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Comparisons of Renoprotective Activities between White Ginseng Radix and Rootlet in Spontaneously Hypertensive Rats with Diabetes

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The renoprotective activities of white ginseng radix and rootlet were compared in spontaneously hypertensive rat (SHR) with diabetes. During oral administration of white ginseng radix (Ginseng Radix Alba, GRA) and white ginseng rootlet (Ginseng Radix Palva, GRP) for four weeks, arterial blood pressure and blood glucose levels were determined at every 10 days. In both GRA- and GRP-treatment groups, arterial blood pressures started to go down after 10 days of administration and maintained throughout the study period. After four weeks administrations of GRA and GRP, diastolic blood pressures were significantly decreased with 17% and 9%, respectively. GRA treatment also decreased blood glucose levels after 10 days of administration when compared with diabetic SHR group. At the end of the experiment, serum creatinine (Scr) and blood urea nitrogen (BUN) were not significantly different between the groups, except 62% higher value of BUN in diabetic SHR group when compared with SHR group. In the diabetic SHR group, the excretion of urinary albumin was increased significantly when compared with SHR. The level of urinary albumin in GRA treated group was markedly reduced when compared with diabetic SHR group (67.8 ± 4.7 *vs.* 131.3 ± 13.5 mg/24 h). To examine the effects of ginseng radices on an overt diabetic nephropathy, index of kidney hypertrophy and transforming growth factor-β1 (TGF-β1) protein levels were evaluated. The glomerular and tubular cells stained positive for TGF-β1 seemed to be more abundant in diabetic SHR than in those with SHR, and GRA treated rats showed somewhat less TGF-β1 protein in glomerular and tubular cells when compared with diabetic SHR. Our results suggest that GRA might be a useful antihypertensive and antidiabetic agent with renoprotective effect.

Key Words: Ginseng Radix Alba, Ginseng Radix Palva, Diabetic nephropathy, Transforming growth factor-β1, Spontaneously hypertensive rats, Urinary albumin excretion

INTRODUCTION

Panax ginseng, commonly known as Korean ginseng, is native to Northern China, Japan and Korea and has been used for thousands of years as a tonic to elevate mood and reduce fatigue (Hallstrom et al, 1982; Sonnenborn & Proppert, 1990). Han et al (1998) evaluated the changes of diurnal blood pressure pattern by 24 hour ambulatory blood pressure monitoring after 8 weeks of red ginseng medication. In 26 subjects with essential hypertension, 24 hours mean systolic blood pressure was significantly decreased, whereas diastolic blood pressure showed only a tendency of decline. Ginseng has also been reported to improve glucose homeostasis and insulin sensitivity (Sonnenborn & Proppert, 1990). Sotaniemi et al (1995) have conducted a double-blind placebo-controlled study to evaluate the effect of ginseng on newly diagnosed type 2 diabetic patients. When 36 type 2 diabetic patients were treated for 8 weeks with ginseng (100 or 200 mg), fasting blood glucose and weight were reduced, and 200 mg dose of ginseng improved glycated hemoglobin when compared with placebo group. Recently, Vuksan et al (2000) also demonstrated that 3 g per day American ginseng reduced postprandial glycemia in 10 type 2 diabetic individuals.

Although there are some reports on the antihypertensive or antidiabetic activity of ginseng, there has been no experiment to compare renoprotective activities 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 the present study was to compare the effects of GRA and GRP on renal functions in spontaneously hypertensive rat (SHR) with diabetes.

Due to the potential roles in inducing cellular differentiation and/or proliferation, transforming growth factor-β (TGF-β) has attracted much attention in diabetic research (Zhang et al, 1991; Flyvbjerg, 1997). TGF-β have been shown to increase synthesis of extracellular matrix pro-

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ABBREVIATIONS: SHR, spontaneously hypertensive rat; GRA, Ginseng radix alba; GRP, Ginseng radix palva; Scr, serum creatinine; BUN, blood urea nitrogen; TGF-β1, transforming growth factor-β1; UAE, urinary albumin excretion.

teins, and simultaneously to block matrix degradation and modulation of extracellular matrix metabolism (Lawrence, 1995). Among three isoforms of TGF-β, renal expression of TGF-β1 protein was immunohistochemically compared between the groups In addition, urinary albumin excretion and index of kidney hypertrophy were compared to elucidate the effect of ginseng radices on diabetic nephropathy present in streptozotocin (STZ)-induced diabetic SHR.

