Antiobesity Effect of Ginsenoside Rg3 involves the AMPK and PPAR-γ Signal Pathways

Jin-Taek Hwang, Myoung-Su Lee, Hyun-Jin Kim, Mi-Jeong Sung, Hye Young Kim, Myung Sunny Kim and Dae Young Kwon*

Food Function Research Center, Korea Food Research Institute, Songnam, Kyoungki-do, 463-746, Republic of Korea

Ginsenosides, the active component of ginseng, exerts anti-diabetic and anticancer effects. This study investigated the molecular basis of ginsenoside Rg3, a red ginseng rich constituent, focusing on its ability to inhibit adipocyte differentiation in 3T3-L1 cells. The data show that ginsenoside Rg3 was effective in the inhibition of adipocyte differentiation. This inhibitory effect of ginsenoside Rg3 on adipocyte differentiation was accompanied by PPAR-γ inhibition in rosiglitazone-treated cells. The study also tested whether AMP-activated protein kinase (AMPK) activation was involved in the inhibitory effects of ginsenoside Rg3. AMPK plays a role in maintaining health in the context of diseases such as type 2 diabetes, obesity and cancer. AMPK was reported to control nutritional and hormonal signal modulating. Rg3 significantly and time-dependently activated AMPK. Taken together, these results suggest that the antiobesity effect of red ginseng rich constituent, ginsenoside Rg3, involves the AMPK signaling pathway and PPAR-γ inhibition. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: ginsenoside Rg3; AMP-activated protein kinase; antiobesity; peroxisome proliferators activated receptor-gamma; adipocyte differentiation.

INTRODUCTION

Obesity is a major obstacle to human health because it predisposes individuals to various diseases, such as type 2 diabetes, cardiovascular disease and cancer. Therefore, overcoming obesity by supplementation with an active compound is important in the prevention of various obesity-related diseases in humans. A previous study indicated that the modulation of obesity-related proteins using naturally occurring components constitutes a novel strategy for overcoming obesity (Hsu and Yen, 2007). Two major proteins regulate adipocyte differentiation, AMP-activated protein kinase (AMPK) and the peroxisome proliferator-activated receptor (PPAR) (Yin et al., 2003; Zhang et al., 2004). Both AMPK and PPAR-γ are major regulatory proteins involved in both obesity and diabetes. PPAR-γ is activated under conditions of adipocyte differentiation (Nedergaard et al., 2005; Lehrke and Lazar, 2005). Therefore, the inhibition of PPAR-γ expression with specific ligands can successfully induce antiobesity effects. Another major protein in obesity regulation, AMPK, plays a role in intracellular energy homeostasis, and shares amino acid sequence homology with yeast metabolic proteins, such as SNF1 (Hong et al., 2003). Under physiological conditions, AMPK is activated by the allosteric binding of AMP when ATP is depleted, and accelerates the generation of ATP by signaling to catabolic pathways. In another study, activated AMPK potentially prevented various diseases after treatment with some natural compounds (Zang et al., 2006). Our recent study demonstrated that the AMPK signaling pathway, which is induced by genistein, epigallocatechin gallate (EGCG) and capsaicin, reduced 3T3-L1 adipocyte differentiation. Moreover, AICAR, a specific activator of AMPK, also inhibited lipid synthesis by controlling β-oxidation-related proteins (Hwang et al., 2005)

This study identified candidate antiobesity compounds in the red ginseng constituent ginsenoside Rg3. Various types of ginseng and its active components, especially the ginsenosides, have emerged as having multiple functions in human diseases (Yun, 2003). In recent studies, ginseng and several ginsenosides that have been used in clinical trials and animal experiments have been shown to influence various physiological conditions (Leung et al., 2006). However, the physiological mechanisms underlying the antiobesity effects of ginseng and ginsenosides are still not clear.

Here, we reasoned that ginsenoside Rg3, rich in red ginseng, might cause a decrease in adipocyte differentiation in 3T3-L1 cells. The involvement of AMPK and PPAR-γ signaling in the antiadipogenic effects of ginsenoside Rg3 in 3T3-L1 adipocytes was tested.

MATERIAL AND METHODS

Cell culture and reagents, 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum in a CO₂ incubator. Specific antibodies that recognize the
phosphorylated forms of AMPK Thr\(^{172}\) and β-actin were from Cell Signaling Technology (Danvers, MA, USA). Insulin, 3-isobutyl-1-methylxanthine (IBMX) and dexamethasone, used to induce adipocyte differentiation, were purchased from Sigma (St Louis, MO, USA). Ginsenoside Rg3 was purchased from Fletton Reference Substance Co., Ltd (Huaishu, Chengdu, China).

