Ginsenosides Increase Secretion of Lipoprotein Lipase by 3T3-L1 Adipocytes

Hiroshi Masuno, Takashi Kitao, and Hiromichi Okuda

Department of Medical Laboratory Technology, Ehime College of Health Science, Takooda, Tobe-cho, Iyo-gun, Ehime 791-21, Japan
*Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu, Onsen-gun, Ehime 791-02, Japan
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Treatment of 3T3-L1 adipocytes with either an oleanolic acid glycoside or a 20(S)-protopanaxatriol glycoside increased the secretion of lipoprotein lipase activity into the medium dose-dependently. At a concentration of 100 μg/ml, ginsenosides Ro, Re, Rg₁, and Rh₁ increased the secretion of lipase activity into the medium by 119, 107, 56, and 32%, respectively. The ratio of lipase activity in the medium to cellular lipase activity was 4.7% in control cells and 8.6% in ginsenoside Ro-treated cells, 8.3% in ginsenoside Re-treated cells, 7.0% in ginsenoside Rg₁-treated cells, and 6.3% in ginsenoside Rh₁-treated cells. Ginsenoside Rb₂, which is a 20(S)-protopanaxadiol glycoside, increased the secretion of lipase activity by 16% at 25 μg/ml, and the ratio of lipase activity in the medium to cellular lipase activity was higher in ginsenoside Rb₂-treated cells than in control cells. However, at 100 and 200 μg/ml, ginsenoside Rb₂ decreased the secretion of lipase activity in parallel with cellular lipase activity. Ginsenoside Rd also decreased the secretion of lipase activity in the same dose-dependent manner. Thus, the effective dose for the secretion of lipoprotein lipase activity with ginsenosides varies with their aglycone structure.

Key words: 3T3-L1 adipocytes; ginsenoside; lipoprotein lipase; secretion

The root of Panax ginseng C. A. Meyer has been used as a remedy for general weakness including diabetes mellitus. Ginsenosides, which are saponins extracted from ginseng, are responsible for various physiological and pharmacological activities of ginseng. One of the activities is to decrease plasma lipid levels. Yamamoto et al. reported that oral administration of ginseng powder to hyperlipemic patients decreased the plasma triacylglycerol and cholesterol levels. They also found using cholesterol-rich diet fed rats as a hyperlipemic model animal that ginsenosides lower plasma lipid levels.

Lipoprotein lipase is a key enzyme in the metabolism of plasma lipoproteins. This enzyme is synthesized in parenchymal cells of the extrahepatic tissues and located on the luminal surface of capillaries of the tissues, where it hydrolyzes triacylglycerols in circulating chylomicrons and very low density lipoproteins to free fatty acids and monoacylglycerols. Yokozawa and Oura reported that intraperitoneal administration of ginsenoside Rb₂ to rats increased adipose tissue lipoprotein lipase activity. However, the mechanism by which ginsenoside increased lipase activity is unknown.

Ginsenosides are classified into three groups based on their aglycone structure: oleanolic acid glycoside, 20(S)-protopanaxatriol glycoside, and 20(S)-protopanaxadiol glycoside. Herein, we examined the effects of ginsenosides on cellular lipoprotein lipase activity and secretion of activity into the medium in 3T3-L1 adipocytes and described the structure–function relationship of ginsenosides.

Materials and Methods

Figure 1 shows the chemical structures of the ginsenosides used in this study. Ginsenoside Rh₁ was dissolved in 75% ethanol and other ginsenosides were dissolved in 90% ethanol. The concentration was 10 mg/ml. Ginsenosides were kindly supplied by Korean Red Ginseng Co., Ltd.

(A) Oleanolic acid glycoside

(B) 20(S)-Protopanaxatriol glycoside

(C) 20(S)-Protopanaxadiol glycoside

Fig. 1. Chemical Structures of Ginsenosides.

* All correspondence should be addressed to Hiroshi Masuno, Ph.D., TEL: 089-958-2111; FAX: 089-958-2177.
Kobe, Japan.

