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Comparison of the Effects of Korean Ginseng and Heat-Processed Korean Ginseng on Diabetic Oxidative Stress

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Abstract: To investigate the effects of Korean ginseng (KG, *Panax ginseng* C.A. Meyer) and heat-processed Korean ginseng (H-KG) on diabetic renal damage, we used the streptozotocin-induced diabetic rat model in this study. The diabetes-induced physiological abnormalities at early-stage were attenuated by KG or H-KG administration through reducing the blood glucose level and improving renal function. The oxidative stress-induced increases in serum and renal thiobarbituric acid-reactive substance levels were significantly reduced by KG and H-KG administrations. Moreover, the protein expressions related to oxidative stress and advanced glycation endproducts were significantly reduced in diabetic rats and/or not significantly increased compared to normal rats by KG or H-KG administration. All of these beneficial effects of H-KG in diabetic rats were stronger than those of KG. Therefore, KG and H-KG may improve diabetic pathological conditions and prevent renal damage associated with diabetic nephropathy, and these protective effects of KG can be improved by heat-processing. This study provides scientific evidence that H-KG may be a potential therapeutic agent for pathological conditions associated with diabetic complications including diabetic nephropathy.

Keywords: Panax Ginseng; Heat-Processed Korean Ginseng; Ginsenoside Rg_3; Ginsenoside Rk_1; Ginsenoside Rg_5; Diabetes; Renal Damage; Oxidative Stress; Advanced Glycation Endproducts.

Introduction

Korean ginseng (KG, *Panax ginseng* C.A. Meyer) has been used as a medicinal plant for more than 2,000 years, being mainly cultivated in Korea and Northeast China. KG has a wide range

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of pharmacological and physiological actions, such as antiaging, immunoenhancement, anti-stress, anti-fatigue, and anti-tumor activities (Han *et al.*, 1984; Sugaya *et al.*, 1988; Hasegawa *et al.*, 2002; Kaneko and Nakanishi, 2004). These medicinal properties of ginseng have been suggested to be closely linked to ginseng's protective effects against free radical attack (Chen, 1996; Lee *et al.*, 1999; Maffei Facino *et al.*, 1999; Kang *et al.*, 2006a). When ginseng extract was administrated to rats, myocardial ischemia-reperfusion damage induced by hyperbaric oxygen was prevented (Maffei Facino *et al.*, 1999), and ginseng extract was reported to have a hepatoprotective effect against oxidative stress induced by exhaustive exercise (Voces *et al.*, 1999). In particular, several investigations strongly support the evidence that ginseng root possesses an anti-diabetic property, such as *via* inhibition of intestinal glucose absorption, an increase in energy expenditure, improving the sensitivity to insulin, and the stimulation of sugar metabolism (Yokozawa *et al.*, 1985; Chung *et al.*, 2001; Xie *et al.*, 2005). Moreover, ginseng root has been shown in clinical studies to have beneficial effects in diabetic patients (Kwan and Wan, 1994; Sotaniemi *et al.*, 1995).

Recent studies have reported that the biological activities of ginseng are improved by heat-processing (Keum *et al.*, 2000; Kim *et al.*, 2000; Kang *et al.*, 2006a). Heat-processed ginseng has been reported to exhibit more potent pharmacological activities, such as vasorelaxation, antioxidant, and anti-tumor activities than conventional ginsengs (Keum *et al.*, 2000; Kim *et al.*, 2000). These enhanced biological activities of ginsengs were thought to be mediated by changes in the chemical constituents such as ginsenosides by heat-processing, because ginsenosides are known pharmacologically as the main active components of ginseng (Park *et al.*, 1998; Attele *et al.*, 1999; Sievenpiper *et al.*, 2004).

