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The Inhibitory Effect of Ginsenoside Rg1 on Glucose and Lipid Production in Human HepG2 Cells

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Ginseng has been widely used for the treatment of various human body disorders. Despite extensive studies, the mechanism of action of ginseng remains to be clarified. In an effort of clarifying the mechanism of gluco-regulatory activity in ginseng, the effects of ginsenosides on glucose metabolism were studied in human hepatoma HepG2 cells. In this study, we showed that Rg1 significantly inhibited glucose production, glycogen synthesis, and lipogenesis. Rg1 also increased the phosphorylation of AKT and AMP-activated protein kinase (AMPK). Further studies indicated that the Rg1-mediated inhibition of glycogen and lipid synthesis was blocked in the presence of PI3K/AKT- and AMPK-specific inhibitors. These results indicate that Rg1 contributes to glucose and lipid metabolism in liver cells through AKT and AMPK pathways. Our findings provide evidence to support that Rg1 may be potential useful in counteracting insulin resistance and diabetes.

Key Words: AKT, AMPK, ginsenoside, glucose metabolism, HepG2 cells

Introduction

Liver is the major organ that coordinates metabolism in human body by maintaining glucose homeostasis (14). Deranged glucose supply and metabolism in liver are linked with insulin resistance and diabetes, in which tissues become less responsive or even resistant to insulin. In muscle and adipose tissues, insulin is crucial for the stimulation of glucose uptake. Insulin also decreases glucose output by inhibiting gluconeogenesis and stimulating glycogen synthesis in liver (35). Hyperglycemia is the primary contributor to many diabetes-associated disorders and can exacerbate defective glucose disposal by interfering with insulin's action in insulin-targeted tissues. Thus, the regulation of glucose metabolism is critical for the treatment of insulin resistance. Current therapeutic agents, however, have shortcomings, such as serious side effects, and their mechanisms of action are not completely understood. The identification of better therapeutic agents is urgently needed. The identification of natural preventive and therapeutic agents with the potential to modulating deranged hepatic metabolism is an interesting topic that has drawn much attention (19).

AMP-activated protein kinase (AMPK) is a major energy sensor in cells which is activated by increased AMP/ATP ratio. AMPK has been shown to be a key regulator of glucose and lipid metabolism. In liver cells, the activation of AMPK leads to increased fatty acid oxidation and simultaneous inhibition of gluconeogenesis, hepatic lipogenesis, and glycogen biosynthesis (18). These metabolic effects of AMPK activation indicate that AMPK activators may be promising agents for the treatment of metabolic disorders, such as diabetes and insulin resistance. In fact, several drugs in clinical use for the treatment of diabetes are potent AMPK activators (18, 36).

Ginseng is one of the most widely used herbal medicines and has a long history in Asian countries. Ginseng demonstrates a wide range of beneficial effects, such as anti-aging, improving cognitive performance, and enhancing metabolic functions (1). The main active components in ginseng are ginsenosides, which are generally believed to contribute to ginseng's pharmacological actions (1). Historically used in treating diabetes, ginseng is known for its prominent hypoglycemic activity (28). Ginseng has been shown to decrease postprandial glycemia in both nondiabetic and type 2 diabetes subjects (33). The aqueous extract of ginseng was shown to be capable of inducing hypoglycemia in both glucose-loaded healthy animals and in animals with experimentally induced diabetes (21). Despite extensive studies, the mechanism of action of ginseng remains to be clarified. Previous studies on ginseng primarily focused on promoting glucose uptake in adipose and muscle tissues, with little attention to

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the modulation of liver cell metabolism (10, 13). In this study, the effect of ginsenoside Rg1 (Fig. 1A) on energy metabolism was investigated in human hepatoma HepG2 cells. We showed that Rg1 significantly inhibited gluconeogenesis, glycogen and hepatic lipid synthesis through the activation of AMPK and PI3K signaling pathways. Our findings provide a molecular basis for the hypoglycemic activity of ginsenoside Rg1, which is a promising candidate in the development of anti-diabetic agents.

Materials and Methods

Extraction and Isolation of Ginsenoside Rg1

Ginsenoside was isolated from the dried roots of Panax notoginseng as described elsewhere (4).

Cell Culture

Human HepG2 cells were purchased from the ATCC (American Type Culture Collection, Manassas, VA, USA) and were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM; Gibco Laboratories, Grand Island, NY, USA) supplemented with 15 mM HEPES, pH 7.4, 26 mM sodium bicarbonate, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% charcoal-dextran-treated fetal bovine serum with 5% CO_2. The growth curve of these cells was determined by counting the viable cells using trypan blue exclusion. The cells were seeded into culture dishes and allowed to attach for 12 h prior to the treatment. Cell proliferation was then assayed by Cell Counting Kit 8 (Dojindo Molecular Technologies, Gaithersburg, MD, USA).

Cell Viability Assay

HepG2 cells were seeded in 96-well plates (1×10^4 cells/well) 24 h prior to Rg1 treatment. Rg1 was added to the media as a concentrated stock in DMSO. The control groups were fed with media containing the same amount of drug-free DMSO. After incubation for 48 h, 10 μl of CCK-8 solution (Dojindo Molecular Technologies) was injected into each well, and cells were further incubated for 3 h. Relative cell viability was obtained by measuring absorbancy at 450 nm using an ELISA microplate reader (Spectra Max 190, Molecular Devices, Sunnyvale, CA, USA).