METHODS

Plant material

Specimens of *Panax Ginseng* C.A. Meyer (Araliaceae) were collected from Kumsan region (Korea) in November, 1999, and identified by Dr. S.H. Park (Head of ILHWA Central Research Institute). Air-dried GRA and GRP were extracted with hot water and subjected to freeze dry, and stored in deep freezer until use.

Experimental design

Male SHR rats (Clea, Tokyo, Japan), weighing 100~150 g, were used. The rats were kept for 1 week in a temperature (25±2°C) and moisture (50%) controlled chamber, and fed *ad libitum* a regular laboratory chow (Samyangsa, Kangwon-Do, Korea) with ready access to water. Diabetic SHR was induced by intraperitoneal injection of SHR with 70 mg/kg streptozotocin (STZ), freshly dissolved in citrate buffer (pH 4.5). Three days after the injection of STZ, blood glucose levels were determined. STZ-induced SHR with a blood glucose level above 250 mg/dl were considered to be diabetic and used in this study.

Animals were divided into four groups, each containing six rats, as follows: Group 1, hypertensive rats drinking tap water (SHR); Group 2, diabetic hypertensive rats drinking tap water (DIABETIC SHR); Group 3, diabetic hypertensive rats administered with 500 mg/kg of GRA (GRA); Group 4, diabetic hypertensive rats administered with 500 mg/kg of GRP (GRP).

Arterial blood pressure and heart rate were determined by non-invasive tail-cuff and pulse transducer system (LE 5002 Pressure Meter, Barcelona, Spain) (Krege et al, 1995). Rats were put in a restriction cage that was placed in a 37°C heated chamber for 15 min. Animals were habituated to the procedure by at least 5 cycles of inflation and deflation. Thereafter, 5 measurements were performed with each animal. The measurement involved several steps, performed automatically in sequence-the cuff pressure increases until the pulse disappeared (SBP) and then decreased until the signal intensity recovered to the initial level (DBP). The mean blood pressure (MBP) was defined as MBP=DBP+0.33 (SBP-DBP). Measurements were performed during the activity period of rats (09.00~12.00 h) at every 10^{th} day.

Blood samples were obtained from the orbital venous plexus using capillary glass tubes without anesthesia. Plasma glucose was measured at every 10^{th} day by the glucose-oxidation method (Trinder, 1969).

Parameters

Rats were transferred to metabolic cages for 24 h and urines were collected for determination of albumin using commercial kit (Yeongdong Pharmaceutical Co., Korea). Serum creatinine was measured by modified Jaffe method (Verhoeven et al, 2000), and blood urea nitrogen levels were measured by enzymatic method with urease using Urea N-E kit (Yeongdong Pharmaceutical Co, Korea).

Immunohistochemistry

Anesthetized animals were perfused with 0.05 mol/L phosphate-buffered saline (PBS, pH 7.4) and subsequently with 4% formaldehyde in PBS. The kidneys were removed, postfixed for 48 h, sectioned at 10 μm on a freezing microtome, and processed for immunohistochemistry. Endogenous peroxidase was blocked with 1% hydrogen peroxide in PBS for 15 min followed by PBS wash and then incubated with rabbit polyclonal anti TGF-β1 antibody (Santa Cruz Biotechnology, USA) at a 1 : 250 dilution overnight at 25C. Slides were washed and incubated with anti-rabbit biotinylated secondary antibody (Vector Laboratories, USA) at a 1 : 300 dilution for 1 h. After further washes with PBS, Avidin Biotin Complex (Vector Laboratories, USA) was applied for 1 h. After washing, sections were developed in 0.01% DAB (3,3'-diaminobenzidine tetrahydrochloride) for 5 min and rinsed in PBS. Slides were dehydrated in alcohol and xylene, and mounted using the Neo-mount (Merck, Germany).

Statistical analysis

All data were expressed as mean±S.E.M. Student's t-test was used to determine significant differences between groups. Mean values were considered significantly different when $P<0.05$.

Table 1. Effect of GRA and GRP on arterial blood pressure and heart rate in SHR with diabetes after 4 weeks of administration

Group	HR (beat/min)	SB (mmHg)	DBP (mmHg)	MBP (mmHg)
SHR	395.7±28.7	183.2±4.4	120.7±7.1	141.3±8.3
DIABETIC SHR	404.8±39.1	188.8±6.0	115.2±8.1	139.5±7.2
GRA	428.6±39.8	166.4±5.6*	95.2±4.6†	118.7±3.5*
GRP	406.4±39.9	169.7±6.7*	99.9±9.0†	122.9±10.6

SBP, Systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. *$P<0.05$, †$P<0.01$ compared to DIABETIC SHR group

RESULTS

Blood pressure

Oral administrations of GRA and GRP at a dose of 500 mg/kg for 4 weeks to diabetic SHR induced a significant decrease in arterial blood pressure (Table 1). In GRA- and GRP-treated groups, arterial blood pressures started to decrease after 10 days of administration and maintained throughout the study period. However, there was no significant difference in antihypertensive activities between GRA- and GRP-treated groups.

