Ginsenoside Re lowers blood glucose and lipid levels via activation of AMP-activated protein kinase in HepG2 cells and high-fat diet fed mice

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Received July 25, 2011; Accepted September 14, 2011

DOI: 10.3892/ijmm.2011.805

Abstract. Ginsenoside Re is a protopanaxatriol-type saponin isolated from *Panax ginseng* berry. Although anti-diabetic and anti-hyperlipidemic effects of Re have been reported by several groups, its mechanism of action is largely unknown until now. Here, we examine anti-diabetic and anti-hyperlipidemic activities of Re and action mechanism(s) in human HepG2 hepatocytes and high-fat diet fed C57BL/6J mice. Re suppresses the hepatic glucose production via induction of orphan nuclear receptor small heterodimer partner (SHP), and inhibits lipogenesis via suppression of sterol regulatory element binding protein-1c (SREBP-1c) and its target gene [fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1)] transcription. These effects were mediated through activation of AMP-activated protein kinase (AMPK), and abolished when HepG2 cells were treated with an AMPK inhibitor, Compound C. C57BL/6J mice were randomly divided into five groups: regular diet fed group (RD), high-fat diet fed group (HFD) and the HFD plus Re (5, 10, 20 mg/kg) groups. Re treatment groups were fed a high-fat diet for 6 weeks, and then orally administered Re once a day for 3 weeks. The *in vitro* results are likely to hold true in an *in vivo* experiment, as Re markedly lowered blood glucose and triglyceride levels and protected against hepatic steatosis in high-fat diet fed C57BL/6J mice. In conclusion, the current study suggest that ginsenoside Re improves hyperglycemia and hyperlipidemia through activation of AMPK, and confers beneficial effects on type 2 diabetic patients with insulin resistance and dyslipidemia.

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Key words: ginsenoside Re, AMP-activated protein kinase, diabetes, hyperlipidemia, HepG2 cells, C57BL/6J

Introduction

The metabolic defects of obesity and type 2 diabetes, characterized by insulin resistance, nonalcoholic fatty liver disease and dyslipidemia, lead to an increased risk of cardiovascular disease (1). It is now becoming evident that the liver has an important role in the control of whole body metabolism of energy nutrients since the liver is the major site for storage and release of carbohydrates and for fatty acid synthesis. Hepatic AMP-activated protein kinase (AMPK) is thought to play a pivotal role in regulating lipid metabolism, glucose homeostasis, and insulin sensitivity (2). As an energy sensor maintaining cellular glucose homeostasis, AMPK significantly inhibits hepatic glucose output by transcriptional control. The sterol regulatory element-binding protein (SREBP) is a key lipogenic transcription factor that is nutritionally regulated by glucose and insulin (3,4). There is an inverse correlation between SREBP and AMPK activities in hepatocytes (5). AMPK activation by polyphenols can explain their beneficial effects on hepatic lipid accumulation, hyperlipidemia, and atherosclerosis in type 1 diabetic LDL receptor deficient ($LDLR^{-/-}$) mice (6).

Panax ginseng is a widely used herbal medicine in Asian countries known to have anti-diabetic and anti-hyperlipidemic activities (7-12). The principle components for these activities are thought to be the ginsenosides, a group of steroidal saponins. Attele *et al* (13) reported that *Panax ginseng* berry extract shows anti-diabetic and anti-obesity effects in C57BL/6J ob/ob mice, and its major constituent is ginsenoside Re. However, it is largely unknown how Re lowers blood glucose and lipid levels. Recently, protopanaxatriol ginsenoside Rg1 (14) and Rg2 (unpublished data) have been reported to suppress the hepatic glucose production through AMPK activation, but protopanaxadiol ginsenosides such as Rb1 and Rg3 have no effects on hepatic glucose production.

Here, we examined whether Re, a main constituent among the protopanaxatriol ginsenosides, suppresses hepatic gluconeogenesis and this effect is associated with the AMPK pathway in human HepG2 hepatocytes. We found that Re suppresses the hepatic glucose production by inhibiting the gene expression

Table I. RT-PCR primer sequence and annealing temperature.

