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Jinan red ginseng extract inhibits triglyceride synthesis *via* the regulation of LXR-SCD expression in hepatoma cells

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Abstract Hypertriglyceridemia is one of the metabolic syndrome that is often observed as a result of lipid abnormalities. It is associated with other lipids, metabolic disorders, cardiovascular disease and liver disease. Korean red ginseng is known to affect obesity, dyslipidemia, liver disease and liver function, but the mechanism of its effect is not clear. This study examined the beneficial effects of hypertriglyceridemia and the mechanism of action of Jinan red ginseng extract (JRG) in hepatoma cells. To measure the levels of triglyceride accumulation, we studied the expression of proteins and mRNAs related to lipidogenesis in hepatoma cells (Huh7 and HepG2). JRG decreases the lipidogenic markers, peroxisome proliferator-activated receptor γ (PPARγ), CCAAT-enhancer-binding proteins α (C/EBPα) and C/EBPβ which are major regulators of triglyceride synthesis in hepatoma cells. We also found that JRG reduced sterol regulatory element binding proteins 1c (SREBP-1c), C/EBPα and C/EBPβ by regulating liver X receptor (LXR) and stearoyl CoA desaturase (SCD) expressions. In addition, the first-limited step of synthesis triglyceride (TG), glycerol-3-phosphate (G3P) is decreased by JRG. These results suggest that the anti-hypertriglyceride effect of JRG in hepatoma cells could be accompanied with the inhibition of lipidogenic transcription factors by regulating LXR and SCD expression.

Keywords: Inhibition of triglyceride synthesis, Jinan red ginseng, hypertriglyceridemia, LXR, SCD

Introduction

Ginseng (*Panax ginseng* Meyer) is a perennial plant and its roots are used as traditional herbal medicine. The roots of ginseng, which are normally difficult to store for a long time, can be easily stored after secondary processing. The processing method affects the efficacy of ginseng and the products are divided into three types according to the technique used. Ginseng is categorized into white ginseng (desiccated roots), black ginseng (dried roots after steaming), and red ginseng (desiccated roots after steaming using water vapor) based on the manufacturing process employed. Red ginseng is effective against hypertension, lipid metabolism (Shin *et al.*, 2018), cardiovascular disease (Jovanovski et al., 2010), and hyperglyceridemia (Lee et al., 2014). Red ginseng is a valuable herb in Asian countries and is used as a crude substance to improve lipid metabolism. Jinan red ginseng extract (JRG) is a substance extracted from red ginseng with water and contains ginsenosides such as Rg1, Rb1, and Rg3. In previous studies, fatty acid synthesis in adipocytes was found to be inhibited by JRG.

Hypertriglyceridemia is associated with other systemic chronic diseases such as abnormal lipid metabolism or metabolic syndrome. Hypertriglyceridemia is characterized by elevated triglyceride (TG) levels (150 mg/g) in the blood and is associated with an increased possibility of cardiovascular disease (Breier et al., 1989; Reiner, 2017), obesity (Mbata et al., 2017), insulin resistance (Paniagua, 2016), diabetes, hypertension, and hyperuricemia (Helgeland et al., 1978; Wakabayashi, 2013).

Biosynthesis of TG in hepatocytes is essential for the maintenance of TG homeostasis in serum. There are two major pathways for TG biosynthesis: the glycerol-3-phosphate (G3P) pathway involved in de novo lipogenesis and the monoacylglycerol (MAG) pathway, which plays a major role in lipid absorption. Glycerol-3-phosphate dehydrogenase (GPDH) production is the rate-limiting step in the glycerol phosphate pathway. GPDH is an enzyme that catalyzes the reversible redox modification of dihydroxyacetone phosphate to sn-G3P. Thus, TG synthesis in mammalian tissues requires G3P as a source of glycerol. TGs are synthesized in the liver primarily from two sources of fatty acids (FA): FA synthesized de novo in the liver and preformed FA. FA synthesis is regulated by CCAAT/enhancer-binding proteins α (C/EBPα), C/EBPβ, and sterol regulatory element-binding protein 1c (SREBP-1c) expression. C/EBPα and β both have a leucine zipper for factor dimerization in their DNA binding region. The C/EBPα factor includes two activation domains (ADs) and one attenuator domain. Three ADs and two repressive domains mediate the function of C/EBPβ (Pei et al., 1991; Williams et al., 1995). C/EBPα and β are essential for adipocyte differentiation and fatty acid synthesis (Nerlov, 2007). C/EBPα and C/EBPβ are transcriptional factors that control stearoyl CoA desaturase (SCD) expression in the liver (Chu et al., 2006; Xu et al., 2016). Therefore, inhibiting this process is essential to control lipid production. To overcome this disease, a lot of research is being conducted on the reduction of preadipocyte differentiation, inhibition of lipid synthesis, and increased lipolysis (Ono et al., 2011). Many drugs are used for these purposes, but prolonged exposure can cause serious side effects. Thus, research