3T3-L1 fibroblasts, obtained from Dr. Keizo Sekiya (Shikoku National Agricultural Experiment Station, Ministry of Agriculture, Forestry, and Fishery, Kagawa, Japan), were grown to confluence with standard medium in 60-mm plates, and then stimulated for 2 days to differentiate into adipocytes in standard medium supplemented with 1 μM dexamethasone, 10 μM of insulin, and 0.5 mM 1-methyl-3-isobutylxanthine. Then the medium was replaced with complete medium and was changed every 2 days. Cells at 8-12 days after confluence were used for experiments. About 80-90% of the cells contained lipid droplets. Standard medium consisted of 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B in Dulbecco’s modified Eagle’s medium. Complete medium consisted of standard medium with 5 μg/ml of insulin.

The medium was replaced with 3 ml of complete medium containing ginsenoside at the indicated concentrations, and cells were incubated for 22 h at 37°C. Then the plates were replenished with 1.5 ml of fresh complete medium containing the appropriate additive, and cells were incubated for 2 h at 37°C. The medium was separated from cells, filtered through a 0.2-μm filter, and used for prompt assay of lipoprotein lipase. Cellular lipoprotein lipase activity was measured in aqueous extracts of acetone/ether powders of cells. Cells were harvested into 1.2 ml of 50 mM NH₄Cl/NH₄OH buffer (pH 8.2) containing 2% bovine serum albumin and 20 μg/ml of heparin, sonicated briefly at 0°C, and used to make acetone/ether powder. The powder was added to ice-cold 50 mM NH₄Cl/NH₄OH buffer (pH 8.2) containing 20 μg/ml of heparin. After 1 h at 0°C, the mixture was sonicated briefly at 0°C, and centrifuged for 10 min at 4°C and 1200×g. The supernatant was decanted for assay of lipoprotein lipase. Lipoprotein lipase activity was assayed in duplicate.

A stock emulsion containing 5 mCi of tritiated oleoylglycerol, 1.13 mmol of trioleoylglycerol, 60 mg of phosphatidylcholine, and 9 ml of glycerol was prepared. One volume of the stock emulsion, 19 volumes of 3% bovine serum albumin in 0.2 M Tris/HCl buffer (pH 8.2) and 5 volumes of heat-inactivated (56°C, 10 min) serum from starved rats were mixed and incubated for 15-30 min at 37°C. For assay, 100 μl of this activated substrate mixture was added to 100 μl of the diluted cell extract and the medium, and incubated for 60 min at 37°C. One millilitre of lipolytic activity represents release of 1 nmol of fatty acid/min at 37°C.

DNA was measured fluorimetrically by the method of Hinegardner, using calf thymus DNA as a standard.

Insulin, dexamethasone, 1-methyl-3-isobutylxanthine, trioleoylglycerol, phosphatidylcholine, and an antibiotic antimycotic solution were obtained from Sigma. Tritiated oleoylglycerol was obtained from Amersham. Fetal bovine serum was obtained from M. A. Bioproducts, Walkersville, MD, U.S.A. Dulbecco’s modified Eagle’s medium was obtained from Nissui Pharmaceutical Co., Tokyo. Bovine serum albumin was obtained from Wako Pure Chemicals, Co., Osaka. All other chemicals were of the highest quality commercially available.

Results

The effects of ginsenosides on cellular lipoprotein lipase activity and secretion of lipase activity into the medium were examined in 3T3-L1 adipocytes. Cells were treated

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**Fig. 2.** Effects of Ginsenosides Ro and Rb₁ on Lipoprotein Lipase Activity in Cells and Medium.

3T3-L1 adipocytes were incubated for 22 h at 37°C in 3 ml of complete medium containing ginsenoside at the indicated concentrations. The medium was replaced with 1.5 ml of fresh complete medium containing the appropriate additive, and cells were incubated for 2 h at 37°C. The medium was separated from cells and filtered through a 0.2-μm filter, and lipoprotein lipase activity in a sample of the medium was measured. Cells were harvested, sonicated briefly at 0°C, and processed to make acetone/ether powder. Cellular lipoprotein lipase activity was measured in a sample of the extract of the powder. (O) cellular LPL activity; (●) LPL activity in the medium. The values given are means ± S.D. of three plates. *p < 0.05, **p < 0.01 (Student’s t-test, compared with control cells.)
for 24 h with ginsenoside and lipoprotein lipase activity secreted to the medium during the last 2h of the 24-h treatment was measured. Cellular lipoprotein lipase activity was measured in extracts of acetone/ether powder of cells. Treatment of cells with ginsenoside Ro, which is an oleanolic acid glycoside, increased lipoprotein lipase activity in the medium in a dose-dependent manner (Fig. 2A). Ginsenoside Ro at 100 μg/ml increased lipoprotein lipase activity in the medium by 119% (Table). The ratio of lipoprotein lipase activity in the medium to cellular lipase activity was 4.7% in control cells and 8.6% in ginsenoside Ro-treated cells. This treatment also increased cellular lipase activity by 42% at 200 μg/ml (Fig. 2A).