Gene	Forward primer	Reverse primer	Annealing temperature, ˚C
hSHP	CAAGAAGATTCTGCTGGAGG	GGATGTCAACATCTCCAATG	58
hPEPCK	GGTTCCCAGGGTGCATGAAA	CACGTAGGGTGAATCCGTCAG	64
hG6Pase	GTGAATTACCAAGAGCCCAG	GCCCATGGCATGGCCAGAGGG	65
hSREBP1	GTGGCGGCTGCATTGAGAGTGAG	AGGTACCCGAGGGCATCCGAGAAT	64
hSCD1	TTGCCAGCTCTAGCCTTTAAATTC	TCCTGGTAGCATTATTCAGTAGTT	60
hFAS	CAAGAACTGCACGGAGGTGT	AGCTGCCAGAGTCGGAGAAC	65
GAPDH	TCCACCACCCTGTTGCTGTA	AGGTACCCGAGGGCATCCGAGAAT	54
mPEPCK	ATGCCTCCTCAGCTGCATA	TTACATCTGGCTGATTCTCTGTT	63
mG6Pase	ACCCTGGTAGCCCTGTCTTT	GGGCTTTCTCTTCTGTGTCG	50
mSREBP1	GCGCTACCGGTCTTCTATCA	TGCTGCCAAAAGACAAGGG	58
mSCD1	CGAGGGTTGGTTGTTGATCTG	ATAGCACTGTTGGCCCTGGA	56
mFAS	GATCCTGGAACGAGAACAC	AGACTGTGGAACACGGTGGT	50
mGPAT	GGTAGTGGATACTCTGTCGTCCA	CAGCAACATCATTCGGT	58
Actin	GGACTCCTATGGTGGGTGACGAGG	GGGAGAGCATAGCCCTCGTAGAT	58

of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) via induction of orphan nuclear receptor small heterodimer partner (SHP) gene expression stimulated by AMPK. Furthermore, Re attenuates hepatic steatosis through inhibition of gene expression of SREBP-1c and its target molecules, such as fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD1) in HepG2 cells. These effects are likely to hold true *in vivo* experiment, as Re markedly lowers blood glucose and triglyceride levels and protects against hepatic steatosis in high-fat diet fed C57BL/6J mice.

Materials and methods

Materials. Ginsenoside Re was kindly obtained from Professor Sung Kwon Ko of Semyung University. Antibodies against AMPK, phospho-AMPK, acetyl-CoA carboxylase (ACC), phospho-ACC were from Cell Signaling Technology (Beverly, MA, USA), and anti-actin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Reverse transcriptase, Taq polymerase and the MTS solution was supplied by Promega (Mannheim, Germany). Compound C (an AMPK inhibitor) was from Calbiochem (Darmstadt, Germany). Protein extraction, easy-BLUE total-RNA extraction and ECL-reagent kits were from Intron Biotechnology, Inc. (Beverly, MA, USA). Other reagents and chemicals were of the highest grade commercially available.

Cell viability assay. Human hepatoma HepG2 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin in an atmosphere of 95% air and 5% CO_2 at 37˚C. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. In brief, HepG2 cells were seeded at $3x10^4$ cells/well in 96-well plates and treated with Re as indicated. Twenty-four hours after treatment, 20 μl of MTS solution was added and the cells were incubated at 37˚C for 1 h. The cytotoxicity of Re was determined by the CellTiter 96® AQ_{ueous} One solution Cell Proliferation Assay kit (Promega, Madison, WI, USA).

Glucose production assay. Cells were cultured on 12-well plates at a density of $3.5x10^5$ cells/well. Twenty-four hours after the attachment period, cells were treated with or without Re in a serum-free medium for 3 h. Cells were washed twice with phosphate-buffered saline (PBS) to remove glucose and were incubated for 3 h in glucose production assay medium (glucose- and phenol red-free DMEM containing sodium pyruvate (2 mM) and sodium lactate (20 mM). The medium was collected at the end of the incubation period and analyzed for glucose secreted into the medium using an Amplex Red Glucose/Glucose Oxidase Assay kit (Invitrogen, Carlsbad, CA, USA). Glucose production was normalized to the protein concentration measured by a Bio-Rad protein assay kit (Hercules, CA, USA). All experiments were performed at least three times and representative data are shown.

Animals. Five-week-old C57BL/6J mice were purchased from G-Bio (Gwacheon, Korea). Mice were housed in environmentally controlled conditions with a 12 h light/dark cycle and free access to food and water under constant room temperature (22±2˚C), humidity (50±10%). Mice were randomly divided into five groups: regular diet fed group (RD), high-fat diet fed group (HFD) and HFD plus Re (5, 10, 20 mg/kg) groups. Mice in the RD group were maintained on the standard diet and other mice were fed a high-fat diet for 6 weeks, and then orally administered Re once a day for 3 weeks.

