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Comparisons between White Ginseng Radix and Rootlet for Antidiabetic Activity and Mechanism in KKAy Mice

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The mechanisms responsible for the antidiabetic activity of both the white ginseng radix (Ginseng Radix Alba, GRA) and the rootlet (Ginseng Radix Palva, GRP) were investigated. After a four week oral administration, the fasting blood glucose levels in the GRA- and GRP-treated groups were lower when compared to the control group. To elucidate the hypoglycemic mechanism(s) of the ginseng radices, glucose absorption from the small intestine, hepatic hexokinase and glucose-6-phosphatase activities, in addition to PPAR-γ expression in adipose tissue were examined. The results strongly suggest that GRA can improve hyperglycemia in KKAy mice, possibly by blocking intestinal glucose absorption and inhibiting hepatic glucose-6-phosphatase, and GRP through the upregulation of adipocytic PPAR-γ protein expression as well as inhibiting intestinal glucose absorption.

Key words: Ginseng radix alba, Ginseng radix palva, Hexokinase, Glucose-6-phosphatase, PPAR-γ

INTRODUCTION

Panax ginseng, commonly known as Korean ginseng, is native to Northern China, Japan and Korea. It has been used for thousands of years as a tonic to elevate mood and reduce fatigue (Hallstrom *et al.*, 1982; Sonneborn and Proppert, 1990). Ginseng has also been reported to improve glucose homeostasis and insulin sensitivity (Sonneborn and Proppert, 1990). Although there are some reports regarding the antidiabetic activity of white ginseng, there is no experimental evidence to compare antidiabetic effects between the unprocessed ginseng root, called white ginseng (ginseng radix alba, GRA), and the rootlet of white ginseng called ginseng radix palva (GRP). The aim of this study was to compare the antidiabetic effects and mechanism(s) of GRA and GRP action using KKAy mice, a spontaneous hyperglycemic and hyperinsulinemic diabetic animal model.

MATERIALS AND METHODS

Materials

The aqueous extracts of GRA and GRP were obtained from ILHWA Central Research Institute and stored at 4° C until use.

Animals

Male KKAy mice (Clea, Tokyo, Japan) weighing 20-25 g were used. The mice were kept for 1 week in a temperature (25 ± 2°C) and moisture (50%) controlled chamber, and provided a regular laboratory chow (SamYang-Sa, Kang Won-Do, Korea) and water *ad libitum*. The animals were divided into four groups containing six to eight mice each as follows: Group 1, diabetic mice drinking tap water; Group 2, diabetic mice administered with 500 mg/kg GRA; Group 3, diabetic mice administered with 500 mg/kg GRP; Group 4, diabetic mice administered with 0.33 mg/kg rosiglitazone. During 28 days of treatment, the body weight and blood glucose level were determined once a week. The glycosylated hemoglobin and insulin levels in the blood were examined at the 28th day. On the last day of treatment, the fat and liver were removed and stored in a deep freezer until peroxisome proliferator activated receptor-γ (PPAR-γ) protein expression and the hexokinase, glucose-6-phosphatase activities were assayed, respectively.

Determination of plasma glucose and insulin

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Blood samples were obtained from the orbital venous plexus using capillary glass tubes without anesthesia. The plasma glucose was measured once per week by the glucose-oxidation method (Trinder, 1969), and the insulin levels were determined at the 28th day of treatment by a radioimmunoassay kit (Coat-A-Count Insulin, DPC, USA) using a double-antibody technique to separate the free and bound insulin.

Assay of hepatic enzymes

The hexokinase and glucose-6-phosphatase activities were determined using the methods reported by Newgard *et al.* and Baginski *et al.*, respectively.