In diabetic SHR, administration of GRA for 4 weeks caused a significant fall in diastolic blood pressure from 115.2±8.1 mmHg to 95.2±4.6 mmHg, while the systolic blood pressure was decreased from 188.8±6.0 to 166.4± 5.6. The antihypertensive activity of GRP was slightly lower than that of GRA, although difference between them was not significant.

In contrast, the oral treatment of diabetic SHR with either GRA or GRP did not induce any significant changes in heart rate after 4 weeks of treatment (Table 1).

Blood glucose

Fig. 1 shows changes in blood glucose levels. Diabetic SHR treated with GRA showed glucose levels lower than diabetic SHR control group after 10 days of treatment, and maintained the hypoglycemic activity until the end of study period. GRP-treated rats also showed a trend to reduce blood glucose level after 20 days of treatment, and there was a significant decrease in glucose level after 4 weeks administration when compared with diabetic SHR group.

Renal hypertrophy

Indices of renal hypertrophy, as measured by the ratio of kidney weight to body weight, after 4 weeks administration of ginseng radices are summarized in Table 2. There was a trend for diabetic SHR to gain less weight than SHR. The average body weight of diabetic rats was 30% lower than that of SHR group, and diabetic kidney was much heavier than SHR group. The index of renal hypertrophy indiabetic rats, therefore, was elevated by 70% compared with SHR. GRA and GRP treatments increased the body weight, especially for GRA, up to the level of SHR group. In GRA treated rats, renal hypertrophy was significantly improved when compared with diabetic SHR group (9.9±1.6 *vs.* 14.6±0.9, $p<0.05$). GRP also prevented the diabetes-induced renal hypertrophy, but its antihypertropic activity was much less than that of GRA (41.7% *vs.* 78.3%).

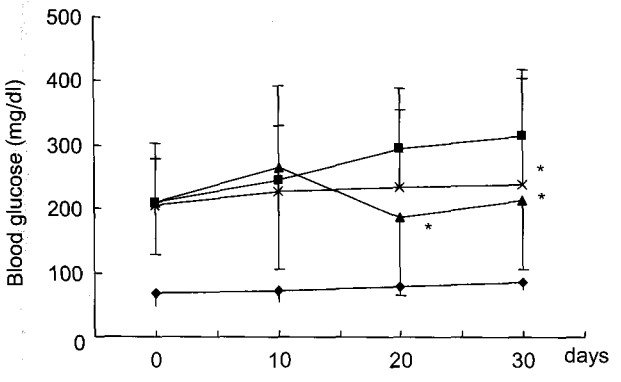

Fig. 1. Effects of Ginseng radix alba (GRA) and Ginseng radix palva (GRP) on blood glucose level of SHR with diabetes. Each point represents mean of six animals. -◆-, SHR; -■-, DIABETIC SHR; -▲-, GRA; -×-, GRP. * $P<0.1$ compared to DIABETIC SHR group.

Renal function

Effects of GRA and GRP on urinary albumin excretion, serum creatinine and blood urea nitrogen are summarized in Table 3. After 4 weeks administration of ginseng radices, there were no significant changes in serum creatinine and blood urea nitrogen between the groups, except 62% higher value of BUN in diabetic SHR, when compared with SHR. In diabetic SHR group, the excretion of urinary albumin was increased significantly when compared with SHR (131.3±13.5 *vs.* 6.8±2.6, $p<0.01$). Treatment with GRA significantly reduced the level of urinary albumin in diabetic SHR (131.3±13.5 *vs.* 67.8±4.7, $p<0.05$). However, GRP did not prevent the urinary albumin excretion in 24 h urine collections.