Western blotting. For preparation of whole cell lysates to detect phosphoproteins, cells were washed with ice-cold PBS and

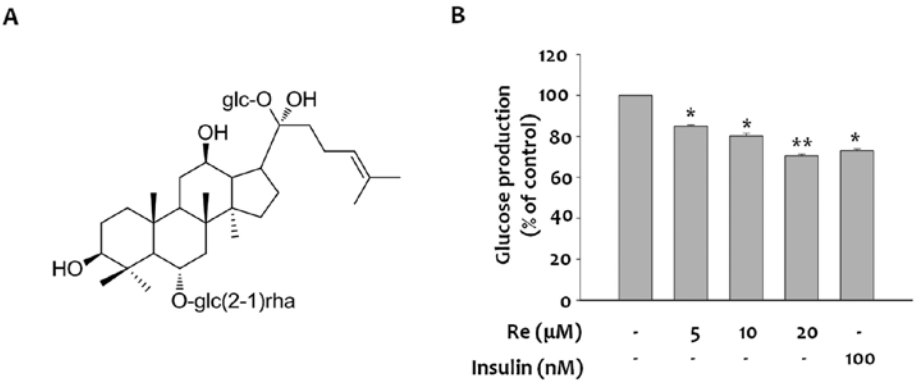

Figure 1. Chemical structure of ginsenoside Re (A) and effect of Re on hepatic glucose production in HepG2 hepatocytes (B). HepG2 cells were treated with ginsenoside Re as indicated for 3 h, and glucose concentrations were measured in culture media using a glucose oxidase assay. Data are represented as the mean ± standard error (SE) of triplicate experiments. *P<0.05; **P<0.01 as compared to the untreated control.

lysed in a protein extraction kit. Insoluble protein was removed by centrifugation at 13,000 rpm for 20 min. Protein concentrations in cell lysates were measured using a Bio-Rad protein assay kit. Equal amounts of protein (40 μg/lane) were resolved by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene fluoride membranes (Millipore, Beverly, MA, USA), and hybridized with primary antibodies (diluted 1:2,000) overnight at 4˚C. After incubation with horseradish-peroxidase-conjugated secondary antibody (diluted 1:2,000) for 2 h at room temperature, protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Uppsala, Sweden), exposed to X-ray film, and then quantified by a densitometric analysis.

Reverse transcription polymerase chain reaction (RT-PCR). Total-RNA was isolated using an easy-BLUE total-RNA extraction kit according to the manufacturer's instructions. Single-strand cDNA synthesis was performed as described previously using 5 μg of RNA, oligo(dt)15 primers and reverse transcriptase in a total volume of 50 μl. PCR reactions were performed in a total volume of 20 μl comprising 2 μl of cDNA product, 0.2 mM of each dNTP, 20 pmol of each primer, and 0.8 units of Taq polymerase. PCR was performed at 95˚C for 30 sec, followed by an annealing procedure as indicated in Table I for 30 sec, and 72˚C for 1 min. The last cycle was followed by a final extension step at 72˚C for 10 min. The RT-PCR products were electrophoresed on 1% agarose gels under 100 V and stained with 0.5 μg/ml ethidium bromide. Scanning densitometry was performed using an i-MAX gel image analysis system (Core-Bio, Seoul, Korea).

Biochemical analyses. Blood samples were collected from overnight fasted mice and centrifuged at 3,000 x g for 15 min at 4˚C. Blood glucose, triglyceride and non-esterified fatty acid (NEFA) concentrations were determined using an automatic chemistry analyzer (Erba SmartLab, Mannheim, Germany) and Stanbio reagents (Boerne, TX, USA). Plasma insulin concentrations were determined using a mouse insulin ELISA kit (Central Lab. Animal, Inc., Seoul, Korea).

Liver histological analysis. Livers were fixed in 10% phosphate-buffered formalin acetate at 4˚C overnight and embedded in paraffin wax. Paraffin sections (5 μm) were cut and mounted on glass slides for hematoxylin and eosin (H&E) staining to visualize the lipid droplets.

Statistical analysis. All data are expressed as a mean ± standard error (SE). Comparison between groups was made by ANOVA variance analysis, and significance was analyzed by the Tukey's test. Differences of P<0.05 were considered to be statistically significant.

Results

Re inhibits hepatic glucose production. To examine the cell viability after Re treatment, HepG2 cells were treated with various concentrations of Re (0-80 μM) for 24 h. Re did not show any cellular toxicity up to 80 μM (data not shown). The hepatic glucose production in response to Re and insulin was examined in human HepG2 hepatocytes. Cells were treated with Re and insulin (positive control) as indicated for 3 h. Re inhibited the hepatic glucose production in a concentration-dependent manner (Fig. 1B). At 20 μM, Re inhibited the hepatic glucose production by 29%, comparable to 100 nM of insulin-treated cells.