Diabetes mellitus is characterized by excessive glucose production. An abnormally elevated blood glucose level causes oxidative stress and the formation of advanced glycation endproducts (AGEs), which have been closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy (Baynes, 1991; Ahmed, 2005). Diabetics are at an increased risk for several types of kidney disease, and the predominant cause of end-stage renal disease in this disorder is diabetic nephropathy (Selby *et al.*, 1990; Held *et al.*, 1991). However, recent clinical trials suggest that there is no effective treatment for diabetic nephropathy without undesirable side-effects or contraindications (The Diabetes Control and Complications Trial Research Group, 1993). Therefore, great effort has been focused on traditional and herbal medicines to find a novel therapeutic agent for diabetic nephropathy without any toxic effects (Yokozawa *et al.*, 2004; Yamabe *et al.*, 2006).

Although the beneficial effects of KG on diabetes and the increase in biological activities of KG by heat-processing are well-defined as mentioned above, a comparison of the effects of KG and H-KG on diabetic oxidative stress has not yet been performed. Therefore, it was carried out in the present study using streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Reagents

Phenylmethylsulfonyl fluoride (PMSF), STZ, Nonidet P 40 (NP-40), and β-actin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dithiothreitol (DTT) was

purchased from BioVision Inc. (Mountain View, CA, USA). Bovine serum albumin (BSA), 2-thiobarbituric acid (TBA), protease inhibitor mixture in DMSO solution, 2-amino-2-hydroxymethyl-1,3-propanediol (Tris(hydroxymethyl)aminomethane), Tween-20, and skim milk powder were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nuclear factor-kappa Bp65 (NF-κBp65), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), receptors for AGE (RAGE), and goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The polyclonal antibody against N^{ε}-(carboxymethyl)lysine (CML) was kindly provided by Dr. Nagai of Kumamoto University. The other chemicals and reagents used were of high quality and obtained from commercial sources.

Plant Material and Its Heat-Processing

Commercial KG (*Panax ginseng*, four years old) was purchased from a ginseng market in Seoul (Korea). Fifty g of KG was ground to pass an 80 mesh sieve and boiled gently in 1,000 ml water 3 times for 1 hour. The solvent was evaporated *in vacuo* to give a water extract with a yield of about 20%, by weight of the original ginseng powder. KG water extract was autoclaved at 120°C and 0.11 MPa for 3 hours, and the product was dried in an oven at 50°C for 3 days to produce the heat-processed KG (H-KG).

Analysis of Ginsenosides

The ginseng extracts were dissolved in MeOH (5 mg/ml), and analyzed with a Hitachi (Tokyo, Japan) L-7100 liquid chromatograph fitted with a C-18, reversed-phase column (5 μm, 25 cm $\times$ 4 mm I.D.; YMC-Pack Pro) utilizing a solvent gradient system (Kwon *et al.*, 2001). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), and the flow rate was 1 ml/min. The detector was a SEDEX 55 ELSD (Sedere, France). The gradient elution was used as follows: 0 min, 15% B; 10 min, 34.5% B; 25 min, 47.5% B; 40 min, 80% B; and 50 min, 100% B. Ginsenosides were identified by a comparison of the retention times with those of authentic samples, which were previously isolated in our laboratory by the reported method (Oura *et al.*, 1975; Park *et al.*, 1998). Results are expressed as the average values (% of each extract) of duplicate analysis. Linearity of the detector responses was tested for all ginsenosides (5–100 μg/ml), and the coefficient of correlation was > 0.99. The relative standard deviation value of intra-day repeatability was lower than 6%, indicating good precision.

Animals and Treatments

The Guidelines for Animal Experimentation, approved by the University of Toyama, were followed in these experiments. Male Wistar rats (120–130 g) from Japan SLC, Inc. (Hamamatsu, Japan) were used. They were kept in a plastic-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 25°C) and humidity (about 60%) were controlled automatically. They were allowed free access to laboratory pellet