Immunohistochemistry

Production of transforming growth factor-β1 (TGF-β1), an important mediator of diabetic nephropathy, was measured to assess the effect of ginseng radices on expression of TGF-β1 by immunohistochemical staining. When glomerular tuft areas were compared between the groups by light microscopy, no significant difference in immunoperoxidase staining was detected between the groups after 4 weeks

Table 2. Effects of GRA and GRP on ratio of kidney to body weight in SHR with diabetes after 4 weeks of administration

Group	n	Body weight (g)	Kidney weight (mg)	Index of kidney hypertrophy (mg/g)
SHR	6	248.0±8.8	2,146±227	8.6±0.6
DIABETIC SHR	6	173.3±11.9[b]	2,523±68	14.6±0.9[c]
GRA	6	234.0±17.5*	2,290±225	9.9±1.6*
GRP	6	223.2±15.3*	2,686±162	12.1±1.4

[b]$P<0.05$, [c]$P<0.01$ compared to SHR group. *$P<0.05$ compared to DIABETIC SHR group

Table 3. Effects of GRA and GRP on UAE, SCr and BUN in SHR with diabetes after 4 weeks of administration

Group	n	UAE (mg/24 h)	SCr (mg/dl)	BUN (mg/dl)
SHR	6	6.8±2.6	0.45±0.04	17.67±2.27
DIABETIC SHR	6	131.3±13.5[b]	0.47±0.02	28.57±3.06
GRA	6	67.8±4.7*	0.45±0.08	26.69±6.62
GRP	6	129.8±6.4	0.38±0.02*	24.86±6.60