Re inhibits hepatic glucose production via the LKB1-AMPK pathway. AMPK is known to be activated by phosphorylation at position 172 of the threonine residue, and the protein levels of the phosphorylated form were determined by immunoblotting with the anti-phospho-Thr172 AMPKα antibody. Re markedly phosphorylated AMPK and ACC (an immediate substrate for AMPK) in a time- and concentration-dependent manner (Fig. 2A and B).

Next, we examine which upstream kinase regulates AMPK in HepG2 cells. A couple of reports demonstrated that the nearest relative kinases in mammals are the liver kinase B1 (LKB1) and the Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) (15,16). The AMPK kinase in these cells seems to be LKB1 since Re significantly phosphorylated LKB1 in a time- and concentration-dependent manner (Fig. 2A and B), but CaMKKβ was not phosphorylated by Re treatment (data not shown). Finally, to test whether the glucose suppressive effect of Re was dependent on activation of AMPK, a pharmacological

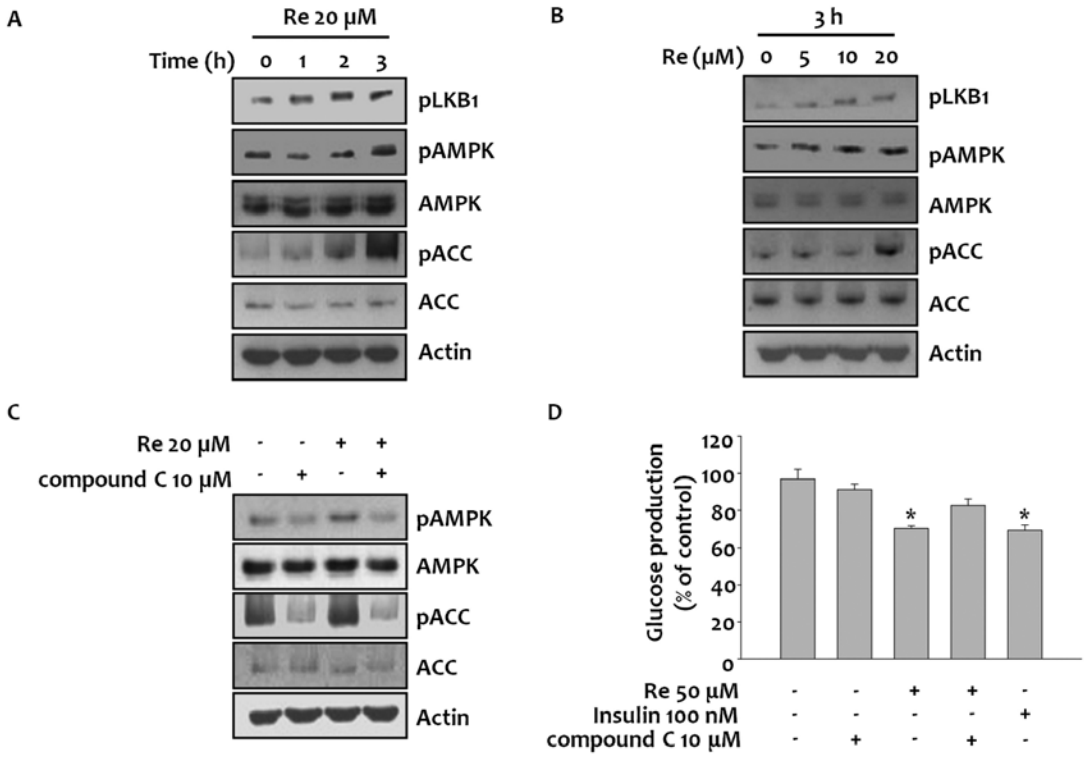

Figure 2. Effects of Re on LKB1, AMPK and ACC phosphorylations in HepG2 cells. Cells were treated with 20 μM of Re for the indicated times (A) or treated with Re as indicated for 3 h (B). Cells were pretreated with 10 μM of Compound C for 2 h, and then treated with Re for 3 h (C). In the same condition, glucose concentrations were measured in culture media using a glucose oxidase assay (D). Data are represented as the mean ± standard error (SE). *P<0.05 as compared with the untreated control.

