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Effect of White, Taegeuk, and Red Ginseng Root Extracts on Insulin-Stimulated Glucose Uptake in Muscle Cells and Proliferation of β-cells

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Recent studies have indicated that β-cell dysfunction and insulin resistance are important factors in the development of type 2 diabetes. The present study investigated the effect of extracts from different parts of white, Taegeuk, and red ginseng root on insulin-stimulated glucose uptake in muscle cells and proliferation of β-cells. Extracts of the fine roots of Taegeuk ginseng significantly enhanced glucose uptake compared with the control. White ginseng lateral root extracts enhanced insulin-induced glucose uptake. Proliferation of β-cells was significantly increased by Taegeuk ginseng main and lateral root extracts and by red ginseng lateral and fine root extracts. In conclusion, different root parts of white, Taegeuk, and red ginseng differentially affect glucose uptake and pancreatic β-cell proliferation.

Keywords: Korean ginseng root, Insulin-stimulated glucose uptake, β-cell proliferation

INTRODUCTION

Ginseng (*Panax ginseng* C.A. Meyer) is a perennial plant belonging to the Araliaceae family, *Panax* species. It has been used alone or as a component of medication to enhance stamina and fight fatigue, and to improve digestion, nervous system function, metabolism, and circulation [1]. Recently, several experimental and clinical studies investigating the anti-stress, anti-fatigue, and anti-atherosclerotic properties of ginseng have reported its beneficial effects in strengthening immunity, fighting cancer and diabetes, controlling blood pressure, and enhancing central nervous system and sexual functions [1-5].

The effect of ginseng on diabetes has been studied for nearly 70 years, and it is well established that ginseng can mediate glucose metabolism and reduce high blood glucose levels [6]. The ginseng root is responsible for the plant's antidiabetic action, and the effect has been reported to differ among ginseng species, owing to differences in the quantity and composition of saponins, the main active compound in ginseng [7]. The quantity and chemical composition of the saponin content differ according to the species as well as the region where the ginseng is grown. The saponin content may also differ within the same species and within a plant; the ginseng root contains high levels of saponin, with the highest concentration in the fine roots, a lower concentration in the lateral roots, and the lowest concentration in the

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main root. Furthermore, saponin composition differs in different parts of the root: protopanaxadiol (PD) saponin and protopanaxatriol (PT) saponin are present in the highest ratio in fine roots. Ginseng with a high saponin content and a high PD/PT ratio has been reported to decrease blood glucose levels in an acute glucose tolerance test in humans [8,9], and fine root, but not main root, extracts of Korean ginseng reduced postprandial blood glucose levels [10]. However, Chung *et al.* [11] reported better hypoglycemic activity of red ginseng, which has a PD/PT ratio lower than that of white ginseng or American ginseng, in an animal study. These findings contradict the results of clinical trials reported by Vuksan *et al.* [12].

The principle active components in ginseng are saponin types of glucosides referred to as ginsenosides. These molecules have a dammarane triterpenoid structure. More than 150 different ginsenosides have been identified and isolated from the roots, stems, leaves, fruits, and flowers of ginseng plants, with more than 30 types in the ginseng root alone [13,14]. Additionally, ginseng contains polysaccharides, which have anticancer and antidiabetic effects and boost the immune system; polyacetylene, which has anticancer effects; phenolic-type substances, which have antioxidant properties; gomisin, which has liver-protective effects; and acidic peptides, which have insulin-like activity [15].

The measured saponin content of ginsengs can differ depending on the method used to process the raw ginseng. In particular, the steaming process used to produce Red ginseng induces chemical changes in some of the components, giving rise to new physiologically active compounds that do not exist in fresh or white ginseng [16]. For example, white ginseng contains an abundant quantity of saponins that have a malonyl residue attached to the glucose unit of the ginsenosides Rb2, Rc, Rd, and Re, whereas the malonyl residue is hydrolyzed upon steam treatment and thus is absent from processed red ginseng [15]. The production of black ginseng, which involves multiple steaming and drying stages, has recently been attempted, with subsequent analysis of the components and physiological activity [17,18]. Ginsengs have been proposed to mediate antidiabetic effects by stimulating insulin secretion, stimulating glucose uptake by adipose tissue and muscle, and inhibiting intestinal absorption of glucose [19]. Further studies are needed to elucidate the underlying molecular mechanisms of ginseng's antidiabetic actions.