Similarly, treatment of cells with ginsenosides Re, Rg1, and Rh2, which are 20(S)-protopanaxatriol glycosides, also increased lipoprotein lipase activity in the medium. At a concentration of 100 μg/ml, ginsenosides Re, Rg1, and Rh2 increased lipoprotein lipase activity in the medium by 107, 56, and 32%, respectively (Table). However, they had no effect on cellular lipase activity. The ratio of lipoprotein lipase activity in the medium to cellular lipase activity was 8.3% in ginsenoside Re-treated cells, 7.0% in ginsenoside Rg1-treated cells, and 6.3% in ginsenoside Rh2-treated cells (Table).

Next, we examined the effects of ginsenosides Rb2 and Rd, which are 20(S)-protopanaxadiol glycosides, on lipoprotein lipase activity. At 25 μg/ml, ginsenoside Rb2 increased lipoprotein lipase activity in the medium by 16% with no effect on cellular lipase activity (Fig. 2B) and the ratio of lipoprotein lipase activity in the medium to cellular lipase activity was higher in ginsenoside Rb2-treated cells than in control cells. At higher concentrations ginsenoside Rb2 decreased lipase activity in the medium in parallel with cellular lipase activity (Fig. 2B). At 100 μg/ml it decreased lipase activity in the medium by 39% (Table). Ginsenoside Rd also decreased both lipase activity in the medium and cellular lipase activity in the same dose-dependent manner (data not shown). Ginsenoside Rd at 25 μg/ml increased lipase activity in the medium by 21%, and at 100 μg/ml decreased it by 29%. At a concentration of 100 μg/ml, the ratio of lipoprotein lipase activity in the medium to cellular lipase activity was 3.6% in ginsenoside Rb2-treated cells and 3.4% in ginsenoside Rd-treated cells (Table).

**Discussion**

It has been reported that oral administration of ginseng powder decreases plasma triacylglycerol levels\(^3\) and that some ginsenosides are responsible for this pharmacological activity.\(^4,9\) Lipoprotein lipase is the key enzyme involved in clearance of triacylglycerols from the circulation. This enzyme is synthesized and secreted by adipocytes. In this study, we found that treatment of 3T3-L1 adipocytes with ginsenosides caused an increase in LPL activity in the medium. Treatment of 3T3-L1 adipocytes with an oleanolic acid glycoside and 20(S)-protopanaxatriol glycosides increased lipoprotein lipase activity in the medium in a dose-dependent manner, while 20(S)-protopanaxadiol glycosides at 25 μg/ml increased lipase activity in the medium but at 100–200 μg/ml decreased it. Moreover, the increase in the secretion of lipase activity into the medium of cells treated with an oleanolic acid glycoside and 20(S)-protopanaxatriol glycosides was greater than that of cells treated with 20(S)-protopanaxadiol glycosides. Thus, the effective dose for the secretion of lipoprotein lipase varies with their chemical structure of the ginsenoside.

Lipoprotein lipase is synthesized in the endoplasmic reticulum, transported from the endoplasmic reticulum to the Golgi apparatus, and finally secreted by the cells.\(^6,7,10–13\) In the cells treated with an oleanolic acid glycoside and 20(S)-protopanaxatriol glycosides, the ratio of lipase activity in the medium to cellular lipase activity was higher than in control cells. The absence of any difference between control and ginsenoside-treated cells in cellular lipoprotein lipase activity indicates that the level of catalytically active lipase in ginsenoside-treated cells was normal. Moreover, when the medium was separated from cells, filtered through a 0.2-μm filter, and incubated again at 37°C, lipoprotein lipase in the medium of ginsenoside-treated cells was inactivated very rapidly with the same half-life (17 min) as that in the medium of control cells (data not shown), indicating that the increase in lipase activity observed in the medium of ginsenoside-treated cells did not result from an increase in the stability of lipase in the medium. Thus, treatment of cells with ginsenoside caused faster trafficking of active lipase in the cells, resulting in the increased accumulation of lipase activity in the medium.