Glucose Production Assay

Glucose production was measured *via* the conversion of lactate and pyruvate to glucose as described elsewhere (5). HepG2 cells were cultured in 6-well plates (5×10^5 cells/well) and incubated in the presence or absence of Rg1 for indicated time period. The cells were washed with PBS to remove remaining glucose and then incubated for 4 h in 100 μl of glucose production medium (glucose-free DMEM containing 2 mM of sodium pyruvate and 20 mM of sodium lactate). Fifty microliters of medium was collected for the measurement of glucose concentration using a glucose assay kit (Cayman Chemical, Ann Arbor, MI, USA). The glucose concentrations were normalized based on cellular protein concentrations afterward.

Assay of Glycogen Synthesis

Glycogen synthesis was measured by estimating the incorporation of D-[^{14}C]-glucose (2 μCi/ml) into glycogen as described elsewhere (17). HepG2 cells were cultured in 24-well plates (1×10^5 cells/well) and incubated in the presence or absence of Rg1 for indicated time period. Glycogen synthesis was allowed to proceed for 1 h after drug treatments. Triplicate assays were performed with 5.5 mM unlabeled glucose. The cells were washed and left lysing in 0.5 N NaOH. The radioactivity incorporated into glycogen was assessed by precipitating glycogen onto glass fiber filters. The filters were washed with 67% ethanol, dried, and analyzed by scintillation counting.

Assay of Lipid Synthesis

HepG2 cells were seeded in 24-well plates (1×10^5 cells/well) and incubated with the appropriate compounds and indicated concentrations for the indicated time periods. The incorporation of [U-^{14}C]-glucose into lipids was analyzed as described previously (32). The assay was terminated with a PBS wash, and then, the cells were scraped to ice-cold methanol. The lipids were extracted by suspending the cells in a final mixture containing chloroform: methanol:H_2O:butylated hydroxytoluene (1:1:0.9:0.0001%). The lipid-containing phase was isolated, dried under a nitrogen stream; and then scintillation counted.

Western Blot Analysis

The cells were plated in 6-cm culture dishes at a density of 1×10^6 cells/dish. The cells were treated with ginsenosides for 24 h. The cells were then washed and left lysing in 0.2 ml of lysis buffer (1% NP-40, 50 mM Tris-Cl, pH 7.4, 180 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM NaF, 10 mM Na_3VO_4) for 30 min at 4°C. The protein concentrations determined, and equal amounts of cell lysate protein (20 μg) were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). Immunoblotting was performed using AKT antibodies, phosphorylated AKT, AMP-dependent kinase (AMPK), phosphorylated

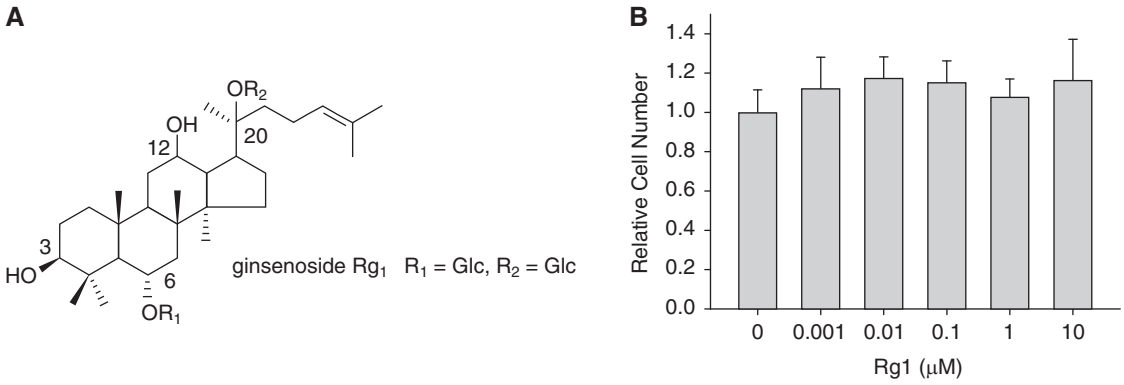

Fig. 1. Rg1 displayed no apparent cytotoxicity in HepG2 cells. (A) Chemical structure of ginsenoside Rg1. (B) The cells were treated with the indicated concentrations of Rg1 for 48 h, and the cell viability was determined. The percent growth was calculated, with 100% representing the growth of control cells treated with 0.1% DMSO. Data represent the mean ± SD (n = 3).

AMPK, acetyl-CoA carboxylase (ACC), phosphorylated ACC, and β-actin. The signals were visualized with an enhanced chemoluminescence kit (ECL, Amersham, Biosciences, Buckinghamshire, UK) followed by exposure of the blots to X-ray film.

Statistical Analysis

All results are representative of at least three independent experiments. Statistical analysis was performed using Windows SPSS 11.0. Analysis of variance (ANOVA) was used to determine the differences between values of various experimental and control groups in an attempt to show statistical significance. The data were expressed as the mean ± SD, and a P value < 0.05 was considered statistically significant.