The aim of the present study was to investigate the stimulation of insulin activity or the exertion of insulin-like effects by the extracts of fine, lateral, and main roots of white, Taegeuk, and red ginsengs, by evaluating the effects of the extracts on glucose absorption by muscle cells and proliferation of pancreatic β-cells.

MATERIALS AND METHODS

Processing of ginseng

Ginseng extracts were made from 5-year-old raw ginseng grown in the same area. White ginseng was prepared by washing unpeeled fresh ginseng and drying it in hot wind and sunlight. Taegeuk and red ginsengs were prepared by first washing the fresh ginseng, and then steaming for 30 minutes at 96°C and 3 hours at 97°C, respectively, followed by drying in hot wind and sunlight.

Ginseng extracts preparation

Processed white, Taegeuk, and red ginseng roots of similar size were selected, and the main, lateral, and fine roots, with average diameters of 17.68 to 19.34 mM, 7.04 to 7.94 mM, and 2.23 to 2.33 mM, respectively, were separated. Each root type was pulverized into a powder, passed through a 40-mesh sieve, and extracted three times with 70% ethanol, using 10 volumes of ethanol for the first extraction and seven volumes each for the second and third extractions. In every case, a reflux condenser was used for continuous extraction at 80°C for 8 hours. The three extracts were combined, filtered, and concentrated at 60°C using a rotary vacuum evaporator. The concentrated extracts was freeze-dried and kept at 4°C until used. Prior to the experiment, the dried extracts was dissolved at an appropriate concentration and filtered through sterile filter paper (0.2 μm; Millipore Corp., Billerica, MA, USA).

Cell culture

Mouse C2C12 muscle cells and INS-1 pancreatic β-cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and RPMI culture medium, respectively, with 10% fetal bovine serum, 50 U/mL penicillin, and 50 mg/mL streptomycin, at 37°C in 5% CO_2.

Muscle cell differentiation

The C2C12 cells were grown to 80% confluence on a 10-cm plate and then inoculated onto 24-well plates, at 5×10^4 cells/well. After incubation for 2 days, muscle cell differentiation was induced with DMEM containing 2% bovine calf serum and 25 mM glucose, and the cells were used for experiments 3 to 4 days later.

Glucose uptake

Differentiated C2C12 cells were cultured for 12 hours in DMEM containing 0.1% free fatty acid-free bovine serum albumin and ginseng extracts. The cells were washed twice with phosphate-buffered saline and cultured for 30 minutes in KRPH buffer (20 mM HEPES-NaOH, pH 7.4, 134 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, and 0.1% bovine serum albumin) to deplete glucose, followed by the addition of 50 nm insulin and incubation for another 30 minutes. The cells were exposed to [^{3}H]2-deoxyglucose (0.1 μCi) for 10 minutes, and the reaction was terminated by the addition of ice-cold phosphate-buffered saline. The cells were lysed with 0.2 N NaOH, and [^{3}H]2-deoxyglucose uptake into the cells was measured using a beta scintillation counter.

β-cell proliferation assay

INS-1 β-cells were inoculated onto a 96-well plate, at 1×10^3cells/well/100 μL, and cultured in the presence of ginseng extracts for 24 hours. The cells were then incubated for 3 hours with 10 μL of Cell Counting Kit-8 (CCK-8) reagent (Dojindo, Kumamoto, Japan), and the absorbance at 450 nm was measured using a microplate reader (ELISA reader, Softmax Pro 5.2).

Statistical analysis

The SPSS ver. 10.0 (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical tests. Analysis of variance with a Bonferroni correction was used to determine statistical significance. Results are expressed as means±SD, and p-values<0.005 were taken to indicate statistical significance.