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is ongoing to find new natural resources related to lipid synthesis and degradation (Park et al., 2015; Lee et al., *2016;* Zhu et al., 2019). During the screening of human SCD inhibitors from natural sources, we found that the distilled water extract from the root of red ginseng strongly inhibited the SCD expression level.

In this study, we assessed the ginsenoside content of JRG and the inhibitory effect of lipid accumulation. Additionally, we investigated the mechanisms of JRG on lipid synthesis and TG accumulation.

Materials and Methods

JRG extract powder

Four-year-old Korean red ginseng was purchased from a commercial supplier (Gansando Agriculture Corporation, Jinan, Korea). The JRG water extract was prepared by the Jinan Red Ginseng Institute (Jinan, Korea) using the following methods. Five liters of distilled water was added to 500 g of crushed red ginseng, and the soluble components were extracted using a reflux condenser (Multi-Purpose Extraction Water Bath, DAIHAN Scientific, Wonju, Korea). The extract was then filtered through a Whatman filter paper (No 2, pore size 8 μm) and condensed under a vacuum (Gast, Gast Manufacturing. INC., Benton Harbor, MI, USA). The JRG concentrate was converted into a powder using a spray-dryer (Spray-dryer, FineFT, Gongju, Korea) with 40% maltodextrin added as a carrier.

High Performance Liquid Chromatography (HPLC) analysis

The analysis of JRG was performed with rapid resolution liquid chromatography (1260 series, Agilent Technologies, Santa Clara, CA, USA) using 4.6×150 mm (3.5 μm) Eclipse plus-C_{18} columns (Eclipse plus-C_{18}, Agilent Technologies, Santa Clara, CA, USA). The gradient solvent was applied at a ow rate of 1.6 mL/min and the mobile phases were water (phase A) and acetonitrile (phase B). Modifications to the solvent gradient were as follows: 0 min, 18% B; 10 min, 20% B; 30 min, 27% B; 40 min, 30% B; 55 min, 51% B; 56 min, 90% B; 61 min, 90% B; 62 min, 18% B; 65 min, 18% B. The column temperature was kept at 30°C, with an injection volume of 10 μL and a wavelength of 203 nm. Ginsenoside standards Rg1 (Ginsenoside standards Rg1, ChromaDex, Irvine, CA, USA), Rb1 (Ginsenoside standards Rb1, ChromaDex), and Rg3 (Ginsenoside standards Rg3, ChromaDex) were dissolved in methanol.

Cell culture

The human hepatoma cell line (HepG2, ATCC, Manassas, VA, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA) and 100 U penicillin and 0.1 mg/mL streptomycin (Penicillin and Streptomycin, HyClone, Logan, UT, USA) at 37°C in a humidified atmosphere of 5% CO_2. Huh7 cells (Huh7, KCLB, Seoul, Korea) were cultured in RPMI 1640 (RPMI 1640, HyClone, Logan, UT, USA) with 10% FBS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin.

Cell Cytotoxicity Detection

After 24 h of cell seeding, the medium was discarded and fresh medium containing 10, 30, 100, 300, and 1,000 μg/mL JRG was added to measure cytotoxicity in the HepG2 cells. Huh7 cells were treated with JRG at concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL. Cell viability was measured by colorimetry using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) 24 h after the cells were cultured. Insoluble formazan crystals were dissolved in dimethyl sulfoxide (DMSO, Junsei, Chuoku, Tokyo, Japan) and measured with a spectrophotometer (Synergy2, BioTek Instruments, Winooski, VA, USA) at 570 nm.