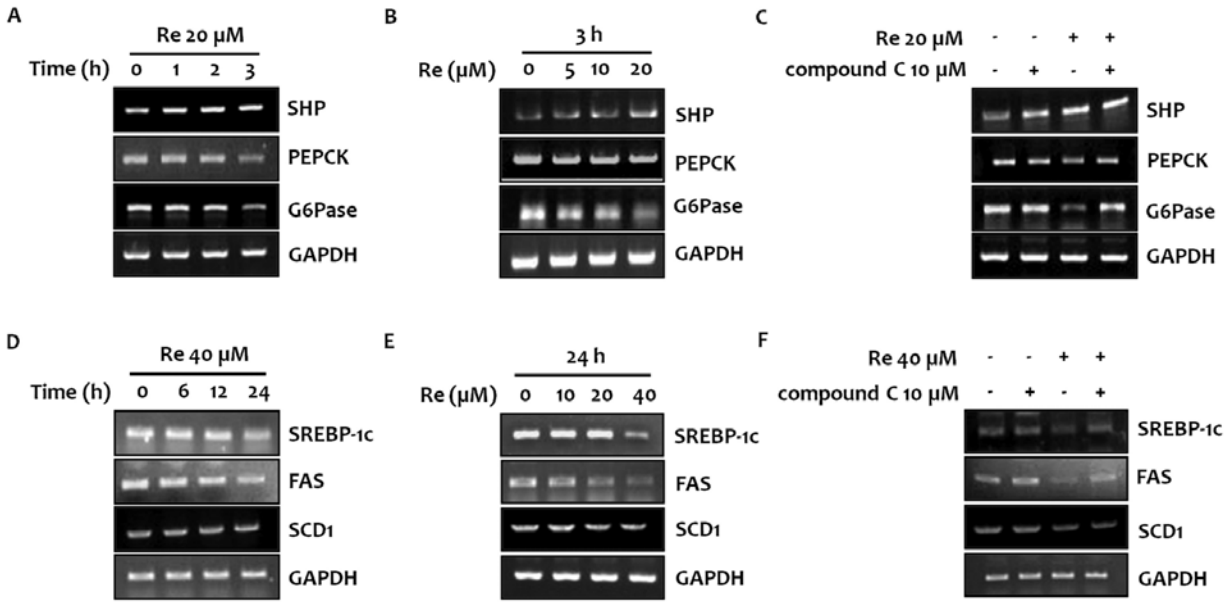

Figure 3. Effects of Re on hepatic gluconeogenic and lipogenic gene expressions in HepG2 cells. Cells were treated with 20 or 40 μM Re for the indicated times (A and D) or treated with the indicated concentrations for 3 h (B) or 24 h (E). HepG2 cells were pretreated with 10 μM of Compound C for 2 h, and then treated with 20 μM Re for 3 h (C) or 40 μM Re for 24 h (F). Total RNA were extracted from HepG2 cells and gene expressions were determined by RT-PCR.

approach using a specific inhibitor of AMPK (Compound C) was conducted. HepG2 cells were pretreated with 10 μM of Compound C for 1 h, and then incubated with 20 μM of Re for 3 h. AMPK and ACC phosphorylations and repression of hepatic glucose production caused by Re were all completely reversed in the presence of Compound C (Fig. 2C and D).

Re inhibits gluconeogenic gene expression via induction of SHP. A recent study demonstrates that AMPK activation causes up-regulation of SHP gene expression, resulting in suppression of hepatic gluconeogenic gene expression in an animal model (17). Being an activator of AMPK, effects of Re on the expression of SHP-dependent gluconeogenic genes were examined