In situ intestinal glucose absorption

According to an *in situ* intestine circulation method described by Barry *et al.* and Yoshiko, the rats starved for 16h were anesthetized by sodium pentobarbital (25-40 mg/kg, i.p.). The abdomen then was opened and the small intestine was cannulated with two tubes; one 2 cm below the duodenum and another about 60 cm from the first. The vein and the mesentery nerve were preserved. A ringer solution (mM: NaCl, 145.4; KCl, 5.4; $CaCl_2$, 1.8; $NaHCO_3$, 2.4; pH 7.5, 37°C) was circulated in the small intestine using a peristaltic pump with a current velocity of 1.5 ml/min. After this was circulated for 30 min, a 10 mM glucose-Ringer solution (23 ml) was circulated for 60 min, to determine the control glucose absorption, and after a 30 min interval with Ringer solution, a 10 mM glucose-Ringer solution (23 ml) containing either GRA or GRP extract was circulated for 60 min. 20 μl of the test solution was taken before and 15, 30, 45 and 60 min after the circulation, and the glucose concentration was measured by the glucose oxidase method using a Trinder reagent (Sigma).

Western blot analysis for PPAR-γ

To determine the PPAR-γ protein level, the adipose tissue was homogenized in an ice-cold lysis buffer containing 0.125 M Tris-HCl (pH 8.0), 3% SDS, 200 μM PMSF, 1 μM leupeptin, 1 μM pepstatin A. and the homogenate was then centrifuged at 20000 g for 20 min at 4°C. The resulting supernatant was removed and the protein concentration was determined using BSA as a standard.

The total protein sample (10 μg) was subjected to SDS-polyacrylamide on a 10% resolving gel. After the completion of electrophoresis, protein was transferred onto nitrocellulose membrane (Hybond N, Amersham Pharmacia Biotech, England) in transfer buffer (50 mM Tris-HCl, pH 7.0, 380 mM glycine, and 20% methanol). The blot was blocked in 5% nonfat dry milk dissolved in phosphate-buffered saline with 0.1% Tween 20, followed by incubation for 2 h with monoclonal mouse anti PPAR-γ antibodies (Santacruz, California, USA), followed by 1 h incubation with anti-mouse IgG conjugated to horseradish peroxidase (Bio-rad, Hercules, USA). The peroxidase luminescence analysis was performed with the enhanced chemiluminescence system (Pierce, Rockford, USA) according to the manufacturer's instructions. The relative amount of positive immunoreactive proteins was quantified with densitometric analysis using a GS-700 imaging densitometer.

Statistical analysis

All data was expressed as a mean ± S.E. and a Student's t-test was used for the statistical analysis. P values<0.05 was considered to be significant.

RESULTS AND DISCUSSION

Ginseng Radix (GR) has been reported to inhibit hyperglycemia. GR has been used to treat diabetes in clinical practice using traditional Chinese medicine. Recently Sotaniemi *et al* conducted a double-blind placebo-controlled study to evaluate the effect of ginseng on newly diagnosed type 2 (non insulin dependent diabetes mellitus) patients (Sotaniemi *et al.*, 1995). In this human trial, ginseng elevated mood, improved psychophysical performance, and reduced fasting blood glucose (FBG) and body weight.

In recent years there has been a phenomenal increase in knowledge regarding the chemical constituents of GR and its phamacological properties. Ng and Yeung reviewed the various hypoglycemic and insulin-like principles such as glycans (panaxans A to E), a peptide with a molecular weight of 1400, adenosine, a carboxylic acid and methanol- or water-soluble substances whose chemical structures have yet to be determined (Ng and Yeung, 1985). The chemical constituents of various ginseng radices have been compared and reported elsewhere (References). A dried and unprocessed white ginseng root (GRA) contains relatively large amounts of free and reduced sugar than the rootlet (GRP). However, GRP contains more than twice the concentration of saponins than GRA. Based upon the different chemical compositions between GRA and GRP, the antidiabetic activities and mechanism(s) between GRA and GRP in a spontaneous hyperglycemic and hyperinsulinemic diabetic animal model were compared.