Adipocyte differentiation. 3T3-L1 preadipocyte cells were plated in 12-well plates, and adipocyte differentiation was induced with a hormone cocktail containing 1 μM dexamethasone, 5 μg/mL insulin and 0.5 mM IBMX (day 0). After 2 days, the medium was changed to normal medium containing insulin (5 μg/mL), and then the cells were treated with the indicated stimuli.

Protein extract and western blotting. The cells were washed twice with ice-cold phosphate-buffered saline (PBS), scraped into lysis buffer (50 mM Tris–HCl [pH 7.4], 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM NaF, 1 μg/mL aprotinin, 1 μg/mL leupeptin and 1 μg/mL pepstatin), and subjected to western blot analysis.

Oil Red O staining. On day 6 after the induction of adipocyte differentiation, the cells were fixed with 3.5% formaldehyde for 20 min, and then the differentiated cells were stained with Oil Red O dye (Sigma) for 1 h. The cells were washed twice with water. Fat droplets in the adipocytes were stained red.

PPAR-γ transcriptional activity assay. The cells were transiently transfected with expression plasmids for PPAR-γ, RXRα, β-galactosidase and a luciferase reporter plasmid with PPAR response element (PPRE). After 24 h, the cells were treated with the indicated stimulus and then the cells were lysed with lysis buffer. The cell lysate was mixed with luciferase assay reagent (Promega, Madison, WI). Luciferase activity was measured by ELISA and then normalized by β-galactosidase activity.

Reverse transcriptase PCR. The cells were treated with the indicated stimuli, and then the total RNA was extracted with Trizol Reagent (Life Technologies, Glasgow, UK). Synthesized cDNA was used for amplification of the specific target.

Statistical analysis. Data are presented as mean ± SEM of at least three independent experiments performed in triplicate. Values of \( p < 0.05 \) were considered to be significant.

RESULTS

Rg3 was effective in inhibiting adipocyte differentiation

It has been proposed that several ginsenosides (ginseng derivatives) could be used in the treatment of various diseases, such as diabetes and cancer (Li et al., 2006). However, the antiobesity effects of ginsenosides have not been clarified. Therefore, first the antiobesity potential of ginsenoside on adipocyte differentiation was tested, especially focusing on the red ginseng rich constituent, ginsenoside Rg3. 3T3-L1 adipocytes were treated with ginsenoside Rg3 at different concentrations (day 0). On day 6, differentiation was terminated and fat drops were detected with Oil Red O staining. As shown in Fig. 1A and B, the treatment of 3T3-L1 cells with ginsenoside Rg3 markedly and dose-dependently inhibited adipocyte differentiation. These results suggest that ginsenoside Rg3 efficiently inhibited adipocyte differentiation in 3T3-L1 cells and have potential antiobesity effects.

Ginsenoside Rg3 activates AMPK

These data suggested that a metabolic master protein, such as AMPK, is a critical factor in the inhibition of obesity under physiological conditions (Luo et al., 2007). Therefore, the study next examined whether the AMPK signaling pathway is involved in the ginsenoside Rg3-induced inhibition of adipocyte differentiation. To this end, the cells were exposed to ginsenoside Rg3 at the indicated concentrations. AMPK activation was examined by western blot analysis. As shown in Fig. 2A, AMPK phosphorylation increased in the 40 μM Rg3 treatment. AMPK was also significantly activated by ginsenoside Rg3 in a time-dependent manner (Fig. 2B). These results suggest that Rg3 activates the AMPK-dependent signaling pathways in the process of adipocyte differentiation inhibition.

PPAR-γ signaling is also involved in the inhibition of adipocyte differentiation by ginsenoside Rg3

A previous report indicated that the suppression of adipogenesis is also associated with the PPAR-γ signaling pathway (Kudo et al., 2004). As adipocyte differentiation progresses, both PPAR-γ activity and expression increase. Thus, PPAR-γ activity is associated with adipogenesis. Therefore, the study next tested whether PPAR-γ inhibition is involved in the inhibition of adipocyte differentiation induced by treatment with ginsenoside Rg3. HEK293 cells were cultured with normal medium. After they had reached 70% confluence, the cells were transfected with a luciferase-tagged PPAR-γ-dependent RXR DNA plasmid. PPAR-γ activation was detected by the analysis of luciferase activity. As shown in Fig. 3A, PPAR-γ transcriptional activity decreased with the addition of ginsenoside Rg3. Independently, 3T3-L1 cells were treated with ginsenoside Rg3, and after 6 days the PPAR-γ mRNA levels were detected by reverse transcriptase PCR (Fig. 3B). These results indicated that ginsenoside Rg3 effectively inhibits both PPAR-γ transcriptional activity and mRNA level.