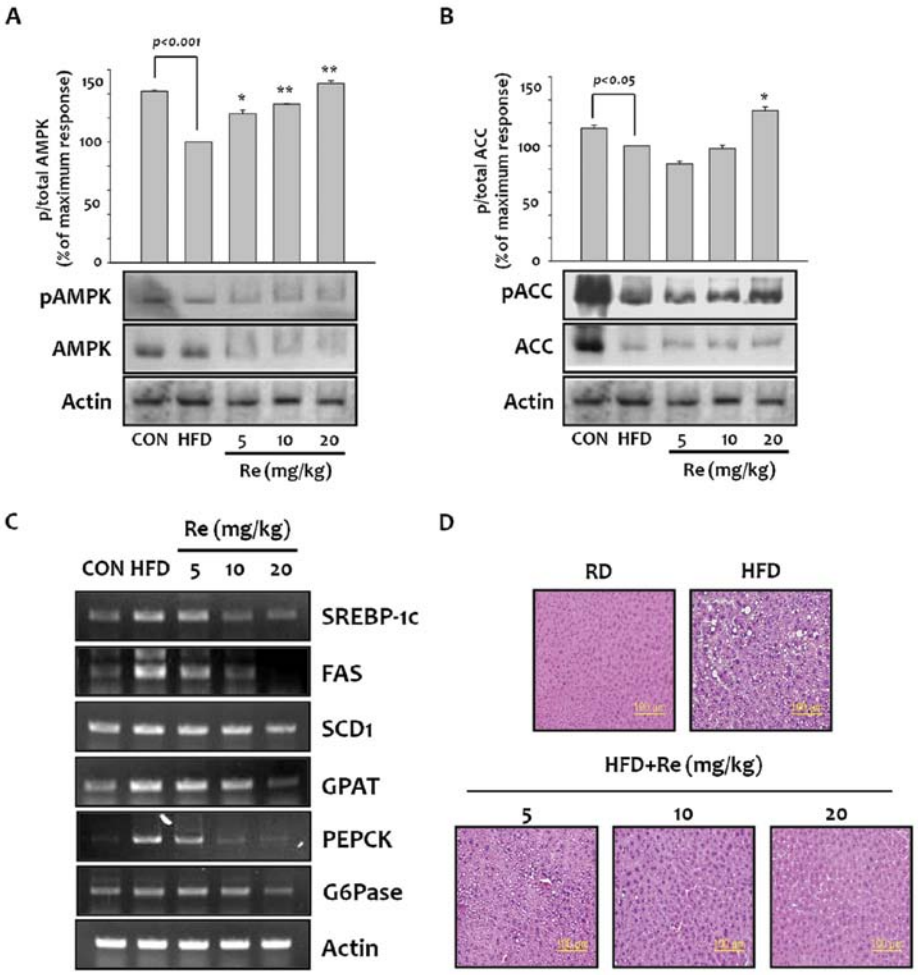

Figure 4. Effects of Re on AMPK (A) and ACC (B) phosphorylation, and lipogenic and gluconeogenic gene expression (C) in the liver of high-fat diet (HFD) fed C57BL/6J mice. Protein expression levels of pAMPK, AMPK, pACC, ACC were determined by Western blotting and transcription levels of lipogenic and gluconeogenic genes were determined by RT-PCR. H&E staining of livers (D). Magnification, x200.

by RT-PCR. Re up-regulated SHP and down-regulated PEPCK and G6Pase gene expressions in concentration- and time-dependent fashions (Fig. 3A and B), and these effects were all reversed in the presence of Compound C (Fig. 3C).

Re inhibits lipogenic gene expression. Li *et al* (18) have demonstrated that AMPK directly phosphorylates SREBP-1c and -2. AMPK stimulates Ser^{372} phosphorylation, suppresses SREBP-1c cleavage and nuclear translocation, and represses SREBP-1c target gene expression in hepatocytes exposed to high glucose, leading to reduced lipogenesis and lipid accumulation. Here, we examined the effect of Re on the gene expression of SREBP-1c and its target lipogenic enzymes, such as FAS and SCD1, by using RT-PCR. HepG2 cells were treated with 10-40 μM of Re for up to 24 h. Re inhibits gene expression of SREBP-1c, FAS and SCD1 in a concentration- and time-dependent manner (Fig. 3D and E). These effects were abolished in the presence of Compound C, indicating that AMPK is necessary for Re to suppress *de novo* lipogenesis through the down-regulation of lipogenic gene transcription in hepatocytes (Fig. 3F).

Re stimulates AMPK phosphorylation, suppresses lipogenic and gluconeogenic gene expression, and eliminates excess fat accumulation in the liver of C57Bl/6J mice. Five-week-old C57BL/6J mice were fed a high-fat diet for 6 weeks, and Re was then administered orally for 3 weeks. After sacrifice, liver tissues were removed, and protein and mRNA were extracted for evaluation of AMPK, ACC, SREBP-1c, FAS, SCD1 and glycerol-3-phosphate acyltransferase (GPAT), respectively. Hepatic phosphorylation of AMPK and ACC was decreased by ~30 and ~20%, respectively, in high-fat diet fed mice and substantially restored by Re treatment (Fig. 4A and B). To determine the functional consequence of AMPK activation, gene expression of key target proteins was assessed by RT-PCR. Expressions of lipogenic and gluconeogenic genes were also markedly enhanced in the high-fat diet control group, compared to the regular diet fed mice, whereas gene expressions in the liver of Re-treated mice were all significantly reduced in dose-dependent manners (Fig. 4C). Administration of Re eliminated excess fat accumulation in hepatic intracellular vacuoles, as determined by H&E staining (Fig. 4D).

