J. Korean Soc. Appl. Biol. Chem. **53**(1), 106-109 (2010)

Short Communication

Effect of Ginsenoside Rg3 and Rh2 on Glucose Uptake in Insulin-resistant Muscle Cells

Hye-Min Lee, Ok-Hwan Lee, and Boo-Yong Lee*

Graduate School of Biomedical Science, CHA University, Sungnam 463-836, Republic of Korea

Received November 13, 2009; Accepted November 25, 2009

Ginsenoside Rg3 and Rh2 possess various biological activities associated with Type 2 diabetes mellitus (T2DM). The insulin resistance of the muscle cells was induced by chronic insulin. The treatment of ginsenoside Rg3 and Rh2 significantly enhanced glucose uptake in the insulin-resistant muscle cells and the relative abundance of AMP-activated protein kinase expression.

Key words: AMPK, ginsenoside Rg3, ginsenoside Rh2, glucose uptake, insulin-resistant muscle cells

Type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, is a major health problem around globe, which characterized by high levels of blood glucose associated with impaired insulin secretion and insulin resistance. Insulin resistance in skeletal muscle is a major contributor to the development of T2DM, which is characterized by a reduced ability of insulin action and stimulation of glucose uptake. Furthermore, it has become an important underlying factor in recent year and been studied primarily by using *in vitro* models of C2C12 muscle cell line. In addition, insulin resistance in skeletal muscle can be induced by treatment with chronic insulin and tumor necrosis factor-α [Kumar and Dey, 2003].

Panax ginseng has been used in traditional medicine for over 2000 years because it is believed to be a panacea and to promote longevity [Attele *et al.*, 1999]. The bioactive compounds of *Panax ginseng* consist mainly of polysaccharides, phenolics, polyacetylenes, and various ginsenosides. Furthermore, ginsenosides have been reported to exhibit various biological activities such as anti-cancer, anti-diabetic, neuroprotective, radioprotective, anti-amnestic and anti-aging effects [Liang and Zhao, 2008]. Ginsenoside Rg3 and Rh2, a protopanaxadiols, is one of the most active ingredients in ginseng ginsenosides, and its pharmacological actions are currently being widely investigated. Although recent studies have shown that ginsenosides improve insulin sensitivity and reinstate blood glucose level in T2DM patients [Xie *et al.*, 2002a; 2002b], the effect of ginsenosides on the glucose uptake in insulin resistant muscle cells has not yet been studied. Thus, this study was to investigate the role of ginsenoside Rg3 and Rh2 in the stimulation of glucose uptake in insulin-resistant C2C12 muscle cells.

C2C12 cells were cultured in Dulbecco's Modified Essential Medium (DMEM) supplemented with 10% FBS and 1% antibiotics (penicillin/streptomycin) in 5% CO_2 at 37°C. The cells were differentiated in an equal mixture of two serum-free media (MCDB201 and Nutrient Mixture F-12 Ham medium) along with 0.05% BSA in the chronic presence of 100 nM insulin for 3 days [Kumar and Dey, 2002; 2003]. The media was changed every 12 h. C2C12 cells were also differentiated in DMEM supplemented with 2% HS for 4 days [Park *et al.*, 2007]. The media was changed every day. Ginsenoside was added during the last 3 h at the indicated concentrations. Ginsenoside Rg3 and Rh2 were obtained from BTGin Co., Ltd (Okcheon, Korea) and the purity was over 99%.

Cell proliferation was detected by the 2,3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt (XTT) (WelGene, Seoul, Korea) assay. When cells were cultured to the log phase, they were seeded on a 96-well plate (1×10^4 cells/well) for 24 h. Cells were divided into a control group and a ginsenoside treatment group at the indicated concentration. Absorbance was detected with an enzyme calibrator at 450 nm. Cell viability=(absorbance of study group/absorbance of control group)×100%. There were five wells for each concentration.