The body weight and plasma glucose level of KKAy mice at 28th day after oral administrations of GRA and GRP are shown in Table I. Although there were no differences in body weight between the water, ginseng radices and rosiglitazone-treated groups, the plasma glucose levels in all three groups, especially in the GRA-treated group, were much lower than the control (40% for GRA,

Table I. Effects of ginseng radices and rosiglitazone on body weight and plasma blood glucose in KKA^{y} mice

	Water	GRA (500 mg/kg)	GRP (500 mg/kg)	Rosiglitazone (0.33 mg/kg)
No. of animal	8	6	6	8
Body weight (g)	40.9 ± 1.5	38.4 ± 3.6	39.0 ± 1.7	35.1 ± 4.8
Blood glucose (mg/dl)	304 ± 53	182 ± 10**	191 ± 10**	193 ± 33**

GRA, Ginseng Radix Alba; GRP, Ginseng Radix Palva
Each value represents the mean ± SD. Significantly different from the control, **P<0.01

37% for GRP, 36% for rosiglitazone; p<0.01).

Fig. 1 and 2 illustrates the effects of the ginseng radices and rosiglitazone on the individual HbA1c and insulin levels n KKAy mice, respectively. Although the HbA1c levels between the groups are not significantly different, the horizontal bar representing the mean HbA1c value in the GRA-treated group was markedly lower than those in both the control and GRP-treated groups (Fig. 1). This suggests that GRA persistently improved the hyperglycemic abnormality. Rosiglitazone, an insulin sensitizer, is known to target the nuclear PPAR-γ, which increases transcription of certain insulin-sensitive genes. As a result, rosiglitazone tends to lower the serum insulin level in a hyperinsulinemic diabetic model, as shown in Fig. 2. The GRA- and GRP-treated groups showed insulin lowering activities comparable to mice treated with rosiglitazone. Improvement in hyperinsulinemia might be clinically beneficial since insulin resistance syndrome can be resolved.

An inappropriately high rate of hepatic glucose production occurs in type 2 diabetics (DeFronzo, 1998; Nielsen *et al.*, 1990; Jeng *et al.*, 1994). Therefore, either the activation of hexokinase or the inhibition of glucose-6-phosphatase appears to be an efficient approach to pharmacologically modulate hepatic glucose production in type 2 diabetics. Therefore, the effects of the ginseng radices on both the hepatic hexokinase and glucose-6-phosphatase activities responsible for glucose metabolic pathways were examined. It has been reported that the administration of ginsenoside Rb_2 fractions to streptozotocin-induced diabetic rats resulted in increased hexokinase activity of the glycokinetic system and decreased glucose-6-phosphatase activity of the gluconeogenetic

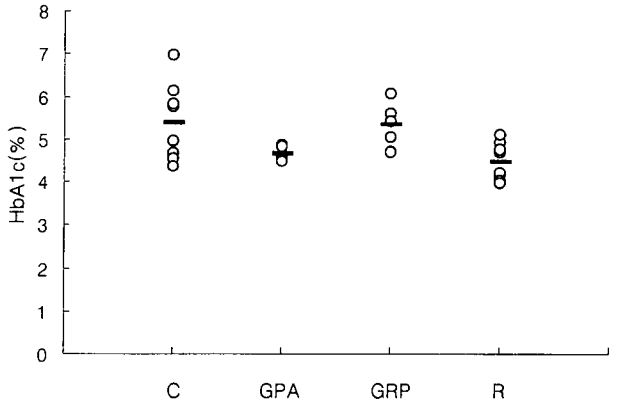

Fig. 1. Effects of ginseng radix alba (GRA), ginseng radix palva (GRP) and Rosiglitazone (R) on individual glycosylated hemoglobin level of KKAy mice. The blood was collected after 28 days of treatment without (C) or with drugs. Each circle represents an individual glycosylated hemoglobin level and the horizontal bar represents the mean value in each group.