[b]$P<0.01$ compared to SHR group. *$P<0.05$ compared to DIABETIC SHR group

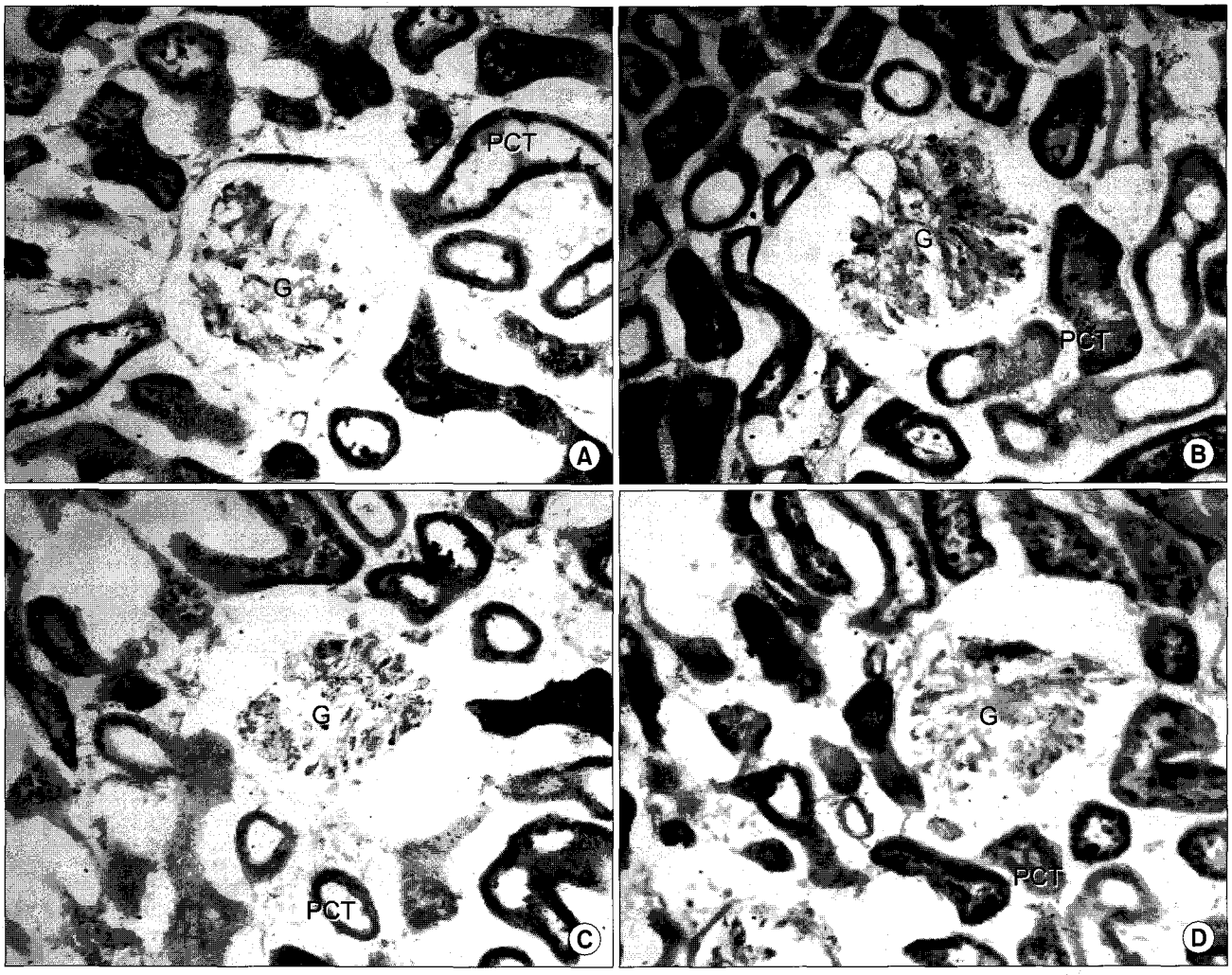

Fig. 2. Immunoperoxidase staining (brown) for TGF-β1 protein in glomeruli and tubular cells of SHR (A), DIABETIC SHR (B), GRA (C) and GRP (D). G, glomerulus, including podocytes and mesangial cells; PCT, proximal convoluted tubules.

administration of ginseng radices: 8,030±530 for SHR, 8,215±558 for diabetic SHR, 7,493±586 for GRA, 8,550± 219 for GRP. Although differences in immunoperoxidase staining between the groups could not be detected, glomerular and tubular cells stained positive for TGF-β1 seemed to be more abundant in diabetic rats (DIABETIC SHR, Fig. 2B) than in those without diabetes (SHR, Fig. 2A), and immunoreactivity of the TGF-β1 in GRA treated rats (Fig. 2C) tended to decrease slightly in the cytoplasm and nuclei of glomerular and tubular cells when compared with diabetic SHR. However, immunoreactivity of the TGF-β1 in GRP treated rats tended to increase surprisingly when compared with diabetic SHR (Fig. 2D).

DISCUSSION

Hypertension and diabetes are interrelated diseases that strongly predispose the patients to renal injuries. Cooper et al. reported that diabetic spontaneously hypertensive rats have features of accelerated nephropathy, and pre-existing hypertension may play an important role in the progression of diabetic nephropathy (Cooper & Navar, 1989).

The streptozotocin (STZ) diabetic rat model has been widely used to study diabetic renal changes (Flyvbjerg et al, 1992). STZ causes insulin deficiency in rats, and the time course and morphological changes in the kidney closely resemble the progression of human disease (Wilson & Letter, 1990), so that the changes up to 30 days of hyperglycemia in the STZ-injected SHR represent changes seen in early incipient kidney disease. In this study, we selected the spontaneously hypertensive rat (SHR) and made them diabetic by intraperitoneal administration of STZ. To the SHR with diabetes, GRA or GRP was administered orally for 4 weeks to compare their renoprotective activities.

Chemical constituents of various ginseng radices have been compared and reported elsewhere. A dried and unprocessed white ginseng root (GRA) contains relatively larger amounts of free and reduced sugar, proteins and peptides than rootlet of white ginseng (GRP), and rootlet

of white ginseng contains saponins more than twice the amount in white ginseng root. Based on the different chemical compositions between GRA and GRP, the present study was designed to examine their effects on kidney protection in spontaneously hypertensive rats with STZ-induced diabetes.

Fig. 1 and Table 1 showed that antihypertensive and antidiabetic activities between GRA and GRP were comparable and not significant different. Antidiabetic activities of GRA and GRP are in agreement with the findings of Chung et al that GRA and GRP markedly reduced blood glucose levels in KKAy mice and their antidiabetic activities were comparable (Chung et al, 2001). However, there were significant differences in renal protection activities of GRA and GRP, as far as kidney failure parameters such as renal hypertrophy, UAE and TGF-β1 protein expression were concerned. In GRA treated rats, renal hypertrophy was significantly improved up to the value of SHR (Table 2). GRA also significantly reduced albuminuria when compared with diabetic SHR group (67.8 ± 4.7 *vs.* 131.3 ± 13.5, $P<0.05$). These findings suggest that reduction in proteinuria by GRA might have been due to improvement of glomerular hypertrophy, as shown in Table 2 and Fig. 2, evidently by blood glucose lowering activity in addition to its antihypertensive effect on systemic hypertension. GRP, however, showed a somewhat less hypoglycemic activity and therefore failed to prevent the renal hypertrophy, evidently by the index of kidney hypertrophy, UAE and immunoreactivity of TGF-β1 protein expression.

In conclusion, it is evident that the administration of GRA ameliorated proteinuria and glomerular expansion in the diabetic SHR, and that GRA had renoprotective effects. Therefore, GRA might be a useful antihypertensive and antidiabetic agent with renoprotective effect.

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