% cyclic stretch alone group, respectively. *KRG* Korean red ginseng extract, *SV* simvastatin

significant (Table 1). A similar result was also observed in SV treatment. The exact reason is unclear, but this issue should be addressed in future experiments to identify the complex pathophysiology of HFD intake.

Leptin levels are highly associated with the body weight (Musaad and Haynes 2007; Francisco et al. 2018); thus, decreased leptin levels (Fig. 3e) caused by KRG supplementation indirectly reflect the reduced body weight (Table 1) in comparison to the HFD group. Insulin resistance causes severe health complications, including fatty liver (Winzell and Ahrén 2004), and is frequently observed in obesity and HFD-fed conditions (Li and Ji 2018; Johnson et al. 2012). In this experimental setting, SV did not suppress HFD-associated increases in leptin and insulin levels despite its strong lipid-lowering activity (Fig. 3). This raises the possibility that a simple lipid-lowering strategy might not be successful in treating HFD-associated disturbances in hormonal homeostasis. Considering the versatile roles of these hormones in metabolism (Li and Ji 2018), the suppressive effects of KRG in the upregulation of leptin and insulin levels caused by HFD may be beneficial in controlling whole-body homeostasis. In the liver (Fig. 5), KRG intake inhibited fat accumulation and impaired liver function. COX-2, the key enzyme in eicosanoid metabolism, contributes to the progression of non-alcoholic fatty liver disease in HFD-induced obesity, but COX-2 inhibitor (e.g., nimesulide) treatment attenuates

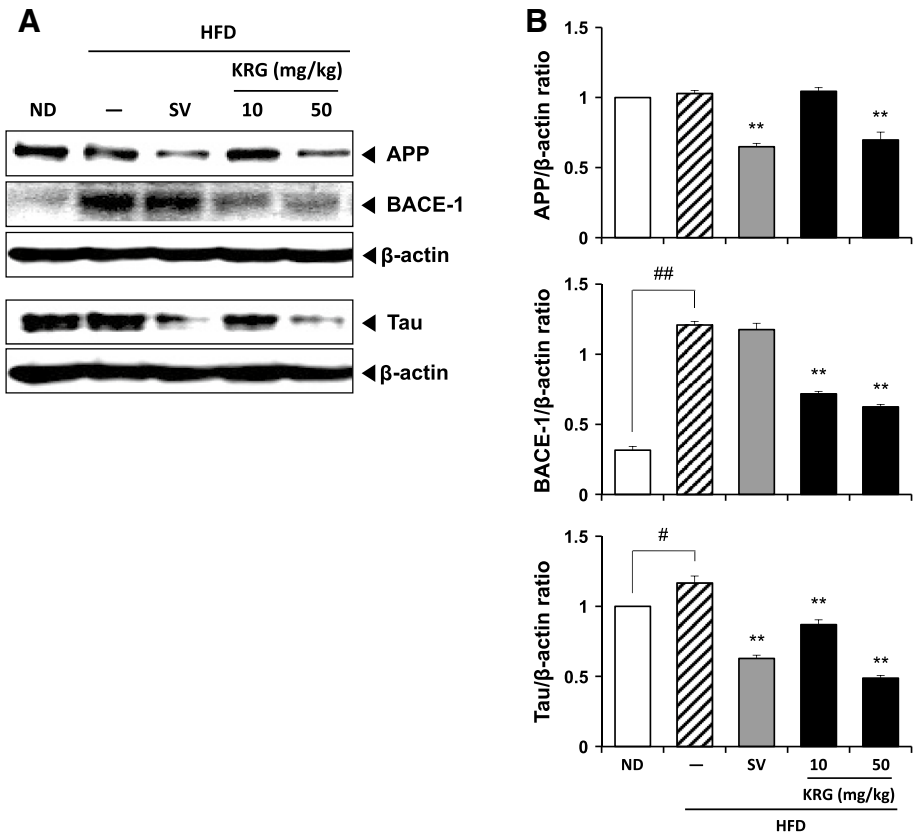

Fig. 8 APP, BACE, and Tau protein expression levels in the brain tissue. Mice were fed with normal chow diet (ND), high-fat diet (HFD; Research Diet D12492; 60% kcal% fat), HFD with KRG (10 or 50 mg/kg), or HFD with SV (10 mg/kg) for 12 weeks. KRG and SV were delivered via gastric gavage. At the end of the experiment, hippocampus were isolated from brain tissues and extracted proteins were subjected to immunoblot analysis. **a** Representative images of immunoblot. **b** Quantified protein levels of APP, BACE-1, and Tau. Data are mean ± SD (each group, $n = 10$) and are expressed as a relative ratio of proteins levels of β-actin. ## and ** indicate $p < 0.01$ compared to ND and HFD alone group, respectively. *KRG* Korean red ginseng extract, *SV* simvastatin