Cell differentiation

Fatty acids were liquefied in ethyl alcohol and mixed with fatty acid-free 0.1% bovine serum albumin (BSA, Pierce Co., Rockford, IL, USA) in fresh media. JRG was dissolved in distilled water and used at a concentration of 100 mg/mL. For the experiments, HepG2 cells were seeded with an initial cell density of 2.5×10^6 cells per cultured dish (diameter 100 mm) and incubated to adhere for 24 h. Afterward, the culture media was exchanged for fresh media, which contained 0.4 mM palmitic acid (Palmitic acid, Sigma-Aldrich) in 0.1% BSA with JRG (0, 30, 100, 300, and 1,000 μg/mL).

Fatty acid/BSA complexes were added as a supplement to the culture dishes after the change of serum-free medium at 24 h, at a concentration of 400 μM PA and 0.1% BSA with or without JRG. Control cell groups were treated with BSA vehicles. With the design of dose-response experiments in mind, the appropriate amount of JRG was added to the culture media for each concentration (30, 100, 300, and 1,000 μg/mL). After incubation for 24 h, the cells were harvested and used for protein extraction and RNA isolation.

TG assay

TG is formed by the combination of glycerol with three fatty acid molecules. To enhance the intracellular TG level, Huh7 cells were seeded in 100 mm cell culture dishes at 2.5×10^6 cells per well containing 30, 100, 300, and 1,000 μg/mL JRG and 400 μM oleic acid (Oleic acid, Sigma-Aldrich, St. Louis, MO, USA) was added for 24 h. The quantity of TG added was determined using the TG assay kit (TG assay kit, Abcam, Cambridge, UK) for various concentrations of JRG-treated cells. The protein concentrations in the cell lysate were determined using a bicinchoninic acid assay (BCA, Pierce Co., Rockford, IL, USA) and the TG level was detected with enzymatic cascade reactions. TG contents were visualized with quinoneimine dye by lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase reactions.

G3P assay

Huh7 cells were washed with cold phosphate buffered saline (PBS, Intron, Seongnam, Korea) three times, and then harvested with a rubber scraper. The G3P contents in the Huh7 cells were measured with a G3P assay kit (G3P assay kit, Abcam, Cambridge,

UK). Cells were lysed with a lysis buffer (G3P assay buffer) and the G3P contents were detected using a spectrophotometer at an optical density (OD) of 450 nm.

Oil Red O staining

HepG2 and Huh7 cells were washed three times with PBS and fixed with 10% formalin (Formalin, Sigma-Aldrich, St. Louis, MO, USA) for 1 h. The cells were permeabilized with 60% isopropanol (Isopropanol, Dae Jung, Siheung, Korea), washed three times with distilled water and stained with 60% filtered Oil Red O solution (Oil Red O, Sigma-Aldrich) for 30 min. Oil Red O was eluted by adding pure isopropanol and the absorbance was measured at 500 nm.

Western blot

Cultured cells were harvested in 200 μL radioimmunoprecipitation assay buffer (RIPA buffer, Thermo Fisher Scientific, Waltham, MA, USA), with 10 μg/mL of phosphatase inhibitor cocktail (Phosphatase inhibitor cocktail, Sigma-Aldrich, St. Louis, MO, USA) and protease inhibitor cocktail (Protease inhibitor cocktail, Sigma-Aldrich, St. Louis, MO, USA). The cell lysate was separated by centrifuging at 13,000×*g* for 30 min at 4°C. The supernatant protein concentration was measured with BCA and a BSA standard curve was plotted. Equal amounts of protein for the β-actin expression level were separated by 8-12% SDS-PAGE gels and were transferred to polyvinylidene fluoride (PVDF, Bio-Rad, Hercules, CA, USA) membranes by electrophoretic elution. After transfer, the nonspecific binding sites in the membranes were blocked with 5% nonfat dry milk (Nonfat dry milk, Difco Laboratories, Detroit, MI, USA) in Tris-buffered solution (15 mM Tris-150 mM NaCl, pH 7.4) with 0.05% Tween 20 (Tween 20, Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 1 h. After the blocking step, the membrane was incubated with anti-Fas (anti-Fas, Cell Signaling, Danvers, MA, USA), anti-C/EBPα (anti-C/EBPα, Cell Signaling, Danvers, MA, USA), anti-C/EBPβ (anti-C/EBPβ, Cell Signaling, Danvers, MA, USA), anti-SCD (anti-SCD, Cell Signaling, Danvers, MA, USA), and anti-liver X receptor (anti-LXR, Cell Signaling, Danvers, MA, USA) antibodies at 4 overnight. Afterward, the membrane was treated with the secondary peroxidase-conjugated anti-rabbit (Secondary peroxidase-conjugated anti-rabbit, Invitrogen Corp., Carlsbad, CA, USA) or anti-mouse (Secondary peroxidase-conjugated anti-mouse, Invitrogen Corp., Carlsbad, CA, USA) IgG at 37°C for 90 min. The protein expression bands were visualized with an electrochemiluminescence reagent (ECL reagent, Pierce Co., Rockford, IL, USA).