RESULTS AND DISCUSSION

Effect of ginseng extracts on glucose uptake in muscle cells

To determine whether the ginseng root extracts had insulin-like activity, we measured glucose uptake in muscle cells after incubation for 12 hours with 1 or 10 μg/mL of each root extracts (Fig. 1A). Control cells treated only with insulin showed a 37% increase in glucose uptake, whereas glucose uptake did not increase in muscle cells treated with white ginseng extracts. The Taegeuk ginseng fine root extracts produced a 57% increase in glucose uptake, which was significantly greater than that with insulin alone ($p=0.002$), but the lateral and main root extracts had no significant effect on glucose uptake. The red ginseng main root extracts increased glucose uptake by 35%, but the effect was not statistically significant.

The effects of the root extracts co-administered with insulin on muscle cell glucose uptake are shown in Fig. 1B. Compared with insulin alone, white ginseng lateral root extracts co-administered with insulin significantly increased glucose uptake in muscle cells (1 μg/mL: 1.47-fold, $p=0.059$; 10 μg/mL: 1.67-fold, $p=0.0003$). Taegeuk ginseng fine root extracts co-administered with insulin also increased glucose uptake compared with insulin alone. Although red ginseng main root extracts co-administered with insulin increased glucose uptake, the increase was not statistically significant. These results indicate that different root parts produce different effect on glucose uptake in C2C12 muscle cells.

Compared with insulin, the extracts of white and red ginseng fine roots had no or only a marginal effect on glucose uptake, whereas Taegeuk ginseng fine root extracts had a greater effect than insulin, suggesting that the method of ginseng processing also influences the effect of the ginseng root extracts on glucose uptake. Taegeuk ginseng fine root, which was processed using a short steaming time, was more effective in stimulating glucose uptake than was the main root of red ginseng, which was processed with a longer steaming time.

The glucose uptake with Taegeuk ginseng fine root extracts co-administered with insulin was similar with the extracts alone. Furthermore, the co-administration of white ginseng lateral root extracts and insulin significantly increased glucose uptake, whereas white ginseng extracts alone was not effective. The ginseng-induced enhancement of insulin-induced glucose uptake in muscle cells may be the result of an increase in muscle cell sensitivity to insulin, possibly owing to modulation of gene expression or signaling involved in glucose uptake.

Sievenpiper *et al.* [10] reported that in a clinical study, a fine root extracts of red ginseng was more effective than main root and whole root extracts in stimulating glucose uptake. An increase in glucose uptake by muscle cells can decrease the blood glucose level. However, in the present study, Red ginseng did not significantly increase glucose uptake by the muscle cell line. The red ginseng-induced decrease in blood glucose reported by Sievenpiper *et al*. [10]. might have occurred via non-muscle tissue glucose uptake, a different physiological mechanism, or the effect of a different active component present in their red ginseng as a result of the manufacturing process used by Sievenpiper *et al*. [10].

Effect of ginseng extracts on β-cell proliferation

The effect of ginseng root extracts on β-cell proliferation was determined by treating INS-1 β-cells with