DISCUSSION

This study examined naturally occurring compounds that have been proposed to exert beneficial effects on human health by controlling physiological signaling molecules. These compounds exert various effects on human diseases, such as preventing diabetes, obesity and cancers (Baur et al., 2006). Ginseng has been used traditionally in Oriental countries to improve health. The ginsenosides, of which there are various types, are active components
of ginseng (Hasegawa, 2004). Although the ginsenosides have been suggested to play roles in various diseases, such as diabetes, cardiovascular injury and cancer, their antiobesity effects are poorly understood.

It has been proposed that the antiproliferative and antiobesity effects of naturally occurring phytochemicals act via the modulation of various signaling pathways, especially those that focus on controlling the proliferative signaling network (Loo, 2003; Auborn et al., 2003). However, the precise target of their antiobesity effects has remained unresolved and our understanding is limited. Here, we present the first evidence that Rg3, a constituent of red ginseng, induces the inhibition of adipocyte differentiation through the activation of AMP-activated protein kinase (AMPK) and the parallel inhibition of peroxisome proliferator-activated receptor gamma (PPAR-γ) transcriptional activity. Previously, it was also proposed that the antiobesity activity of the ginsenoside Rh2 is accompanied by AMPK activation in adipocytes (Hwang et al., 2007). According to our proposed mechanism, AMPK is responsible for the antiobesity effects of ginsenosides; thus, AMPK may be a key regulator of the antiobesity effects induced by naturally occurring compounds such as ginsenosides.

AMPK is a well-known metabolic master switch, activated by various stimuli, including exercise, hypoxia...
and reactive oxygen species (Towler and Hardie, 2007). Once activated, AMPK blocks anabolic pathways and promotes catabolic pathways, and protects the cells from various stress stimuli (Dolinsky and Dyck, 2006).

Our previous study suggested that several naturally occurring compounds, such as genistein, EGCG and capsaicin, activate AMPK in a dose-dependent manner, leading to the inhibition of adipocyte differentiation (Hwang et al., 2005). Although the role of AMPK in adipocyte differentiation was identified while investigating the actions of genistein, EGCG and capsaicin, the molecules downregulated by AMPK in its antiobesity capacity were not identified. Therefore, this study focused on finding the molecules regulated by AMPK in ginsenoside Rg3-induced antiadipogenesis, because AMPK signaling has been postulated to respond to intracellular levels of AMP or the AMP: ATP ratio under conditions of cellular stress (Hardie et al., 2006). Several previous reports have indicated that ginsenosides exert pro-apoptotic effects on cancer cells via stress signal induction (Wang et al., 2006). The processes of intracellular apoptosis are induced by various stress response proteins and induce apoptotic marker proteins (Tsurutani et al., 2005).

Therefore, it was deduced that the antiobesity effects of AMPK might be related to the negative regulation of proliferation key molecules in adipogenesis, such as PPAR-γ.

At the initiation step of adipocyte differentiation, various types of proteins are expressed, such as PPAR-γ and C/EBPα (Wagatsuma, 2006). These two transcription factors play a role in the initiation of adipocyte differentiation, and once bound to DNA, they induce the synthesis of various adipogenic proteins (Zuo et al., 2006). A previous study has demonstrated that the transcriptional inhibition of PPAR-γ by specific antagonists caused potent antiproliferative effects in many hematopoietic and epithelial cancer cell lines (Burton et al., 2007). However, it is still unclear how these antiproliferative effects of PPAR-γ inhibition, induced by various stimuli, are exerted.

Although it was found that AMPK and PPAR-γ signaling were involved in the antiobesity effects exerted by the ginsenoside Rg3, no direct correlation was found between the AMPK and PPAR-γ signaling pathways, both of which are modulated by the ginsenoside Rg3. Therefore, the possible connection between the AMPK and PPAR-γ-signaling pathways should be investigated in future studies.

The present study is the first to suggest that the activation of AMPK and the inhibition of PPAR-γ are necessary for the inhibition of adipogenesis in 3T3-L1 cells, that AMPK activation and PPAR-γ inhibition are induced by the ginsenoside Rg3, and that AMPK primarily targets antiobesity.

Acknowledgement

This study was supported by the Inter-Institutional Collaboration Research Program under the Korea Research Council for Industrial Science and Technology (KOI) and Korea Science and Engineering Foundation (KOSEF) for Bio Food Research Program, under the Ministry of Science and Technology.

REFERENCES


Tsurutani J, West KA, Sayyah J et al. 2005. Inhibition of the transcriptional inhibition of PPAR-γ by specific antagonists caused potent antiproliferative effects in many hematopoietic and epithelial cancer cell lines (Burton et al., 2007). However, it is still unclear how these antiproliferative effects of PPAR-γ inhibition, induced by various stimuli, are exerted.

Acknowledgement

This study was supported by the Inter-Institutional Collaboration Research Program under the Korea Research Council for Industrial Science and Technology (KOI) and Korea Science and Engineering Foundation (KOSEF) for Bio Food Research Program, under the Ministry of Science and Technology.