Results

In this study, human hepatoma HepG2 cells were used to study metabolic adaptation of liver cells in the presence of ginsenosides. HepG2 cells have been widely used as an *in vitro* hepatic model system as they retain their morphology and most of the functions of hepatocytes in culture (29). These cells have been extensively used in the study of hepatic energy metabolism and modulation of insulin pathways (17, 20). The cytotoxicity of Rg1 was first examined. No apparent adverse effect was found regarding the morphology or viability of Rg1-treated HepG2 cells at concentrations up to 10 μM in this study (Fig. 1B). These results suggest that Rg1 is relatively non-toxic to HepG2 cells.

Rg1 Inhibits Glucose Production in HepG2 Cells

The effect of Rg1 on glucose production was investigated. Treatment of HepG2 cells with Rg1 for 1 h significantly suppressed glucose production. As shown in Fig. 2, Rg1 significantly inhibited glucose production in HepG2 cells in a concentration-dependent manner from 83% to 70% over the concentration range of 0.001 to 1.0 μM. Insulin also inhibited glucose production in HepG2 cells as expected. These results indicate that Rg1 suppressed glucose production in HepG2 cells in an insulin-independent manner.

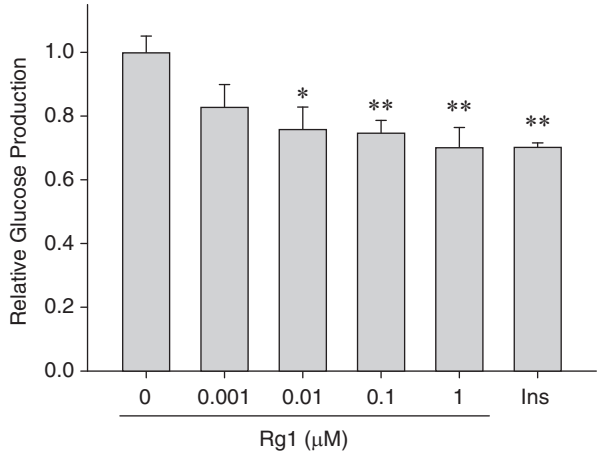

Fig. 2. Rg1 decreased glucose production in HepG2 cells. The cells were treated with the indicated concentrations of Rg1 or with 100 nM insulin (Ins) for 1 h. The cells were then analyzed for glucose production as described in the Materials and Methods. The data represent the mean ± SD (n = 3). *, P < 0.05 *vs*. the sample without Rg1.

Rg1 Downregulates Glycogen and Lipid Synthesis in HepG2 Cells

The effects of Rg1 on lipid and glycogen synthesis were studied for full understanding. Glycogen synthesis and lipid synthesis were assessed by respectively measuring the incorporation of glucose carbon atoms into glycogen particles or chloroform extractable lipids (32). The results shown in Fig. 3A indicate that Rg1 treatment significantly inhibited glycogen synthesis

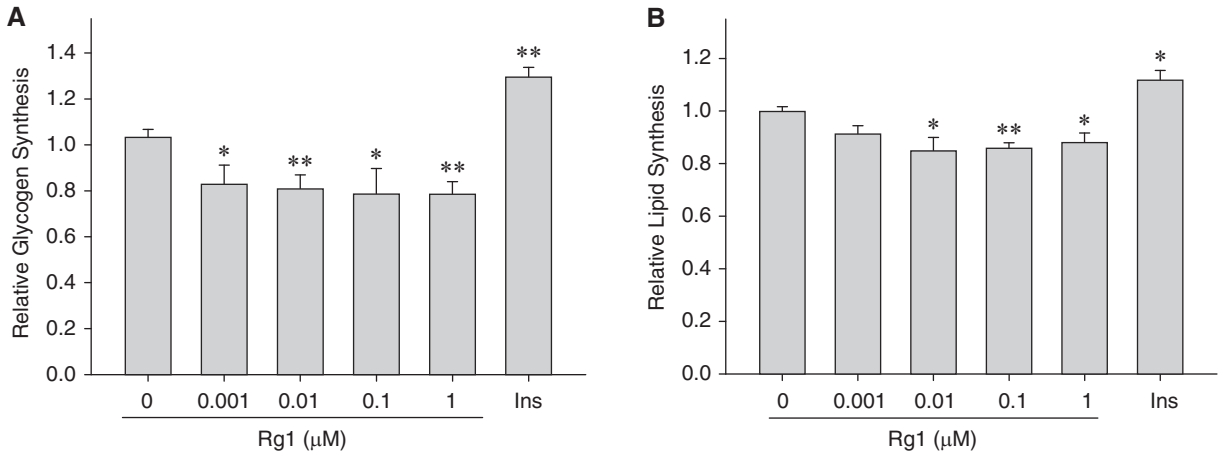

Fig. 3. Rg1 inhibited glycogen and lipid synthesis in HepG2 cells. The cells were subcultured in 24-well plates and incubated with serum-free medium for 24 h. The cells were then treated with the indicated concentrations of Rg1 or with 100 nM insulin (Ins) for 1 h before ^{14}C(U)-D-glucose was added, after which the cells were incubated for 3 h. The amounts of ^{14}C-incorporated into glycogen (A) and lipids (B) were analyzed as described in the Materials and Methods. The results represent the mean ± SD (n = 3), *$P < 0.05$, **$P < 0.01$ *vs*. the sample without Rg1.