chow (CLEA Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipids, and 60.5% carbohydrate) and water. After several days of adaptation, STZ dissolved in citrate buffer (10 mM, pH 4.5) was injected intraperitoneally at a dose of 50 mg/kg body weight following overnight fasting. Ten days after the injection, the glucose level in the blood taken from the tail vein was determined. The rats were divided into 3 groups (8 rats/group), avoiding any inter-group differences in blood glucose levels. The control group was given water (vehicle), while the other two groups were orally administered the KG or H-KG extract at a dose of 100 mg/kg body weight daily using a stomach tube, respectively. The dose was determined based on our previous short-term toxicity assessments of KG and H-KG in rats (Kang *et al.*, 2007a). Rats that underwent a sham injection of citrate buffer without STZ were also used as a normal group ($n = 5$). After administration for 20 consecutive days, urine was collected from metabolic cages and blood samples were collected from the abdominal aorta. The serum was immediately separated from the blood samples by centrifugation. Subsequently, the renal arteries of each rat were perfused with ice-cold physiological saline (0.9% NaCl, pH 7.4), and the kidneys were removed, quickly frozen, and kept at $-80°C$ until analysis.

Assays of Serum and Urine Samples

Serum glucose and creatinine (Cr) were determined using commercial reagents (Glucose CII-Test Wako obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan; CRE-EN Kainos obtained from Kainos Laboratories Inc., Tokyo, Japan). The serum TBA-reactive substance levels were determined using a previously described method (Naito and Yamanaka, 1978). Urine components were determined as follows: protein by the sulfosalicylic acid method (Sakagishi, 1968), and Cr by a commercial reagent (CRE-EN Kainos, Kainos Laboratories Inc.). Creatinine clearance (Ccr) was calculated on the basis of urinary Cr, serum Cr, urine volume, and body weight using the following equation: Ccr (ml/min/kg body weight) = [urinary Cr (mg/dl) × urine volume (ml)/serum Cr (mg/dl)] × [1,000/body weight (g)] × [1/1,440 (min)].

Determination of Renal TBA-Reactive Substance Levels

TBA-reactive substance levels in the kidney were determined by the method of Uchiyama and Mihara (1978), and the protein level was evaluated by the micro-biuret method (Itzhaki and Gill, 1964) with BSA as the standard.

Western Blotting

Renal cortical sections were homogenized with ice-cold lysis buffer (pH 7.5) containing 137 mM NaCl, 20 mM Tris-HCl, 1% Tween-20, 10% glycerol, 1 mM PMSF, and the protease inhibitor mixture in DMSO solution. Samples were then centrifuged at 2,000 × g for 10 min at 4°C. The protein concentration in tissue was determined using a Bio-Rad protein assay kit and BSA as a standard. For Western blot analysis, each sample (30 μg protein/lane) was denatured by boiling in Laemmli sample buffer and stored at $-80°C$ until assaying.

Nuclear extract was obtained from the renal cortex in experimental rats according to the method of Rangan *et al.* (1999) with minor modifications. In brief, cortical renal tissue (100 mg) was homogenized in 200 μl of ice-cold hypotonic buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT, 1 mM PMSF, and protease inhibitor mixture in DMSO solution. Then, 65 μl of 2% (v/v) NP-40 was added and the mixture was vortexed for 1 min, incubated for 10 min, and centrifuged at 1,000 $\times$g for 10 min at 4°C. Supernatant fractions were discarded and crude nuclear pellets were rinsed twice with hypotonic buffer and resuspended in 60 μl of hypertonic buffer containing 50 mM HEPES (pH 7.9), 50 mM KCl, 300 mM NaCl, 10% (v/v) glycerol, 0.5 mM DTT, 1 mM PMSF, and protease inhibitor mixture in DMSO solution. It was then vortexed twice for 1 min each, and the mixture was centrifuged at 13,000 $\times$g for 10 min at 4°C to yield a supernatant containing extracted nuclear proteins. The protein concentration was determined as described above and equal amounts of protein (30 μg) were used in Western blot analysis with anti-NF-κB antibody.