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was obtained using an easy-spin™ (DNA free) Total RNA Extraction Kit (Total RNA Extraction Kit, Intron, Seongnam, Korea). Reverse transcription was performed with the PrimeScript ™RTase (PrimeScript ™RTase, TaKaRa, Kusatsu, Shiga, Japan) using 1 μg of total RNA with the Oligo dT primer sand dNTP mixture. The action mixture (containing Oligo dT primer, dNTP mixture, and template RNA) was incubated for 5 min at 65°C and placed on ice. The reaction mixture was prepared by adding Prime Script Buffer, RNase inhibitor, and Prime Script RTase for 1 cycle at 30°C for 10 min, 42°C for 60 min, and 95°C for 10 min at 4°C. PCR was performed with the Accu Power® PCR Pre Mix (Accu Power® PCR Pre Mix, Bioneer, Daejeon, Korea). Pre-denaturation was performed at 95°C for 5 min and 30 cycles of 15 sec at 94°C, 40 sec at 55°C, and 45 sec at 72°C, with a final extension of 7 min at 72°C. The PCR reaction was conducted with the Veriti 96-well Thermal Cycler (Veriti 96-well Thermal Cycler, ABI, Foster city, CA, USA). The PCR amplicons were electrophoresed in 1%-agarose (Agarose, AMRESCO, Radnor, PA, USA) gels containing Tris Acetate Ethylenediaminetetraacetic acid (EDTA) buffer (TAE buffer, Intron, Seongnam, Korea).

Statistics

Data are expressed as means±standard errors of the mean (SEMs). Differences between groups were evaluated using the t-test with the Bonferroni post hoc test or by calculation of the Spearman's rank correlation coefficient, as appropriate, using Prism 5.03 (Prism 5.03, GraphPad Software Inc., San Diego, CA, USA). The following p-values indicated significance differences among the group: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Results and Discussion

Cytotoxicity of JRG

To establish whether the application of JRG to HepG2 and Huh7 cells has value for medical use without toxicity, the cells were treated with different concentrations of JRG (30, 100, 300, and 3,000 μg/mL) for 24 h and cell viability was evaluated with an MTT assay. The application of 3,000 μg/mL of JRG was signicantly toxic to HepG2 and Huh7 cells. In contrast, 30, 100, 300, and 1,000 μg/mL of JRG caused no substantial decrease in cell viability and were used for further investigations, as shown in Fig. 1.

Ginsenoside Rg1, Rb1, and Rg3 concentrations in JRG

The ginsenoside-Rg1, Rb1, and Rg3 contents were analyzed using HPLC with the Eclipse plus C_{18} column. The ginsenoside content was computed in comparison with a standard solution (Table. 1). The sum of Rg1, Rb1, and Rg3 in the JRG was 6.88 mg/g (Rg1 1.54 mg/g, Rb1 4.71 mg/g, and Rg3 0.63 mg/g).

Effect of JRG on lipid accumulation in HepG2 and Huh7 cells

HepG2 and Huh7 cells were incubated in the mixture with PA or OA for 24 h, which can lead to lipid accumulation simultaneously in hepatocytes. The cells were stained with Oil Red O solution for 30 min. The HepG2 and Huh7 cells were treated with 0.4 mM PA or OA for 24 h to induce hepatic steatosis. Cells treated with 1% BSA were used as a control. Lipid droplet accumulation in cells treated with PA or OA was significantly reduced by 1.8±0.7% (30 μg/mL JRG), 9.1±0.7% (100 μg/mL JRG), 39.0±0.6% (300 μg/mL JRG), and 50.5±0.8% (1,000 μg/mL JRG) in the Huh7 cells, as shown in Fig. 2. Further, lipid accumulation was decreased by −1.3±5.0% (30 μg/mL JRG), 8.7±4.8% (100 μg/mL JRG), 21.7±4.1% (300 μg/mL JRG), and 57.1±2.3% (1,000 μg/mL JRG) in the