in HepG2 cells. Rg1 significantly inhibited glycogen synthesis maximally to approximate 80% of the control level ($P < 0.05$) at the concentration of 0.01 μM, higher concentrations of Rg1, up to 1 μM, resulted in similar inhibition. The results shown in Fig. 3B indicate that Rg1 significantly inhibited lipid synthesis in HepG2 cells, with the/ a maximum inhibition reached at 0.1 μM, which is approximately 85% of the level compared to untreated control cells ($P < 0.05$). Higher doses of Rg1 up to 1 μM also displayed similar inhibitory effects on lipid synthesis. The results shown in Fig. 3 also demonstrate that in HepG2 cells, the incorporation of glucose into glycogen and lipids was significantly increased assisted by treatment with insulin.

Rg1 Activates the PI3K and AMPK Pathways in HepG2 Cells

Direct evidence of the activation of PI3K and AMPK signaling pathways was provided by measuring the phosphorylation levels of AKT and AMPK in Rg1-treated HepG2 cells. The results shown in Fig. 4 indicate that the phosphorylation levels of AKT (pAKT) and AMPK (pAMPK) were significantly increased in Rg1-treated HepG2 cells. The results shown in Fig. 4A demonstrate that Rg1 increased the phosphorylation of AKT protein, with a maximum at 0.001 μM for pS473-Akt (142% of the control, $P < 0.05$). It should be noted that Rg1 induced a slight, though not significant, increase in the pT308-Akt at all concentrations studied (110-125% of the control).

The incubation of HepG2 cells with Rg1 significantly increased the pAMPK level in a dose-and time-dependent manner, with a maximum increase at 1 μM (177% of the control for pAMPK, $P < 0.05$, Fig. 4B) over the concentration range studied. The pAMPK level reached a maximum after Rg1 treatment for 12 h (195% of the control for pAMPK, $P < 0.01$, Fig. 4C). The phosphorylation of acetyl-CoA carboxylase (pACC), the direct downstream target of AMPK, was also examined as further confirmation of AMPK activation in HepG2 cells. AICAR was included as a positive control for AMPK activation. These results suggest that the activation of PI3K/AKT and AMPK signaling pathways might be involved in insulin-independent mechanism of ginsenoside Rg1's effects on energy metabolism in hepatocytes.

The PI3K and AMPK Pathways are Involved in Rg1-Mediated Inhibition of Glycogen and Lipid Synthesis

With the given result that PI3K and AMPK are activated in cells treated with Rg1, the effect of Rg1 on glycogen and lipid biosynthesis was examined in the presence of specific inhibitors for PI3K and AMPK. Considering the Rg1 concentration to cause significant inhibition in Fig. 3 and the stimulatory effect of Rg1 on p-AMPK level is much more significant than p-Akt, we used 1 μM of Rg1 for the assays with inhibitors. As shown in Fig. 5, Rg1 alone significantly reduced glycogen (Fig. 5A) and lipid (Fig. 5B) synthesis to 76% ($P < 0.01$) and 83% ($P < 0.05$) of the control levels, respectively. The pretreatment of HepG2 cells with the PI3K inhibitor LY294002 or the AMPK inhibitor compound C markedly blocked Rg1-mediated inhibition of glycogen synthesis (92% and 85% of the control levels, respectively, compared with 76% of Rg1 alone). Similarly, the Rg1-mediated inhibition of lipid biosynthesis was also greatly suppressed in the presence of