The differentiated C2C12 cells were incubated with low glucose serum-free media for the indicated time. After incubation, the cells were washed twice with KRPH

*Corresponding author
Phone: +82-31-725-8371; Fax: +82-31-725-8350
E-mail: bylee@cha.ac.kr

doi:10.3839/jksabc.2010.018

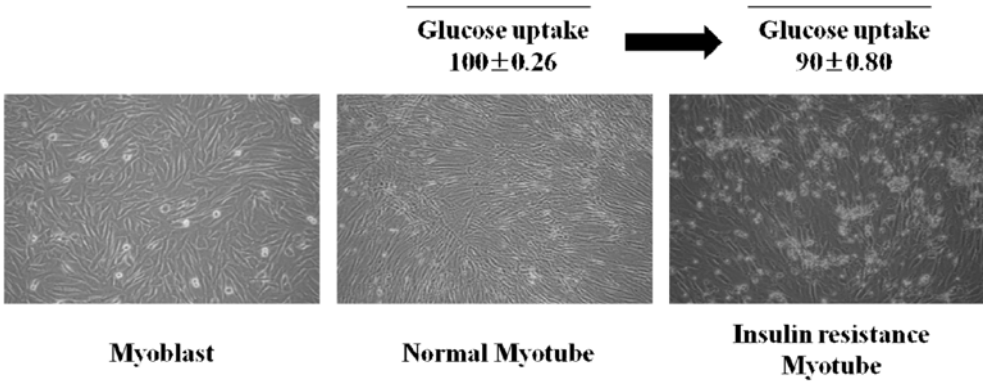

Fig. 1. Changes of C2C12 muscle cell during differentiation in the presence or absence of chronic insulin. Cells were differentiated in an equal mixture of two serum-free media (MCDB201 and Nutrient Mixture F-12 Ham medium) along with 0.05% BSA in presence or absence of chronic insulin at 100 nM (denoted as insulin resistance) for 3 days. Normal myotubes in 2% HS containing differentiation medium (denoted as normal). Glucose uptake was measured as described in Materials and Methods. The data are presented as mean±SD of the three different experiments. Statistical analysis was performed using the Student's T-test ($p<0.05$).

buffer (pH 7.4, 20 mM Hepes, 5 mM KH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 136 mM NaCl, 4.7 mM KCl) containing 0.1% bovine serum albumin (BSA). The cells were then incubated with Krebs-Ringer-phosphate-Hepes (KRPH) buffer containing 1 mM 2-Deoxy-Glucose (DG) and 0.1% BSA for the indicated time at 37°C in 5% CO_2. After incubation, the cells were washed twice with KRPH buffer containing 0.1% BSA and then 0.1 N NaOH was added. To degrade NAD(P)H, $NAD(P)^+$ and any enzymes in the cells, the culture plate was subjected to one freeze-thaw cycle and incubated at 85°C for 40 min on a dry oven. The components in the wells were then neutralized by the addition of 0.1 N HCl. The uptake of 2DG into the cells was measured by the enzymatic fluorescence assay described above.

Total RNA was extracted from C2C12 cells using the Qiagen RNeasy Kit (Qiagen, Valencia, CA), and then cDNA synthesis was performed with 400 ng of total RNA. The sequence of the oligonucleotide primers were as follows: GAPDH, sense (5'-AACTTTGGCATTGTGG AAGG-3') and antisense (5'-ACACATTGGGGGTAGG AACA-3'); AMP-activated protein kinase (AMPK), sense (5'-TTGTAGAGGCAAGCAG-3') and antisense (5'-AGTAGCAGTCCCTGATTTG-3'). PCR products were run on 1.5% agarose gels, stained with ethidium bromide and photographed.

Quantitative analysis of the RT-PCR results was conducted to determine if the expression of gene was increased or decreased by treatment with ginsenoside Rg3 and Rh2, and related gene was then quantified by real-time RT-PCR. To confirm the results, quantitative real-time PCR with SYBR Green PCR master mix (Applied Qiagen, Foster, CA) was conducted using the Rotor-Gene 3000 Sequence Detection System (Applied Corbett Research, Concorde, NSW, Australia).