Since plant saponins have been reported to cause hemolysis,\(^14\) the decrease in lipoprotein lipase activity by ginsenoside Rb2 may have resulted from the damage of cells. However, this is unlikely because the amount of DNA in the cultures of ginsenoside Rb2-treated cells was similar to that of the control cells (control cells, 181 ± 4 μg/plate; ginsenoside Rb2-treated cells, 185 ± 3 μg/plate at 100 μg/ml and 171 ± 3 μg/plate at 200 μg/ml; mean ± S.D. of three plates), and cells attached to the bottom of the plates excluded trypan blue. Thus, the decrease in cellular lipo-
protein lipase activity is attributed to the decreased synthesis of lipase in ginsenoside Rb2-treated cells. Ginsenoside Rd also decreased cellular lipase activity. This decrease in lipase activity may have resulted from damage to cells, because treatment of cells with 100 and 200 µg/ml of ginsenoside Rd reduced the DNA contents of the cultures to 85 and 75%, respectively, of that of control cells. Thus, ginsenoside Rd may be toxic for 3T3-L1 adipocytes at concentrations of over 100 µg/ml.

Takino’s group reported the absorption and metabolism of ginsenosides after oral administration to rats, since the ginseng powder had been administered orally as a crude drug. They found that in the case of ginsenoside Rb2, 3.7% of the dose was absorbed from the gastrointestinal tract, but its amount incorporated into the adipose tissue was very low. In the case of ginsenoside Rg1, 1.9% of the dose was absorbed, but there has been no report on its incorporation into the adipose tissue. Therefore, which of the ginsenosides are more responsible for in vivo clearance of triacylglycerol from the circulation has been unknown.

Ginsenoside Rg1 was detected in the serum as early as 15 min after oral administration to rats, and its serum concentration reached the maximum 30 min after administration and then decreased. After 6 h, ginsenoside Rg1 was not detected in the serum. Although ginsenoside Rb2 also appeared in the serum after oral administration, its serum concentration reached the maximum 6 h after administration. Ginsenoside Rb2 was detectable in the serum even 12 h after administration. Thus, ginsenoside Rg1 has more rapid turnover rate in the serum than ginsenoside Rb2. These results suggest that ginsenosides Rb2 and Rg1 may show the physiological and pharmacological activities in vivo with different courses.

There are some reports that the biological and pharmacological actions of ginsenosides vary with their aglycone structures. Mitsuma et al. reported that intraperitoneal administration of ginsenoside Rb2 to cholesterol-rich diet fed rats decreased both plasma triacylglycerol and cholesterol levels. Ginsenoside Rg1 decreased slightly, but not significantly, the plasma triacylglycerol level, and significantly increased the plasma cholesterol level. Sekiya et al. also reported the relationship between structure and function of ginsenosides. They found that 20(S)-protopanaxadiol glycosides increased the differentiation of 3T3-L1 fibroblasts into adipocytes in the presence of insulin or dexamethasone and 1-methyl-3-isobutylxanthine, but 20(S)-protopanaxatriol glycosides and an oleanolic acid glycoside did not show this action. Takimoto et al. reported that some 20(S)-protopanaxadiol glycosides, but not 20(S)-protopanaxatriol glycoside, potentiated nerve fiber production induced by nerve growth factor.

In summary, treatment of 3T3-L1 adipocytes with ginsenoside increased the secretion of lipoprotein lipase activity into the medium. An oleanolic acid glycoside and 20(S)-protopanaxatriol glycosides increased the secretion of lipase activity in a dose-dependent manner, but 20(S)-protopanaxadiol glycosides increased it only at a low concentration. Thus, the effective dose for the secretion of lipoprotein lipase activity varies with the aglycone structure.

References