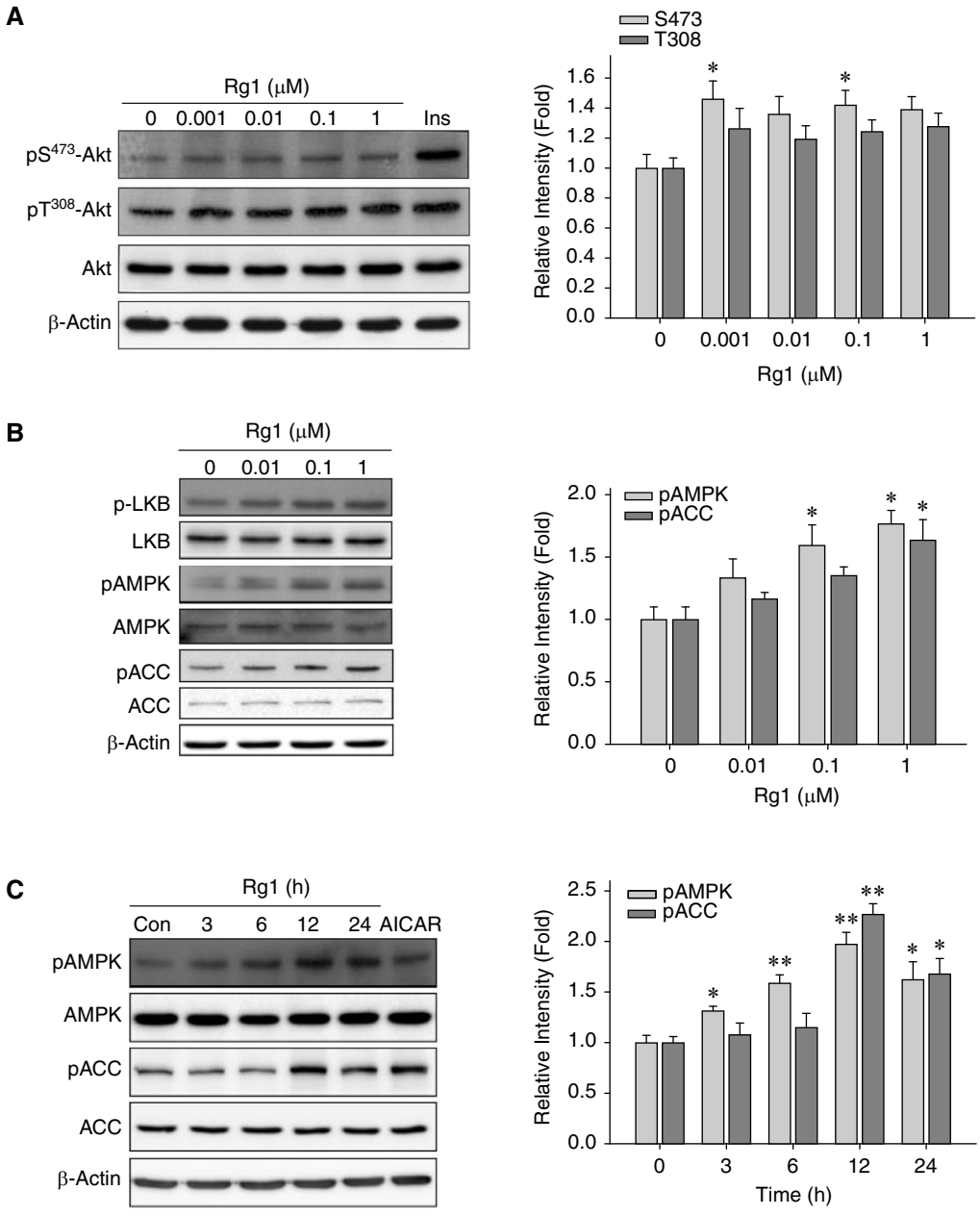

Fig. 4. Rg1 increased PI3K and AMPK phosphorylation in HepG2 cells. The cells were treated with the indicated concentrations of Rg1 or insulin for 12 h and analyzed to determine the phosphorylation levels of PI3K (A), AMPK, and ACC (B). For the time-course study, the cells were treated with 1 μM Rg1 for the indicated time periods (C). The right panels of each figure display the results of the quantitative analysis of relative levels of pAKT, pAMPK, and pACC. The results represent the mean ± SD (n = 3), $*P < 0.05$, $**P < 0.01$ *vs*. the sample without Rg1.

LY294002 or compound C (97% and 93% of the control level, respectively, compared with 83% of Rg1 alone). These results suggest that ginsenoside Rg1 demonstrates its metabolic effects in HepG2 cells through the PI3K and AMPK pathways.

Discussion

The use of ginseng is popular worldwide; however, scientific data supporting the claimed benefits are not always available. Because ginseng is a classic adaptogen, the hypoglycemic activity of ginseng is one of the most studied effects. Although numerous *in vitro* and animal studies have reported the hypoglycemic effects of ginseng, evidence demonstrating the efficacy of ginseng is still inconclusive (26, 27). It has been shown that different species of ginseng or even different batches of the same species display quite vari-

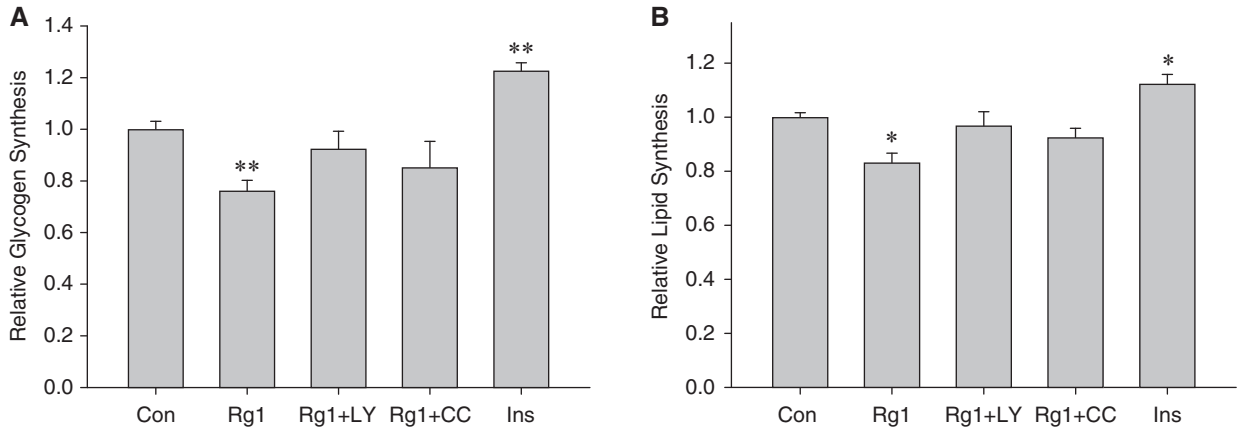

Fig. 5. The PI3K and AMPK pathways are involved in the Rg1-mediated effects in HepG2 cells. The cells were subcultured in 24-well plates and incubated with serum-free medium for 24 h. The cells were then treated with 1 μM Rg1 or 100 nM insulin (Ins) alone or in combination with 10 mM compound C (CC) or LY294002 (LY) for 1 h before ^{14}C(U)-D-glucose was added, after which the cells were incubated for 3 h. The amounts of ^{14}C-incorporated into glycogen (A) and lipids (B) were analyzed. The results represent the mean ± SD (n = 3), *$P < 0.05$, **$P < 0.01$ *vs*. the sample without Rg1.