The results are expressed as a mean±S.D. The effects were assessed using the one-way ANOVA (analysis of variance) or the Student's T-test. We used Tukey's HSD test (Honestly Significant Differences) to make comparisons. All calculations were performed using SAS software (SAS Inst., Cary, NC).

To induce insulin resistance, the cells were differentiated in an equal mixture of two serum-free media (MCDB201 and Nutrient Mixture F-12 Ham medium) along with 0.05% BSA in the chronic presence of 100 nM insulin for 3 days [Kumar and Dey, 2002; 2003]. The insulin resistance induced myotube cells demonstrated lower 2-deoxy glucose uptake (~10%) than that of normal cells (Fig. 1). These results indicate that C2C12 myotubes displayed induced insulin resistance by treatment with chronic insulin.

We examined whether cytotoxicity of ginsenoside Rg3 and Rh2 in C2C12 cells were treated with various concentrations (1 to 40 μM) for 24 h. Ginsenoside Rg3 and Rh2 treated cells did not show any cytotoxicity up to 40 μM (Fig. 2). Thus, we employed less than 40 μM of ginsenoside Rg3 and Rh2 in the following experiments.

To determine whether ginsenoside Rg3 and Rh2 improves glucose uptake in insulin-resistant muscle cells, C2C12 cells were treated with chronic insulin in the presence or absence of ginsenoside for 3 h. The C2C12 myotubes treated with ginsenoside Rg3 and Rh2 enhanced glucose uptake at a concentration of both 10 and 40 μM (Fig. 3). These results suggest that ginsenoside Rg3 and Rh2 may enhance 2-deoxy glucose uptake in insulin-resistant muscle cells via glucose transport systems associated with the AMPK pathways. We further examined the effect of ginsenoside Rg3 and Rh2 on

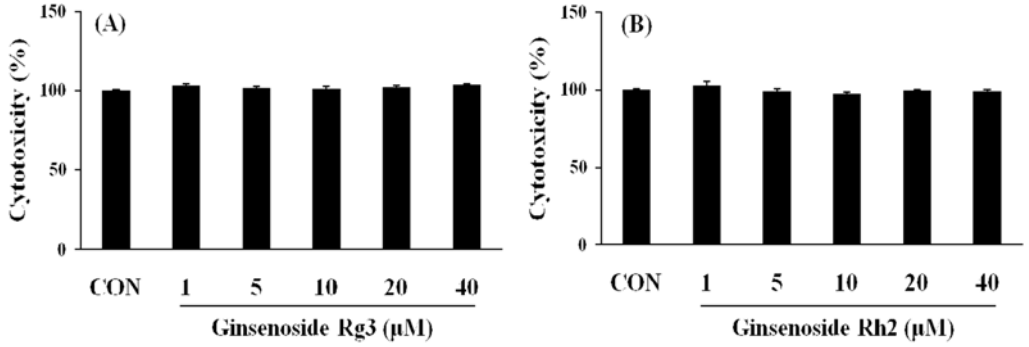

Fig. 2. Effects of ginsenoside Rg3 (A) and Rh2 (B) on cytotoxicity using the XTT assay. The exponentially growing cells were plated into 96-well micro plates at a density of 1×10^4 cells/well in DMEM/FBS medium and incubated for 24 h prior to treatment at 37°C in 5% CO_2. Cells were divided into a control group and a ginsenoside treatment groups in indicated concentration. The data presents the means±SD of the three different experiments. Statistical analysis was performed using the one-way ANOVA ($p<0.05$).