Re decreases blood glucose and lipid levels. Blood glucose, insulin, triglyceride and NEFA levels of high-fat diet fed mice were increased by 25.0, 21,5, 38.6 and 35,7%, respectively, when compared to those in regular diet fed mice (Table II). Blood glucose levels were significantly lowered by 18.9% when

Table II. Blood parameters in regular diet fed and high-fat diet fed C57BL/6J mice treated with or without Re.

Parameters	RD	HFD	HFD + Re (mg/kg)		
			5	10	20
Glucose (mM)	7.6±0.2	9.5±0.4[c]	9.5±0.4	9.4±0.2	7.7±0.3[f]
Insulin (μU/ml)	272.7±6.3	331.2±17.9[a]	313.8±17.6	295.1±12.6	278.1±8.6[d]
HOMA-IR	91.7±2.0	139.2±7.7[b]	133.1±11.0	123.0±7.2	94.9±2.8[e]
Triglyceride (mg/dl)	56.2±3.7	77.9±8.2[a]	74.1±7.8	69.1±6.1	54.9±3.8[d]
NEFA (mEq/l)	641.4±24.8	870.2±33.6[c]	787.0±21.7	742.5±28.4[e]	707.5±28.3[f]

Values represent the mean ± SE (n=6). Animals were fed a regular diet (RD) or a high fat diet (HFD). The homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as insulin (μU/ml) x glucose (mM)/22.5. [a]P<0.05, [b]P<0.01, [c]P<0.001 vs. RD; [d]P<0.05, [e]P<0.01, [f]P<0.001 vs. HFD. NEFA, non-esterified fatty acid.

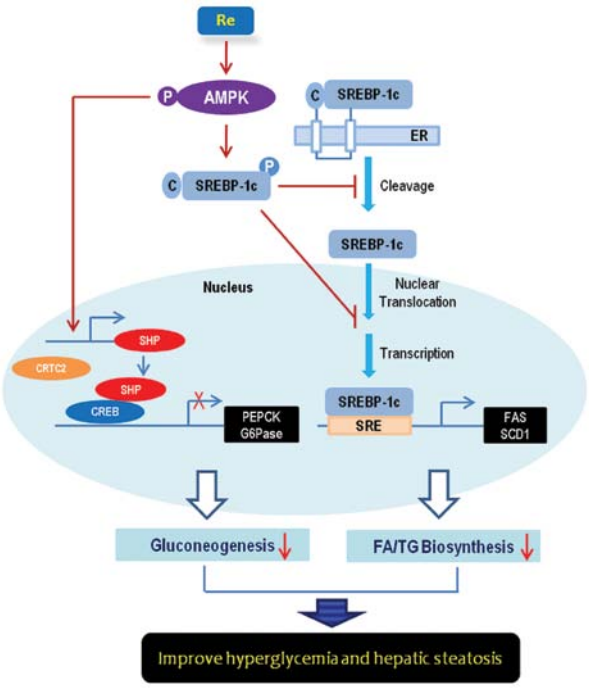

Figure 5. Proposed model for Re to suppress gluconeogenesis and lipogenesis through SHP induction and SREBP-1c regulation, respectively, via AMPK activation: potential therapeutic implication in diabetes and hepatic steatosis.

the mice were treated with 20 mg/kg dose of Re for a 3 week period. A similar pattern was observed for blood insulin levels. Having decreased glucose and insulin levels, HOMA-IR (insulin resistance index) of the mice treated with 20 mg/kg of Re was significantly decreased by 31.8%, compared to that of the HFD control group. Blood triglyceride levels were also dose-dependently lowered by 4.9, 11.3 and 29.5% when the mice were treated with 5, 10 or 20 mg/kg dose of Re for a 3 week period, respectively. Blood NEFA concentrations were also decreased in a dose-dependent manner, compared to that of the HFD control group.

Discussion

Ginsenoside Re is one of the major bioactive protopanaxatriol-type saponins found in ginseng root and berry. There are some reports demonstrating various pharmacological activities of Re, such as enhancing viability of human CD4(+) T cells through regulation of IFN-γ-dependent autophagy (19), enhancing an immune response to the influenza vaccine (20), suppressing electromechanical alternans in cardiomyocytes by opening ryanodine receptors (21), reducing insulin resistance in adipocytes of high-fat diet rats through inhibition of c-Jun NH_2-terminal kinase and NF-κB (22) and possessing antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats (23). Since 2000, Yuan and colleagues have focused on the evaluation of the pharmacological activities of ginseng (especially American ginseng) and they have demonstrated the anti-diabetic and anti-obesity activities of ginsenoside Re (13,24). However, the anti-diabetic and anti-hyperlipidemic mechanism of Re remains largely unknown. Herein for the first time, we demonstrate the anti-hyperglycemic and anti-hyperlipidemic effects of Re. We show that activated AMPK is the key mediator through which Re suppresses the glucose production and lipogenesis in the liver.