ous gluco-regulatory effects (26, 27). A possible explanation for diverse variable hypoglycemic effects of different ginseng preparations is the marked difference in the profile of ginsenosides (8). To avoid any ambiguity, purified ginsenosides were used in our studies. In our study, we investigated the beneficial activity of ginsenoside Rg1 on glucose metabolism in hepatic-derived HepG2 cells. No apparent cytotoxicity of Rg1 at doses up to 10 μM toward HepG2 cells was found. We used submicromolar concentrations (0.01 to 1.0 μM) of ginsenoside Rg1, which are much lower than the concentrations used in other studies of ginsenosides from different sources (12, 13). The concentrations used in this study are important because lower concentrations could decrease side effects or other unwanted activities of Rg1. In addition, considering that red ginseng contains about 0.1% of Rg1 (3) and the oral bioavailability of Rg1 is around 10-20% (34), these Rg1 concentrations should fit well with dietary doses of normal subject in traditional use of dried ginseng slices.

Liver plays a key role in maintaining blood glucose concentration through glucose metabolic pathways, including gluconeogenesis, glycogen synthesis and degradation, as well as the conversion of glucose to fatty acids. The net effect is to remove glucose from blood circulation after meals (14). The control of glucose metabolism in hepatocytes is important because there is a strong correlation between abnormal glucose metabolism and the development of dysregulated metabolism in the whole body (22). In diabetes, the aberrantly activated hepatic gluconeogenic pathway supplies a relatively larger amount of glucose into blood circulation (14). Abnormal glucose production, impaired glucose uptake, and impaired glycogen synthesis in liver are main causes of insulin resistance and type 2 diabetes. Thus, the suppression of hepatic glucose production or facilitating glucose uptake and glycogen synthesis could be beneficial for the prevention and control of insulin resistance.

Traditional herbal medicines have been employed in the treatment of diabetes. The compounds isolated form Moutan cortex and Astragalus extract were shown to be capable of restoring the impaired glucose uptake and glycogen synthesis under high glucose condition in hepatic cells, suggesting the potential beneficial activity of these natural products (7, 38). In this study, using normal glucose concentrations, we found that Rg1 significantly inhibited glucose production in HepG2 cells, with a potency comparable to that of insulin (Fig. 2). In addition, we also demonstrated that acute exposure of Rg1 induced significant decreases in glycogen and lipid biosynthesis (Fig. 3). The activation of PI3K is involved in the ability of many ginsenosides to facilitate glucose uptake (2, 10, 25). The pentacyclic triterpenoid ursolic acid has been shown to act similarly to insulin, activating the PI3K signaling pathway (11). In addition to PI3K pathway, AMPK has been shown to regulate glucose transport in muscle, adipose, and liver cells *via* an insulin-independent mechanism (9, 10). AMPK is an important therapeutic target for regulating energy balance in human body (15). Compounds that activate AMPK have hypoglycemic effects by inhibiting gluconeogenesis in liver cells (5, 6, 12, 30, 36). It has been shown that many compounds or drugs with beneficial effects on metabolic syndrome activate AMPK, with metformin being the prominent example (37). In liver cells, the activation of AMPK signals a status of energy demand that inhibits energy-consuming anabolic processes, such as glucose production, glycogen synthesis, and lipogenesis. Thus, our results suggest that

the metabolic effect of ginsenoside Rg1 is primarily mediated through the activation of AMPK pathway.

To confirm the signaling pathways involved in Rg1-mediated activities, the phosphorylation levels of AKT and AMPK were analyzed in cells treated with Rg1. As it has been shown that full activation of AKT requires phosphorylation at T308 and S473, the phosphorylation level of both sites were determined (23, 24). Here, we showed that Rg1 increases the pS473- and pT308-AKT levels. In addition, Rg1 significantly increased the phosphorylation levels of pAMPK and pACC in HepG2 cells in a time- and dose-dependent manner (Fig. 4). Furthermore, we showed that the Rg1-mediated inhibition of glycogen and lipid biosynthesis was substantially abrogated by specific AKT and AMPK inhibitors (Fig. 5). These results indicate that PI3K/AKT and AMPK-ACC signaling pathways are involved in Rg1-mediated activities. In our previous study, ginsenosides CK and Rg1 were shown to activate PI3K/AKT and AMPK pathways in 3T3-L1 adipocytes (10). Cocoa flavonoids have been shown to improve insulin signaling *via* PI3K/AKT and AMPK pathways in HepG2 cells (6). It should be noted that our results, shown here, are somewhat different from those of previous studies of ginsenosides in HepG2 cells, which indicated that CK and Rg1 induced AMPK, phosphorylation, but not AKT phosphorylation (12, 13). This discrepancy might be resulted from different concentrations of ginsenoside used, as much lower doses of Rg1 were used in this study. In addition, it has also been reported that the increased phosphorylation of AKT can be induced by the activation of AMPK signaling (16, 31). Thus, it is also possible that the mild increase in the phosphorylation of AKT might have been resulted from AMPK activation as our study pointed out.