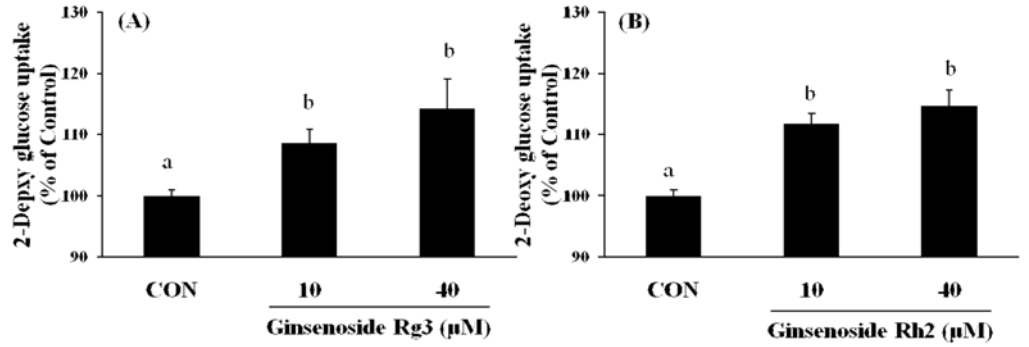

Fig. 3. Effects of ginsenoside Rg3 (A) and Rh2 (B) on 2-deoxy glucose uptake. (A) C2C12 cells exposed to ginsenoside Rg3 for 3 h. (B) C2C12 cells exposed to ginsenoside Rh2 for 3 h. Glucose uptake was measured as described in Materials and Methods. The data presents the means±SD of the three different experiments. Statistical analysis was performed using the one-way ANOVA. ($p<0.05$). a and b were statistically significant differences.

molecular mechanisms involving the AMPK that leads to increase glucose uptake. Because glucose uptake of ginsenoside Rg3 and Rh2 showed no significant difference between 10 and 40 μM (Fig. 3), we tested the effect of ginsenoside Rg3 and Rh2 (10 μM) on AMPK gene expression. Our results show that ginsenoside Rg3 and Rh2 treatments were increased AMPK expression (Fig. 4). These results indicate that ginsenoside Rg3 and Rh2 may enhance glucose uptake associated with AMPK expression.

Insulin resistance in skeletal muscle is associated with the development of metabolic diseases such as hyperglycemia and obesity. Recent studies have attempted to discover natural bioactive compounds for the improvement of insulin resistance associated with T2DM. Ginsenoside Rg3 an active component derived from *Panax ginseng;* it have emerged as having multiple functions in human disease [Yun, 2003] and ginsenoside Rh2 exerts anti-diabetic, anti-inflammatory, and anti-cancer effects by modulating various signal pathways [Oh *et al.*, 2005; Choi *et al.*, 2007]. However, in insulin resistant muscle cells, the anti-diabetic effects of ginsenoside Rg3 and Rh2 have not yet been reported. Thus, we evaluated the molecular mechanism of ginsenoside Rg3 and Rh2 on glucose uptake in insulin-resistant C2C12 myotubes.

In this study, ginsenoside Rg3 and Rh2 increased glucose uptake at a concentrations of both 10 to 40 μM (Fig. 3). The AMPK pathway is one of the glucose metabolisms, which is activated by stimuli such as exercise and hypoxia. Initially, AMPK was proposed to directly stimulate GLUT4 translocation [Krook *et al.*, 2004]. Ginsenoside Rg3 and Rh2 treatment increased the expression of AMPK in insulin-resistant C2C12 cells. These results indicate that the enhancement of glucose uptake by ginsenoside Rg3 and Rh2 are at least in part caused by an increase in the AMPK pathway. These results are consistent with other study by Park *et al.* [2007], who demonstrated that resveratrol increases glucose uptake by mediating AMPK signaling. Furthermore, berberine has also been shown to increase glucose uptake via AMPK activation [Yin *et al.*, 2008].