hepatic inflammation (Hsieh et al. 2009). The SV treatment suppressed HFD-associated increases in lipid profiles significantly but failed to reduce COX-2 expression in the liver. Although SV was less effective in reducing the HFD-related increase in COX-2, KRG supplementation significantly suppressed the increase in COX-2 mRNA expression. In the HFD treatment, significant suppression of lipid profiles aimed at the inhibition of HMGR was not sufficient to correct the pathological phenomena caused by HFD intake. Therefore, KRG intake may independently contribute to alleviating the unfavourable hepatic pathological transition. In the aorta tissue, inflammatory processes in the endothelial cells will be influenced by the interactions of cellular adhesion molecules such as ICAM-1 and VCAM-1 (Gangoda et al. 2018; Fotis et al. 2012). The thickening of the aortic wall and an increase in protein expression of ICAM-1 were efficiently improved in the presence of SV and KRG in the HFD group (Fig. 6). Obesity is also highly associated with hypertension, which further impairs vascular health in susceptive tissues. Treatment with KRG also suppressed the excessive mechanical stretch-associated upregulation of ICAM-1 and VCAM-1 in cerebral vascular endothelial cells (Fig. 7). The reason why KRG suppresses the cyclic stretch-associated increases in ICAM-1 and VCAM-1 expression is not clear. Various compounds that exist in KRG have antioxidant and anti-inflammatory activities, as reviewed elsewhere (Li and Ji 2018). This may contribute, at least in part, to the suppression of ICAM-1 and VCAM-1 in lipotoxicity-associated inflammatory conditions. In the brain, HFD may disrupt intracellular cholesterol dynamics (Koudinov and Koudinova 2005) and play a role in cognitive deficits and neuropathological alterations (Zhu et al. 2019; Mastrocola et al. 2011). HFD mice showed significantly increased expression of BACE1, which contributes to a build-up of amyloid-β proteins in the brain (Fig. 8), but this increase in BACE1 could not be reversed by the lipid-lowering drug SV. Considering the promising roles of BACE inhibitors in the suppression of amyloid-β production (Burki 2018), KRG intake may be effective in the suppression of the upregulated expression of BACE1 in HFD mice, validating its applicability for the prevention of amyloid-β production and neurodegenerative and neurological disorders (Kim et al. 2018). In addition, KRG at 50 mg/kg decreased the production of APP and Tau like the SV treatment did. These suppressive effects of KRG on the upregulation of Alzheimer-associated proteins could be promising in the prevention of cognitive impairment induced by lipotoxicity in obesity.

Our study has several limitations. Firstly, only male mice were included in the experiment because there are some experimental limitations in including female mice due to complex behaviours of hormones. If most available parameters such as sex and age are included in the experimental setting, the results will be closer to the truth. In a future study, female groups should be included to convincingly justify the results. Secondly, the thymus, spleen, and liver are the major immune organs and are sensitive to the effects of lipotoxicity in the HFD model. Thus, we measured the changes in the weights of these organs. Unfortunately, we did not measure the weights of the heart, lung, and brain because the changes in the weights of these organs are not critically associated with lipotoxicity. Thirdly, we did not investigate the combination effect of SV and KRG on the changes in HMGR activity. This issue should be resolved in a future study to obtain the information on whether KRG supplementation provides an additive or cumulative effect on HMGR activity. Fourthly, we only measured the mRNA expression levels of COX-2. Because, in contrast to the cell-based assay, we cannot fully exclude the massive degradation of COX-2 protein during sample preparations from the euthanised mice. In addition, the accumulation of fat in the liver (Fig. 4) and liver function tests (Fig. 5a, b) were significantly affected by KRG supplementation. Finally, we observed decreased levels of APP, BACE1, and Tau after KRG supplementation in the HFD group. However, direct evidence showing the decreased levels of amyloid-β in the KRG treated group was not present in this experiment. Considering previous reports (Kasi et al. 2018; Chu et al. 2014), KRG may attenuate the deposition of amyloid-β directly. In a future study, the suppressive effect of KRG on the deposition of amyloid-β should be demonstrated directly in HFD conditions.

Conclusion

HFD intake causes inflammatory responses of the liver, aorta, and the brain, which are associated with lipotoxicity. The supplementation of KRG will be beneficial to reduce the multi-organ inflammation mediated by HFD intake. However, the underlying molecular mechanisms of KRG involved in the suppression of lipotoxicity should be further investigated.

Acknowledgements This work was supported by the 2016 Grant from the Korean Society of Ginseng and funded by the Korea Ginseng Corporation.

Author contributions S-AJ, SN, SRL and E-HS designed the research study. S-AJ, SN, JWL, YP, GS, SHK, M-JK, K-HJ, APA, SVSG, HJK, MKK and SCK performed the experiments and analysed data. S-AJ, SN, SRL and E-HS interpreted data and wrote the manuscript.

Compliance with ethical standards

Conflict of interest Seon-A Jang, Seung Namkoong, Sung Ryul Lee, Jin Woo Lee, Yuna Park, Gyeongseop So, Sung Hyeok Kim, Mi-Ja Kim, Ki-Hyo Jang, Alberto P. Avolio, Sumudu V. S. Gangoda, Hyun Jung Koo, Myung Kyum Kim, Se Chan Kang and Eun-Hwa Sohn declare that they have no conflict of interest.