In conclusion, we showed that ginsenoside Rg1 effectively decreased glucose production in HepG2 cells. We also showed that Rg1 inhibited glycogen and lipid biosynthesis, primarily through AKT and AMPK-ACC pathways in HepG2 cells. Although further research is necessary to elucidate the detailed mechanisms of action of these compounds, our findings provide evidence to support the possibility of Rg1 serving as the treatment of insulin resistance and diabetes. This potential use of Rg1 is important, as the regulation of glucose production would be beneficial for glucose homeostasis and present an effective way to control hyperglycemia or insulin resistance.

Acknowledgments

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Conflict of Interests

The authors declare that there are no conflicts of interest

References

1. Attele, A.S., Wu, J.A. and Yuan, C.S. Ginseng pharmacology: multiple constituents and multiple actions. *Biochem. Pharmacol*. 58: 1685-1693, 1999.
2. Attele, A.S., Zhou, Y.P., Xie, J.T., Wu, J.A., Zhang, L., Dey, L., Pugh, W., Rue, P.A., Polonsky, K.S. and Yuan, C.S. Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component. *Diabetes* 51: 1851-1858, 2002.
3. Barnes, J., Anderson, L.A. and Phillipson, J.D. Herbal medicines. Grayslake, IL., USA. 2007, Pharmaceutical Press, 3rd ed., pp. 325-336.
4. Chang, T.-C., Huang, S.-F., Yang, T.-C., Chan, F.-N., Lin, H.-C. and Chang, W.-L. The effect of ginsenosides on glucose uptake in human Caco-2 cells is mediated through altered Na^+/coupled glucose transporter 1 expression. *J. Agri. Food Chem*. 55: 1993-1998, 2007.
5. Collins, Q.F., Liu, H.Y., Pi, J., Liu, Z., Quon, M.J. and Cao, W. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. *J. Biol. Chem*. 282: 30143-30149, 2007.
6. Cordero-Herrera, I., Martín, M.A., Bravo, L., Goya, L. and Ramos, S. Cocoa flavonoids improve insulin signalling and modulate glucose production *via* AKT and AMPK in HepG2 cells. *Mol. Nutr. Food Res*. 57: 974-985, 2013.
7. Ha, D.T., Tuan, D.T., Thu, N.B., Nhiem, N.X., Ngoc, T.M., Yim, N. and Bae, K. Palbinone and triterpenes from Moutan Cortex (Paeonia suffruticosa, Paeoniaceae) stimulate glucose uptake and glycogen synthesis *via* activation of AMPK in insulin-resistant human HepG2 Cells. *Bioorg. Med. Chem. Lett*. 19: 5556-5559, 2009.
8. Harkey, M.R., Henderson, G.L., Gershwin, M.E., Stern, J.S. and Hackman, R.M. Variability in commercial ginseng products: an analysis of 25 preparations. *Am. J. Clin. Nutr*. 73: 1101-1106, 2001.
9. Huang, S. and Czech, M.P. The GLUT4 glucose transporter. *Cell Metab*. 5: 237-252, 2007.
10. Huang, Y.C., Lin, C.Y., Huang, S.F., Lin, H.C., Chang, W.L. and Chang, T.C. Effect and mechanism of ginsenosides CK and Rg1 on stimulation of glucose uptake in 3T3-L1 adipocytes. *J. Agri. Food Chem*. 58: 6039-6047, 2010.
11. Jung, S.H., Ha, Y.J., Shim, E.K., Choi, S.Y., Jin, J.L., Yun-Choi, H.S. and Lee, J.R. Insulinmimetic and insulin-sensitizing activities of a pentacyclic triterpenoid insulin receptor activator. *Biochem. J*. 403: 243-250, 2007.
12. Kim, D.Y., Yuan, H.D., Chung, I.K. and Chung, S.H. Compound K, intestinal metabolite of ginsenoside, attenuates hepatic lipid accumulation *via* AMPK activation in human hepatoma cells. *J. Agric. Food Chem*. 57: 1532-1537, 2009.
13. Kim, S.J., Yuan, H.D. and Chung, S.H. Ginsenoside Rg1 suppresses hepatic glucose production *via* AMP-activated protein kinase in HepG2 cells. *Biol. Pharm. Bull*. 33: 325-328, 2010.
14. Klover, P.J. and Mooney, R.A. Hepatocytes: critical for glucose homeostasis. *Int. J. Biochem. Cell Biol*. 36: 753-758, 2004.
15. Lage, R., Dieguez, C., Vidal-Puig, A. and Lopez, M. AMPK: a metabolic gauge regulating whole-body energy homeostasis. *Trends Mol. Med*. 14: 539-549, 2008.
16. Leclerc, G.M., Leclerc, G.J., Fu, G. and Barredo, J.C. AMPK-induced activation of Akt by AICAR is mediated by IGF-1R dependent and independent mechanisms in acute lymphoblastic leukemia. *J. Mol.*