Using an *in vitro* experiment, we found that the suppressive effect of Re on hepatic glucose production was dependent on AMPK activation although the molecular mechanism by which Re activates AMPK is not yet known (Fig. 2). These *in vitro* results were confirmed in animal experiment using high-fat diet fed C57BL/6J mice. Blood glucose levels of 20 mg/kg Re-administered mice were lowered to the level of regular diet fed mice (Table II), and AMPK was dose-dependently phosphorylated in Re-treated mice (Fig. 4). AMPK achieves its downstream effects by direct phosphorylation of immediate substrates as well as long term effects on gene expression. Recently, as a molecular mechanism underlying AMPK-mediated PEPCK gene suppression, the phosphorylation of CRTC2, a co-activator of CREB protein, by AMPK was reported. Phosphorylation of CRTC2 at Ser^{171} by AMPK confers binding of the protein 14-3-3 and sequestration of CRTC2 out of the nucleus, resulting in inhibition of gluconeogenic gene expression (25), but Re failed to phosphorylate CTRC2 (data not shown). On the other hand, Lee *et al* (26) have demonstrated that SHP decreases the CREB-dependent induction of gluconeogenic gene expression and hepatic glucose production via disruption of the CREB·CRTC2 complex due to direct interaction with CREB. When HepG2 cells were treated with

Re, the SHP gene was overexpressed in a time- and concentration-dependent manner and these effects were blunted in the presence of Compound C (an AMPK inhibitor), indicating that Re suppresses the hepatic gluconeogenesis by inducing SHP gene expression via AMPK activation. The delayed response of Re antagonized the stimulatory effects of the CREB•CRTC2 complex-mediated gluconeogenesis through induction of SHP gene expression. SHP directly interacts with CREB, which subsequently inhibits CREB-dependent transcription of the subsequent gluconeogenic gene expression including PEPCK and G6Pase via competition with CRTC2 (Fig. 5).

Activated AMPK is a master regulator of cellular responses to low energy states, coordinating the changes in the activity of enzymes of lipid metabolism and modulating partitioning of fatty acids between the oxidative and biosynthetic pathways. Using *in vitro* and *in vivo* experiments, we demonstrate for the first time that Re inactivates lipogenic enzymes by inducing phosphorylation of ACC, as well as by down-regulating transcription factors and enzymes associated with lipid metabolism, such as SREBP-1c, FAS, SCD1 and GPAT via the LKB1-AMPK pathway (Figs. 2-4). Interestingly, specific deletion of LKB1 kinase in a murine liver model, which results in complete loss of phosphorylated AMPK, leads to a dramatically increased expression of hepatic lipogenic enzymes (27). Recently, Li *et al* (18) demonstrated that AMPK interacts with and directly phosphorylates SREBP. Ser^{372} phosphorylation of SREBP-1c by AMPK is necessary for inhibition of proteolytic processing and the transcriptional activity of SREBP-1c. Although we did not determine the phosphorylated form of SREBP-1c in the liver, we observed the suppressive effects of Re on the gene expression of SREBP-1c and its target enzymes, which result in improvement of hepatic steatosis. To support the hypothesis that AMPK is an upstream kinase that negatively regulates hepatic SREBP activity, we demonstrated that impaired hepatic AMPK signaling caused by HFD feeding was restored by Re treatment (Fig. 4).

In summary, we suggest that Re could antagonize the stimulatory effects of the CREB•CRTC2 complex-mediated hepatic gluconeogenesis through induction of SHP expression, suggesting that SHP has an important role in the anti-hyperglycemic effect of Re (Fig. 5). In addition, Re inhibits *de novo* biosynthesis of triglyceride in the liver through down-regulation of SREBP-1c and lipogenic enzymes associated with lipid metabolism. Our study provides a novel insight into the molecular mechanisms by which Re affects hepatic gluconeogenesis and steatosis. Further studies are needed to elucidate how Re activates AMPK in the liver and to provide therapeutic strategies to combat insulin resistance, dyslipidemia and hepatic steatosis prevalent in type 2 diabetics.

Acknowledgements

We acknowledge the support of the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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