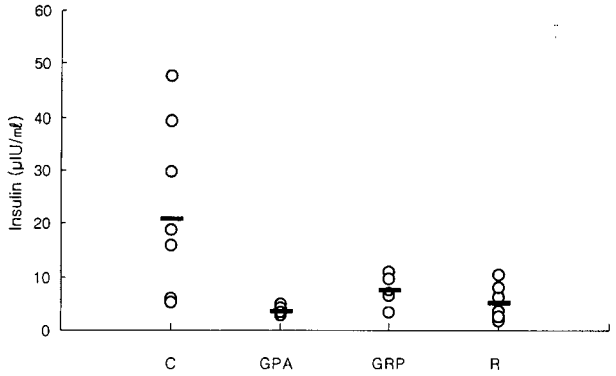

Fig. 2. Effect of GRs (GRA, GRP) and Rosiglitazone (R) on individual immunoreactive insulin (IRI) level of KKAy mice. The blood was collected after 28 days of treatment without (C) or with drugs. Each circle represents an individual immunoreactive insulin level and the horizontal bar represents the mean value in each group.

Table II. Effects of ginseng radices and rosiglitazone on hepatic hexokinase and glucose-6- phosphatase in KKA^{y} mice

Group	No. of animal	Hexokinase (mol glucose phosphorylated/mg protein/min)	Glucose-6-phosphatase (nmol phosphate/mg protein/min)
Control	8	25.7 ± 15.9	113.6 ± 2.2
GRA	6	16.0 ± 2.4	60.8 ± 5.7**
GRP	6	24.7 ± 4.7	90.4 ± 3.1*
R	8	15.9 ± 6.2	65.8 ± 8.3**

GRA, Ginseng Radix Alba; GRP, Ginseng Radix Palva; R, Rosiglitazone
Each value represents the mean ± SD. Significantly different from the control, **P<0.01; *P<0.05

system (Yokozawa *et al.*, 1985). As shown in Table II, the GRA- and GRP-treated groups markedly inhibited the hepatic glucose-6-phosphatase activity (46% and 20%, respectively; $p<0.01$). This is in agreement with Yokozawa's report except on hexokinase activity.

Fig. 3 and 4 shows the effects of the ginseng radix extracts on the *in situ* intestinal absorption in normal SD rats. GRA and GRP both exhibited inhibitory effects on glucose absorption in a concentration dependent manner. At a 5 mg/ml concentration, both extracts completely blocked glucose absorption from the small intestine. This result is consistent with Onomura *et al.* who demonstrated that GR inhibited sugar absorption in the small intestine possibly by blocking the actions of the Na^+/glucose cotransporter, SGLUT1 (Onomura *et al.*, 1999).

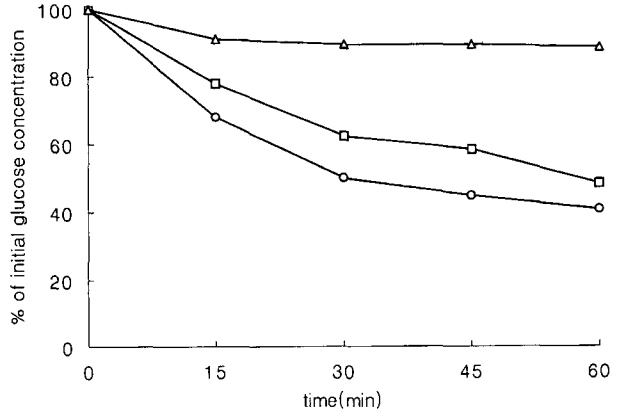

Fig. 3. Effect of ginseng radix alba on intestinal glucose absorption in SD rats. 10 mM glucose-Ringer solution without (○) or with 1 (□) or 5 mg/ml ginseng radix alba (△) was circulated in small intestine of SD rats fasted for 16 h, and the glucose concentration in the circulation fluid was measured at the indicated times.