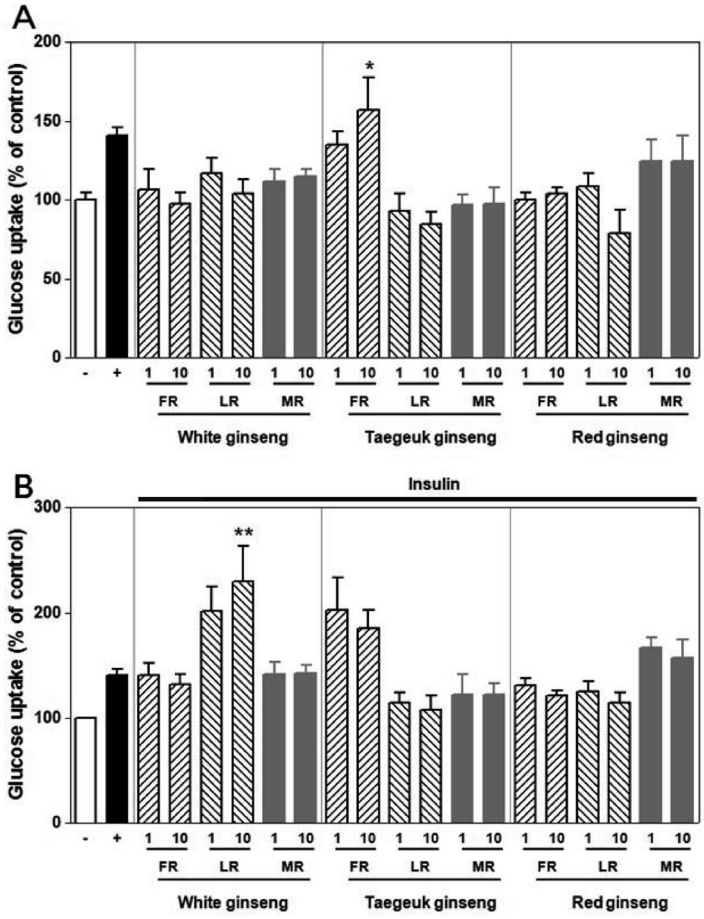

Fig. 1. Effect of white, Taegeuk, and red ginseng root extracts on insulin-induced glucose uptake in C2C12 myotubes. C2C12 cells were incubated with 1 or 10 μg/mL of ginseng root extracts for 12 hours. The medium was replaced with KRPH buffer (A) or KRPH buffer containing 50 nM insulin (B), and the cells were incubated for 30 minutes. Glucose incorporation into the cells was measured. The results are expressed as percentage of control (untreated). Data are the means±SD of three independent experiments. FR, fine root; LR, lateral root; MR, main root. *$p < 0.005$, **$p < 0.001$ *vs*. insulin (–) (A) or insulin (+) (B).

ginseng root extracts (0, 2.5, 5, 10, 20, and 40 μg/mL) for 24 hours and then measuring cell proliferation using a CCK-8 assay (Fig. 2). None of the white ginseng root extracts stimulated β-cell proliferation. Taegeuk lateral root extracts (2.5 to 40 μg/mL) significantly increased β-cell proliferation, by 6 to 8%, compared with the untreated control cells; Taegeuk main root extracts at 40 μg/mL also increased β-cell proliferation, by 8%. Red ginseng fine root and lateral root extracts, each at 2.5 μg/mL, significantly increased β-cell proliferation, and the higher extracts concentrations further stimulated β-cell proliferation (fine root extracts, 9 to 11% increase; lateral root extracts, 9 to 12% increase). The ginseng root processing method had an effect on the ability of the extracts to increase β-cell proliferation. Generally, the composition of saponins and other chemical compounds differed among the fine, lateral, and main roots, and each root type displayed unique antidiabetic properties. Chung *et al.* [11] showed that white ginseng (main root with lateral roots attached) and white fine root ginseng (lateral and fine roots) decreased blood glucose levels in KKAy diabetic male mice by 40 and 37%, respectively, compared with the levels in untreated controls ($p < 0.01$), and the decrease was not significantly different between the two root preparations. Based on our results of ginseng effect on glucose uptake in muscle cells, no difference on blood glucose levels with two root extracts might resulted from lateral roots contained in both of extracts.

The antidiabetic effect of ginseng is mediated by saponins such as G-Rb2 [20] and G-Re [21], as well as by acidic polysaccharides, adenosine, and pyroglutamic acid [22], which have anti-lipolytic activity and are separated from the water-soluble extracts comprising the insulin-like action. Panaxans such as the glucan components [23,24] and glucopeptide [25] have been reported to have an antidiabetic effect. Suzuki and Hikino [26] studied the actions of Panaxan A, B, C, D, and E and showed that the major glucans, Panaxan A and B, de-