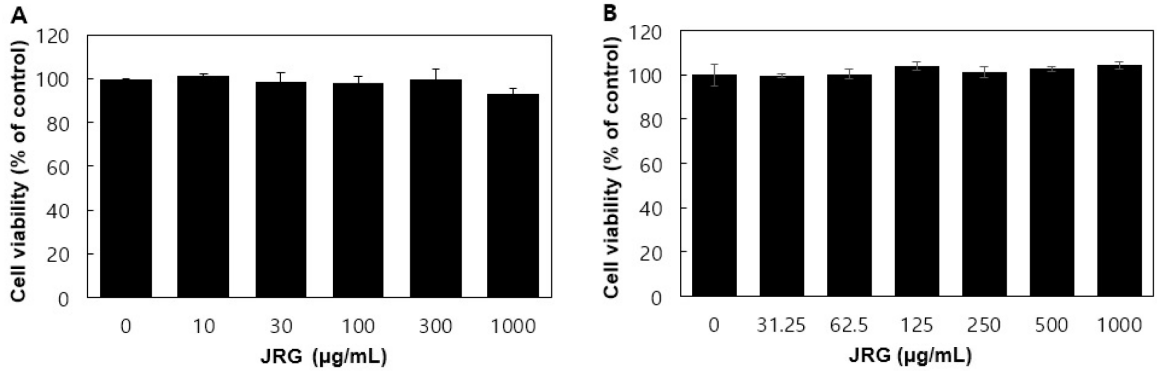

Fig. 1. Effect of JRG of cell viability and growth of hepatoma cells. (A) HepG2 cells were treated with JRG (10, 30, 100, 300, 1,000 μg/mL). (B) Huh7 cells were treated with JRG (31.25, 62.5, 125, 250, 500, 1,000 μg/mL). Cell viability was determined with MTT assays. *p*-values were calculated with Bonferroni's test (**p*<0.05).

Table 1. Ginsenoside content (Rg1, Rb1, Rg3) of red ginseng concentrate powder

Red ginseng concentrate powder	Rg1	Rb1	Rg3(s)	Total ginsenoside content
A	1.53	4.67	0.62	6.82
B	1.58	4.72	0.62	6.92
C	1.53	4.74	0.64	6.91
Average content (mg/g)	1.54±0.03	4.71±0.04	0.63±0.01	6.88±0.06

HepG2 cells. The optical density of the extracted Oil Red O decreased depending on the concentration of JRG (Fig. 2). These results showed that JRG reduced lipid accumulation in HepG2 and Huh7 cells induced with PA or OA.

Effects of JRG on fatty acid synthase, C/EBPβ, and SREBP-1c

To determine whether the decreased fatty acid synthesis was regulated by fatty acid synthase, we assessed the Fas expression level using western blotting. Fas expression was significantly downregulated by JRG in both HepG2 and Huh7 cells (Fig. 2). We also assessed the expression of C/EBPα, C/EBPβ, and SCD1 (Fig. 3). C/EBPβ and SCD1 protein expression was significantly suppressed in the JRG-treated group compared to that in the control group (Fig. 3A). In addition, JRG treatment reduced the mRNA levels of C/EBPβ and SREBP-1c, the key enzymes involved in fatty acid synthesis (Fig. 3C). These results indicate that JRG inhibits the expression of major lipid synthesis factors in hepatocytes under high fatty acid conditions.