Signal. 5: 15, 2010.
17. Lin, C.L. and Lin, J.K. Epigallocatechin gallate (EGCG) attenuates high glucose-induced insulin signaling blockade in human HepG2 hepatoma cells. *Mol. Nutr. Food Res*. 52: 930-939, 2008.
18. Mihaylova, M.M. and Shaw, R.J. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol*. 13: 1016-1023, 2011.
19. Moller, D.E. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* 414: 821-827, 2001.
20. Nakajima, K., Yamauchi, K., Shigematsu, S., Ikeo, S., Komatsu, M., Aizawa, T. and Hashizume, K. Selective attenuation of metabolic branch of insulin receptor down-signaling by high glucose in a hepatoma cell line. HepG2 cells. *J. Biol. Chem*. 275: 20880-20886, 2000.
21. Ohnishi, Y., Takagi, S., Miura, T., Usami, M., Kako, M., Ishihara, E., Yano, H., Tanigawa, K. and Seino, Y. Effect of ginseng radix on GLUT2 protein content in mouse liver in normal and epinephrine-induced hyperglycemic mice. *Biol. Pharm. Bull*. 19: 1238-1240, 1996.
22. Postic, C., Dentin, R. and Girard, J. Role of the liver in the control of carbohydrate and lipid homeostasis. *Diabetes Metab*. 30: 398-408, 2004.
23. Saltiel, A.R. and Kahn, C.R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799-806, 2001.
24. Sarbassov, D.D., Guertin, D.A., Ali, S.M. and Sabatini, D.M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098-1101, 2005.
25. Shang, W., Yang, Y., Zhou, L., Jiang, B., Jin, H. and Chen, M. Ginsenoside Rb1 stimulates glucose uptake through insulin-like signaling pathway in 3T3-L1 adipocytes. *J. Endocrinol*. 198: 561-569, 2008.
26. Sievenpiper, J.L., Arnason, J.T., Leiter, L.A. and Vuksan, V. Variable effects of American ginseng: a batch of American ginseng (*Panax quinquefolius* L.) with a depressed ginsenoside profile does not affect postprandial glycemia. *Eur. J. Clin. Nutr*. 57: 243-248, 2003.
27. Sievenpiper, J.L., Arnason, J.T., Leiter, L.A. and Vuksan, V. Decreasing, null and increasing effects of eight popular types of ginseng on acute postprandial glycemic indices in healthy humans: the role of ginsenosides. *J. Am. Coll. Nutr*. 23: 248-258, 2004.
28. Sotaniemi, E.A., Haapakoski, E. and Rautio, A. Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care* 18: 1373-1375, 1995.
29. Sussman, N.L., Gislason, G.T., Conlin, C.A. and Kelly, J.H. The hepatix extracorpeal liver assist device–initial clinical experience. *Artifical Organs* 18: 390-396, 1994.
30. Tan, M.J., Ye, J.M., Turner, N., Hohnen-Behrens, C., Ke, C.Q., Tang, C.P., Chen, T., Weiss, H.C., Gesing, E.R., Rowland, A., James, D.E. and Ye, Y. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. *Chem. Biol*. 15: 263-273, 2008.
31. Tao, R., Gong, J., Luo, X., Zang, M., Guo, W., Wen, R. and Luo, Z. AMPK exerts dual regulatory effects on the PI3K pathway. *J. Mol. Signal*. 5: 1-9, 2010.
32. Van Epps-Fung, M., Hardy, R.W., Williford, J., Gupta, K. and Wells, A. Epidermal growth factor induces glucose storage in transgenic 3T3-L1 adipocytes overexpressing epidermal growth factor receptors. *Diabetes* 45: 1619-1625, 1996.
33. Vuksan, V., Sievenpiper, J. L., Koo, V. Y., Francis, T., Beljan-Zdravkovic, U., Xu, Z. and Vidgen, E. American ginseng (Panax quinquefolius L) reduces postprandial glycemia in nondiabetic subjects and subjects with type 2 diabetes mellitus. *Arch. Intern. Med*. 160: 1009-1013, 2000.
34. Xu, Q.F., Fang, X.L. and Chen, D.F. Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from Panax notoginseng in rats. *J. Ethnopharmacol*. 84: 187-192, 2003.
35. Zaid, H., Antonescu, C.N., Randhawa, V.K. and Klip, A. Insulin action on glucose transporters through molecular switches, tracks and tethers. *Biochem. J*. 413: 201-215, 2008.
36. Zhang, B.B., Zhou, G. and Li, C. AMPK: An emerging drug target for diabetes and the metabolic syndrome. *Cell Metab*. 9: 407-416, 2009.
37. Zhou, G.., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M., Goodyear, L. and Moller, D. Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest*. 108: 1167-1174, 2001.
38. Zou, F., Mao, X.Q., Wang, N., Liu, J. and Ou-Yang, J.P. Astragalus polysaccharides alleviates glucose toxicity and restores glucose homeostasis in diabetic states *via* activation of AMPK. *Acta Pharmacol. Sin*. 30: 1607-1615, 2009.