For determination of NF-κBp65, COX-2, iNOS, CML, and RAGE protein expressions in the kidney, 30 μg of protein from each sample was electrophoresised through 8, 10, and 12% sodium dodecyl sulfate-polyacrylamide gels. Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 3 hours at 4°C, and then incubated with primary antibodies overnight at 4°C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP conjugated secondary antibody for 90 min at room temperature. Each antigen-antibody complex was visualized using ECL Western blotting detection reagents (Amersham, New Jersey, USA) and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Japan). Band densities were determined by Scion image software (Scion Corporation, Frederick, MD, USA) and quantified as the ratio to β-actin.

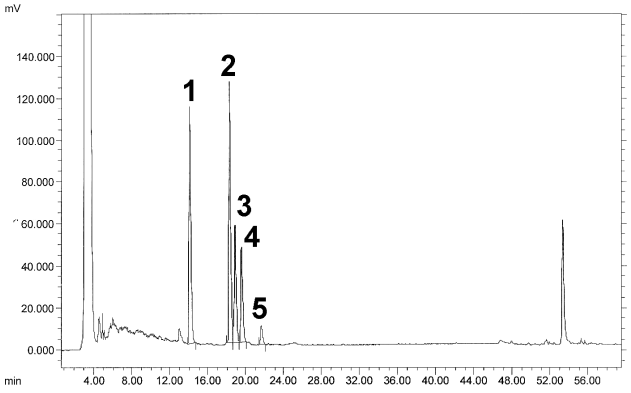
Data Analysis

The results for each group are expressed as mean $\pm$ SE values. The effect on each parameter was examined using one-way analysis of variance. Individual differences between groups were evaluated by Dunnett's test, and those with $p < 0.05$ were considered to be significant.

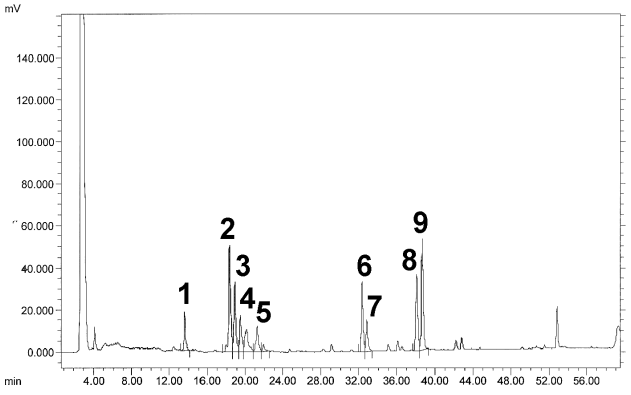
Results

Changes in Contents of Ginsenosides

Figure 1 shows the HPLC-ELSD chromatograms of KG and H-KG water extracts. The major components of KG were ginsenoside Re, Rb_1, Rc, and Rb_2. In H-KG, the contents of these polar ginsenosides (peaks 1–4) were reduced, and the contents of less-polar ginsenosides, such as ginsenoside 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 (peaks 6–9), were increased (Table 1).



(a)



(b)

Figure 1. HPLC-ELSD chromatograms of (a) Korean ginseng and (b) heat-processed Korean ginseng water extracts. (1) Re, (2) Rb_1, (3) Rc, (4) Rb_2, (5) Rd, (6) 20(*S*)-Rg_3, (7) 20(*R*)-Rg_3, (8) Rk_1, and (9) Rg_5.

Table 1. Changes in Contents of Ginsenosides

	Re	**Rb_1**	**Rc**	**Rb_2**	**Rd**	**20(*S*)-Rg_3**	**20(*R*)-Rg_3**	**Rk_1**	**Rg_5**
KG	0.32	0.34	0.17	0.15	0.04				
H-KG	0.05	0.16	0.11	0.06	0.02	0.10	0.05	0.11	0.15

Data are expressed as % of sample extract.