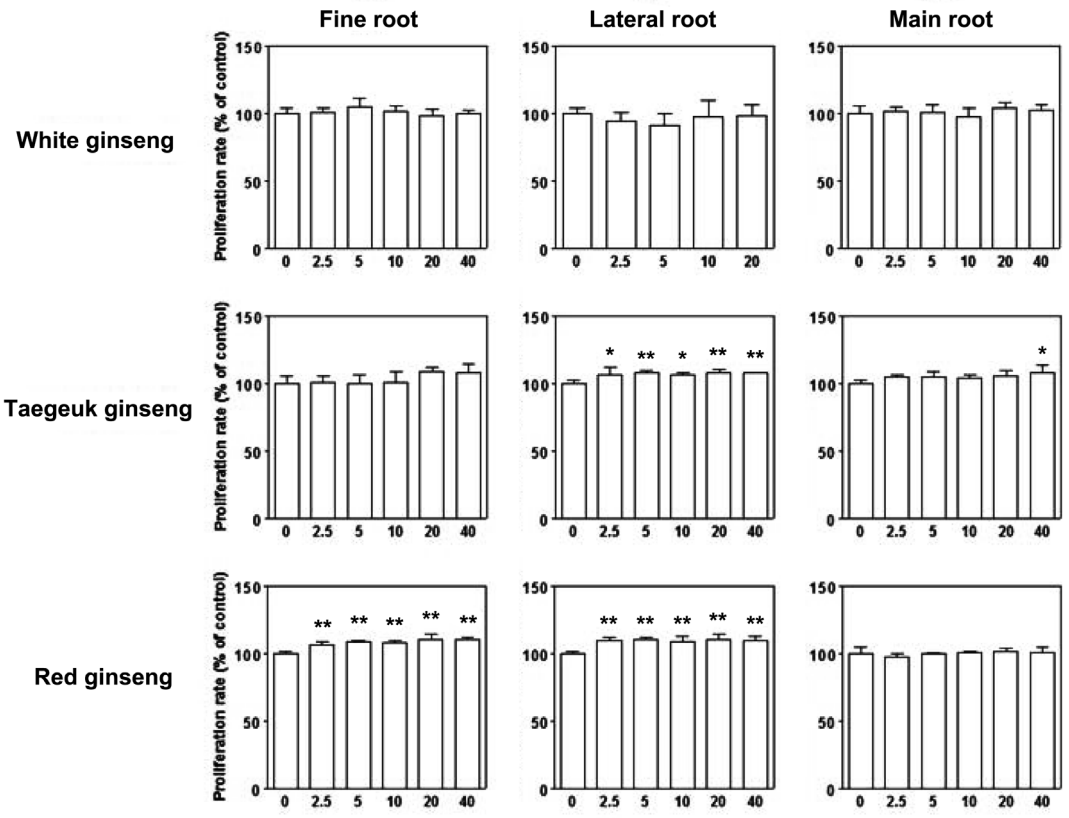

Fig. 2. Effect of white, Taegeuk, and red ginseng root extracts on the proliferation of pancreatic β-cells. INS-1 β-cells were incubated with the indicated concentrations of the ginseng root extracts for 24 hours. Cell proliferation was measured using a Cell Counting Kit-8 assay. The experiments were repeated three times, and the results are expressed as percentage of control (untreated). Data are the means±SD of three independent experiments. $*p < 0.005$ and $**p < 0.001$ *vs*. 0 μg/mL of each extract.

creased blood glucose to different levels. Although their chemical structures are similar, the glucans had different mechanisms of action: Panaxan B increased the insulin content and sensitivity, whereas Panaxan A increased glucose utilization in the liver.

A major component of red ginseng, G-Rg3, was recently reported to activate AMP-activated protein kinase, which acts as an important switch in glucose and lipid metabolism to promote insulin secretion in HIT-T15 cells [27]. Moreover, treatment of goat red blood cells with certain ginsenosides [PPT, (20R)-PPD, Rg1, Rc, Rd, Re, Rf, Rg2, Rh1, Rb1, and Rb2] stimulated the uptake of 2-deoxy-D-[^{3}H]glucose (2-DG) by glucose transporter 1, G-Rg3 inhibited 2-DG uptake, and Rd, Ro, and Rh2 did not affect 2-DG uptake [28]. Thus, the antidiabetic effect of ginseng varies according to its composition.

In the present study, the effect of ginseng extracts on glucose uptake and β-cell proliferation differed depending on which part of the root was extracted. However, we could not determine the chemical composition of each extracts and thus could not identify the components that produced the antidiabetic effect.

The chemical composition of ginseng plants grown in the same area may differ, and the antidiabetic effect of ginseng varies depending on the species, the processing method, and the part of the root used [9]. The varied pharmacological effects of ginseng may result from differences in ginsenosides, which are the principle active components in ginseng, or differences in non-saponin components. Further study of the quantity and chemical composition of the active components in ginseng extracts is necessary to fully understand the relationship between ginseng components and ginseng effects.

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