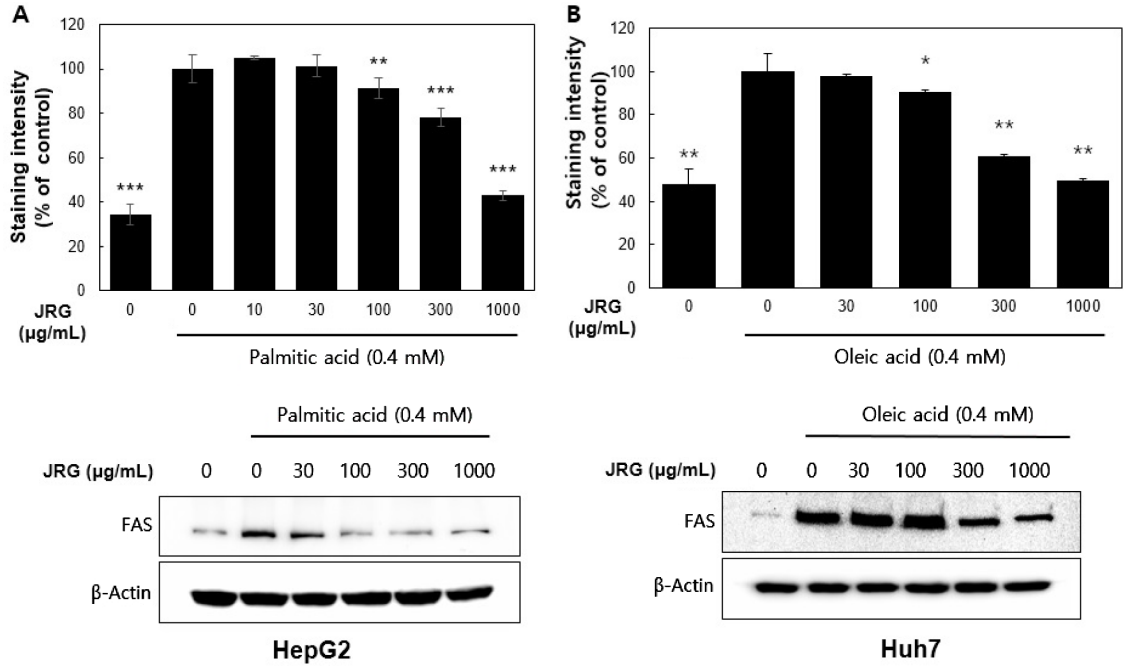

Fig. 2. The effect of JRG on lipid accumulation in hepatoma cell lines. (A) Relative lipid content quantified via Oil Red O staining and Fas expression levels are changed by JRG in HepG2 cells. (B) Relative lipid content quantified via Oil Red O staining and Fas expression levels is changed by JRG in Huh7 cells. Data represent the mean±SD of six independent experiments. The p values were calculated with t-tests (**p*<0.05; ***p*<0.01; ****p*<0.001).