The thiazolidinediones (TZDs), represented by rosiglitazone and pioglitazone, have recently been introduced in the USA and Korea as a treatment for type 2 diabetics (Day, 1999). These agents improve the sensitivity to insulin by binding to the nuclear peroxisome proliferator activated receptor (PPAR-γ), which acts in conjunction with the retinoid X receptor by de-repression to increase transcription of certain insulin sensitive genes (Spiegelman, 1998). In the adipose tissue, where PPAR-γ is most strongly expressed, TZDs stimulation promotes the expression of genes encoding lipoprotein lipase (LPL), the fatty acid transporter protein (FATP), the adipocyte fatty acid binding protein (aP2), fatty acyl-CoA synthase, the malic enzyme and the insulin-sensitive glucose transporter isoform GLUT4. Weaker PPAR-γ expression in the skeletal muscle and liver, and possibly other TZDs actions, facilitate the ability of TZDs to enhance actions of insulin in these tissues, collectively increasing fatty acid uptake, lipogenesis and glucose uptake, improving the glucose-fatty acid (Randle) cycle and reducing gluconeogenesis.

Reduced PPAR-γ expression may have a dominant role in producing peripheral insulin resistance in type 2 diabetics. Fig. 5 shows the effects of GRA, GRP and rosiglitazone on PPAR-γ protein expression in adipose tissue. Surprisingly, the GRP-treated group revealed the adipocytic PPAR-γ expression level to be comparable to the group treated with rosiglitazone. However, no increase in PPAR-γ protein expression was observed in the GRA-treated group.

In conclusion, the data strongly suggests that GRA can improve the hyperglycemia in KKAy mice possibly through blocking intestinal glucose absorption and inhibiting hepatic glucose-6-phosphatase. Furthermore, GRP may improve the

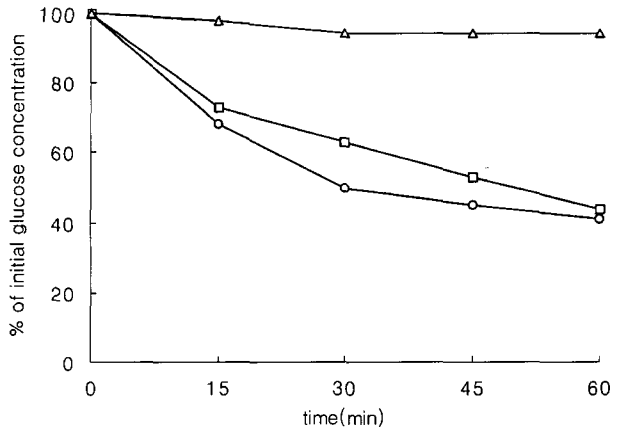

Fig. 4. Effect of ginseng radix palva on intestinal glucose absorption in SD rats. 10 mM glucose-Ringer solution without (○) or with 1 (□) or 5 mg/ml ginseng radix palva (△) was circulated in small intestine of SD rats fasted for 16 h, and the glucose concentration in the circulation fluid was measured at the indicated times.

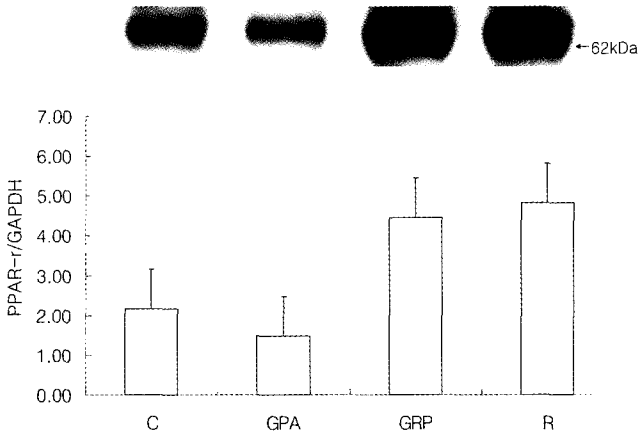

Fig. 5. Western blot analysis of the PPAR-γ protein in epididy-mal fat from the control, ginseng radix alba (GRA), ginseng radix palva (GRP) and rosiglitazone (R) treated KKAy mice. The epididymal fat was removed after 28 days of treatment without (C) or with drugs. 10 μg of protein was separated by SDS-gel electrophoresis and western blotted.

hyperglycemia in KKAy mice through the upregulation of adipocytic PPAR-γ protein expression in addition to blocking intestinal glucose absorption.

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