Human and animal rights The experimental animal facility and study protocols were approved by the Animal Care and Use Committee of Kangwon National University (KW-161129-1). All experimental procedures were undertaken in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publications no. 8023, revised 1978) and the National Animal Welfare Law of the Republic of Korea.

References

Acosta A, Dayyeh BKA, Port JD, Camilleri M (2014) Recent advances in clinical practice challenges and opportunities in the management of obesity. Gut 63:687–695

Burki T (2018) Alzheimer's disease research: the future of BACE inhibitors. Lancet 391:2486

Carris NW, Tipparaju SM, Magness DJ, Chapalamadugu KC, Magness RR (2017) Pleiotropic effects of metformin to rescue statin-induced muscle injury and insulin resistance: a proposed mechanism and potential clinical implications. Med Hypotheses 107:39–44

Choi J, Kim TH, Choi TY, Lee MS (2013) Ginseng for health care: a systematic review of randomized controlled trials in Korean literature. PLoS One 8(e59978):59971–59974

Chu S et al (2014) Ginsenoside Rg5 improves cognitive dysfunction and beta-amyloid deposition in STZ-induced memory impaired rats via attenuating neuroinflammatory responses. Int Immunopharmacol 19:317–326

Duan Y et al (2018) Inflammatory links between high fat diets and diseases. Front Immunol 9:2649

Engin A (2017) Eat and death: chronic over-eating. Adv Exp Med Biol 960:53–80

Fotis L et al (2012) Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 at the early stages of atherosclerosis in a rat model. Vivo 26:243–250

Francisco V et al (2018) Obesity, fat mass and immune system: role for leptin. Front Physiol 9:1–20

Gadde KM, Martin CK, Berthoud H-R, Heymsfield SB (2018) Obesity: pathophysiology and management. J Am Coll Cardiol 71:69–84

Gangoda SVS et al (2018) Pulsatile stretch as a novel modulator of amyloid precursor protein processing and associated inflammatory markers in human cerebral endothelial cells. Sci Rep 8:1689

Haslam DW, James WPT (2005) Obesity. Lancet 366:1197–1209

Hsieh PS et al (2009) COX-2-mediated inflammation in fat is crucial for obesity-linked insulin resistance and fatty liver. Obesity (Silver Spring, Md.) 17:1150–1157

Hussain SS, Bloom SR (2011) The pharmacological treatment and management of obesity. Postgrad Med 123:34–44

Johnson AR, Milner JJ, Makowski L (2012) The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunol Rev 249:218–238

Kasi PB et al (2018) The inhibitory effect of panax ginseng extract on amyloid-like fibril formation of trypsin in aqueous ethanol. Protein Pept Lett 25:253–259

Kim SN et al (2007) Simultaneous quantification of 14 ginsenosides in Panax ginseng CA Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control. J Pharm Biomed Anal 45:164–170

Kim HJ et al (2018) Panax ginseng as an adjuvant treatment for Alzheimer's disease. J Ginseng Res 42:401–411

Koudinov AR, Koudinova NV (2005) Cholesterol homeostasis failure as a unifying cause of synaptic degeneration. J Neurol Sci 229–230:233–240

Langevin HM et al (2006) Fibroblast spreading induced by connective tissue stretch involves intracellular redistribution of alpha- and beta-actin. Histochem Cell Biol 125:487–495

Li Z, Ji GE (2018) Ginseng and obesity. J Ginseng Res 42:1–8

Lichtenstein AH et al (1998) Dietary fat consumption and health. Nutr Rev 56:S3–19 **(discussion S19-28)**

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408

Mastrocola R et al (2011) Dysregulation of SREBP2 induces BACE1 expression. Neurobiol Dis 44:116–124

Musaad S, Haynes EN (2007) Biomarkers of obesity and subsequent cardiovascular events. Epidemiol Rev 29:98–114

Schaffer JE (2003) Lipotoxicity: when tissues overeat. Curr Opin Lipidol 14:281–287

So S-H, Lee JW, Kim Y-S, Hyun SH, Han C-K (2018) Red ginseng monograph. J Ginseng Res 42:549–561

Weksler BB et al (2005) Blood-brain barrier-specific properties of a human adult brain endothelial cell line. Faseb J 19:1872–1874

Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119

Winzell MS, Ahrén B (2004) The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 53:S215–S219

Zhang H et al (2015) Content and activity of human liver microsomal protein and prediction of individual hepatic clearance in vivo. Sci Rep 5:1–12

Zhu Q, Zhu YY, Wang WN (2019) TRUSS inhibition protects against high fat diet (HFD)-stimulated brain injury by alleviation of inflammatory response. Biochem Biophys Res Commun 511:41–48

Zuckerman M, Greller HA, Babu KM (2015) A review of the toxicologic implications of obesity. J Med Toxicol 11:342–354