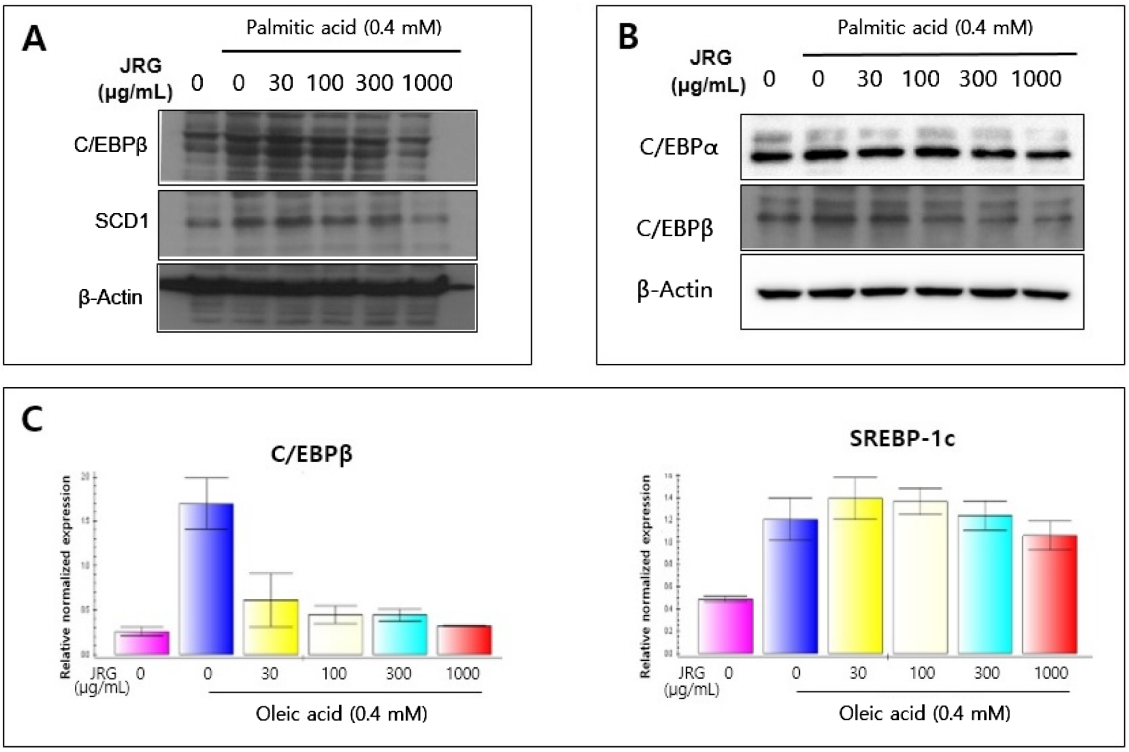

Fig. 3. The effect of JRG on major lipid synthesis factors in hepatoma cells. (A) The expression levels of C/EBPβ and SCD1 proteins were confirmed after JRG (30, 100, 300, 1,000 μg/mL) treatment in hepatoma cells with hepatic steatosis induced by PA. (B) Expression levels of C/EBPα and C/EBPβ proteins were confirmed after JRG (30, 100, 300, 1,000 μg/mL) treatment in hepatoma cells with hepatic steatosis induced by OA. (C) Expression levels of C/EBPβ and SREBP-1c mRNA were confirmed after JRG (30, 100, 300, 1,000 μg/mL) treatment in hepatoma cells with hepatic steatosis induced by OA.

Effects of JRG on expression levels of TG synthesis-regulating proteins, LXR and SCD

We measured the protein expression levels of LXR and SCD, which are lipid synthesis-regulating proteins, to determine the effect of JRG. The expression of LXR and SCD was lower in the JRG treatment group compared to that in the untreated group (Fig. 4).

Effects of JRG on TG accumulation in HepG2 and Huh7 cells

TG was accumulated as lipid droplets in the OA-treated cells compared to no accumulation in the untreated group. The TG level was reduced by JRG in a dose-dependent manner (Fig. 5). The TG inhibition rate was 4.0±3.3, 10.9±3.6, 13.9±2.9, and 58.1±3.3% with 30, 100, 300, and 1,000 μg/mL JRG in Huh7 cells.

Inhibition of G3P activation by JRG

TG is synthesized via a series of enzyme reactions in liver cells, and the product of the first reaction is converted to G3P by G3P transferase. Thus, we next assessed the effect of JRG on G3P levels in Huh7 cells. Fig. 5B shows that G3P was reduced by JRG in a dose-dependent manner compared to the controls.

Red ginseng is the most widely recognized plant used in traditional medicine and plays a major role in herbal health care. We were particularly interested in the fact that red ginseng exerts effects that improve lipid metabolism homeostasis. Red ginseng is

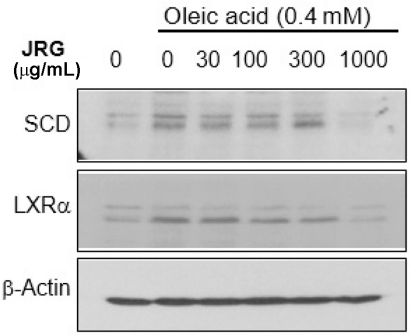

Fig. 4. The effect of JRG on LXR and SCD1 expression levels in hepatoma cells. Expression levels of LXR and SCD1 in differentiated Huh7 cells following treatment with the JRG. Cells were treated with JRG (30, 100, 300, 1000 μg/mL) and fatty acid as described in the materials and methods. Protein expression levels were measured by western blot analysis.

used as a medicinal herb to reduce TG in the blood, but its mechanism of action is not completely clear. Red ginseng is made through steaming and drying white ginseng and is more commonly used as herbal medicine than white ginseng. Red ginseng contains various ginsenosides, which are triterpenoidic saponins. The composition of ginsenosides in red ginseng is different from that in unprocessed ginseng (Jin et al., 2015; Kim et al., 2014). A previous study showed that red ginseng has a higher content of