In conclusion, ginsenoside Rg3 and Rh2 in insulin-

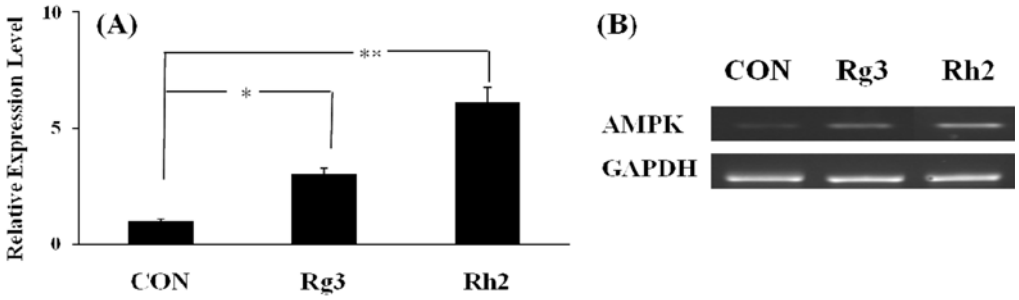

Fig. 4. Effects of ginsenoside Rg3 and Rh2 on AMPK gene expression. (A) C2C12 cells were treated with ginsenoside Rg3 and Rh2 at 10 μM for 3 h. The relative expression levels of AMPK mRNA were then calculated using the comparative C_t. Total RNA was subjected to Real-Time PCR as described in Materials and Methods. The data are presented as mean±SD of the three different experiments. Statistical analysis was performed using the Student's T-test. (*p<0.05, **p<0.01). (B) C2C12 cells were treated with ginsenoside Rg3 and Rh2 at 10 μM for 3 h. Total RNA was subjected to semi-quantitative RT-PCR as described in Materials and Methods.

resistant muscle cells stimulates glucose uptake as well as AMPK expression. These results suggest that ginsenoside Rg3 and Rh2 might be natural bioactive compounds with anti-diabetic properties and may also be potentially useful agents for the prevention of insulin resistance in T2DM.

References

Attele AS, Wu JA, and Yuan CS (1999) Ginseng pharmacology: multiple constituents and multiple actions. *Biochem Pharmacol* **58**, 1685-1693.

Choi K, Kim M, Ryu J, and Choi C (2007) Ginsenosides compound K and Rh(2) inhibit tumor necrosis factor-alpha-induced activation of the NF-kappaB and JNK pathways in human astroglial cells. *Neurosci Lett* **421**, 37-41.

Krook A, Wallberg-Henriksson H, and Zierath JR (2004) Sending the signal: molecular mechanisms regulating glucose uptake. *Med Sci Sports Exerc* **36**, 1212-1217.

Kumar N and Dey CS (2002) Metformin enhances insulin signalling in insulin-dependent and-independent pathways in insulin resistant muscle cells. *Br J Pharmacol* **137**, 329-336.

Kumar N and Dey CS (2003) Development of insulin resistance and reversal by thiazolidinediones in C2C12 skeletal muscle cells. *Biochem Pharmacol* **65**, 249-257.

Liang Y and Zhao S (2008) Progress in understanding of ginsenoside biosynthesis. *Plant Biol (Stuttg)* **10**, 415-421.

Oh JI, Chun KH, Joo SH, Oh YT, and Lee SK (2005) Caspase-3-dependent protein kinase C delta activity is required for the progression of Ginsenoside-Rh2-induced apoptosis in SK-HEP-1 cells. *Cancer Lett* **230**, 228-238.

Park CE, Kim MJ, Lee JH, Min BI, Bae H, Choe W, Kim SS, and Ha J (2007) Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. *Exp Mol Med* **39**, 222-229.

Xie JT, Aung HH, Wu JA, Attel AS, and Yuan CS (2002a) Effects of american ginseng berry extract on blood glucose levels in ob/ob mice. *Am J Chin Med* **30**, 187-194.

Xie JT, Zhou YP, Dey L, Attele AS, Wu JA, Gu M, Polonsky KS, and Yuan CS (2002b) Ginseng berry reduces blood glucose and body weight in db/db mice. *Phytomedicine* **9**, 254-258.

Yin J, Gao Z, Liu D, Liu Z, and Ye J (2008) Berberine improves glucose metabolism through induction of glycolysis. *Am J Physiol Endocrinol Metab* **294**, E148-156.

Yun TK (2003) Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. *Mutat Res* **523-524**, 63-74.