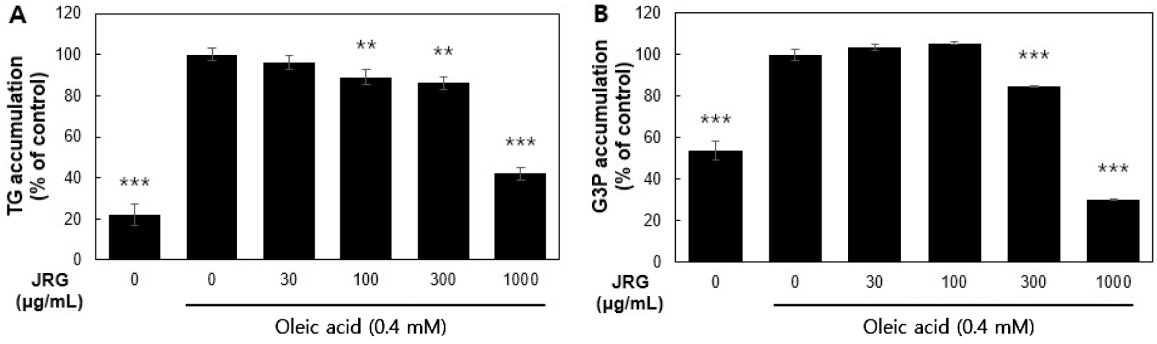

Fig. 5. The effect of JRG on triglyceride (TG) and glycerol-3-phosphate (G3P) expression levels in liver cancer cells. Expression levels of (A) TG and (B) G3P in Huh7 cells following treatment with the JRG. Cells were treated with JRG (30, 100, 300, 1000 μg/mL) and fatty acid as described in the materials and methods. TG and G3P levels were measured using the enzymatic method with an assay kit (*p<0.05, **p<0.01, ***p<0.001).

ginsenosides (Rb1, Rb2, Rc, Rd, Rg1, Rg2, Rg3, Rh1) than white ginseng and Taegeuk ginseng (Choi et al., 2010). In this study, we demonstrated that JRG contains 6.8 mg/g of Rg1, Rb1, and Rg3. It was previously reported that these ginsenosides have a unique mode of action in modulating various signaling cascades and networks in different tissues. These ginsenosides also attenuate signaling pathways in various tissues related to cancer (Lu et al., 2008; Shan et al., 2014), diabetes (Hwang et al., 2010; Shen et al., 2015), cardiovascular diseases (Chan et al., 2009; Kwok et al., 2012), and neurodegenerative disorders (Chen et al., 2008; O'Brien et al., 2011).

Hypertriglyceridemia refers to the elevation of TG level (a type of lipid) in the bloodstream, a condition that increases the risk of coronary artery disease. In our blood, TGs are acquired from dietary sources and synthesized in the body and liver for use as an energy source. Recent studies reported that the ethanol extract of Heshouwu (HSWE), composed of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside and anthraquinones, induces apoptosis and suppress lipid synthesis by inhibiting SREBP1 in human hepatocellular carcinoma cells (Lin et al., 2015; Li et al., 2016). In our previous studies, fatty acid synthesis in adipocytes was inhibited by JRG. TG is produced by three molecules of free fatty acid and one molecule of glycerol in the liver. Thus, we suggest that JRG affects TG synthesis in hepatoma cells. TG synthesis is inhibited by JRG in the liver and adipocytes (Fig. 5). Fatty acid synthesis is regulated by lipogenic transcriptional factors, such as C/EBPα, C/EBPβ, and Fatty Acid-Binding Protein 4 (FABP4). JRG inhibited the expression of C/EBPα, C/EBPβ, and FABP4 at the protein and mRNA levels in hepatoma cells (Fig. 2). C/EBP is an SREBP-1c factor that directly impacts lipogenesis by controlling the early induction of the key lipogenic enzyme SCD during adipogenesis (Ferre et al., 2007; Payne et al., 2009). The activation of LXR downstream of PPAR α activates SREBP-1c and induces the transcription of genes encoding SCD1 and SCD2 (Hellemans et al., 2009). In this study, LXR was downregulated by JRG treatment (Fig. 3), inducing a decrease in SCD. These findings indicate that TG synthesis is downregulated by JRG. Thus, JRG may have potential as a treatment for hypertriglyceridemia.

Conclusion

In this study, JRG reduced the transcriptional factors C/EBPα, C/EBPβ, and PPAR in lipid metabolism such as TG synthesis. Additionally, JRG regulated the expression of SCD and LXR, which are regulators of lipid metabolism. We demonstrated that TG synthesis is reduced by the regulation of C/EBPs and PPAR expression by JRG.

Acknowledgments

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