Changes in Physico-Metabolic Symptoms

The effects of KG and H-KG on the changes in physico-metabolic symptoms with diabetes over the experimental period are shown in Table 2. The body weight gain by STZ-induced diabetic rats was significantly lower than that of normal rats, but it was slightly increased by the H-KG administration. The increased kidney weight under diabetes was significantly reduced by the H-KG administration. The levels of food and water intake, and urine excretion

Table 2. Physico-Metabolic Symptoms

Group	Dose (mg/kg body weight/day)	Body Weight			Kidney Weight (g/100 g of body weight)	Food Intake (g/day)	Water Intake (ml/day)	Urine Volume (ml/day)
		(Initial, g)	(Final, g)	(Gain, g)				
Normal	—	213.3 ± 7.5	291.2 ± 8.2	71.8 ± 12.8	0.65 ± 0.01	18.8 ± 2.2	33.4 ± 1.9	14.1 ± 1.6
Diabetic								
Control	—	187.8 ± 4.4^{a}	210.8 ± 7.9^{b}	23.0 ± 4.1^{b}	1.09 ± 0.02^{b}	29.3 ± 1.6^{b}	156.7 ± 11.0^{b}	122.3 ± 6.3^{b}
KG	100	187.8 ± 7.6^{a}	207.7 ± 7.0^{b}	22.7 ± 5.8^{b}	1.07 ± 0.03^{b}	29.4 ± 1.3^{b}	149.8 ± 4.1^{b}	116.0 ± 4.6^{b}
H-KG	100	187.8 ± 4.0^{a}	216.0 ± 8.1^{b}	29.5 ± 8.5^{a}	$1.02 \pm 0.03^{b,c}$	29.1 ± 2.2^{b}	143.5 ± 7.7^{b}	105.5 ± 9.0^{b}

Data are expressed as the mean ± SE. $^{a}p < 0.05$, $^{b}p < 0.01$ compared with normal rats, $^{c}p < 0.05$ compared with diabetic control rats.

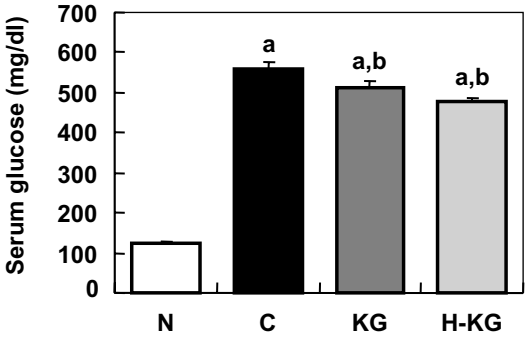


Figure 2. The effects of Korean ginseng and heat-processed Korean ginseng water extracts on serum glucose levels. N, normal rats; C, diabetic control rats; KG, diabetic rats treated with Korean ginseng (100 mg/kg body weight/day); H-KG, diabetic rats treated with heat-processed Korean ginseng (100 mg/kg body weight/day). [a] $p < 0.01$ compared with normal rats, [b] $p < 0.01$ compared with diabetic control rats.

were markedly elevated in diabetic control rats; however, there were no significant ameliorations in these parameters by KG or H-KG administration.

Biochemical Features of Serum and Urine

Figure 2 shows the effects of KG and H-KG on serum glucose levels. The diabetic control rats (561 mg/dl) showed a markedly higher blood glucose level than that of normal rats (123 mg/dl), while the elevated serum glucose level was significantly reduced to 511 and 480 mg/dl by the KG and H-KG administrations, respectively.

The effects of KG and H-KG on renal function parameters are shown in Table 3. There were no significant changes in the serum Cr level among the normal, diabetic control, and KG- or H-KG-administered groups. However, the urinary protein level was increased from 10.1 mg/day in normal rats to 13.1 mg/day in diabetic control rats, and it was significantly reduced by 100 mg/kg body weight/day of KG or H-KG administration. In addition, the slightly decreased Ccr level in diabetic control rats was significantly increased in H-KG-administered rats.

Table 3. Renal Function Parameters

Item	Normal	Diabetic		
		Control	KG (100 mg)	H-KG (100 mg)
Serum creatinine, mg/dl	0.30 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.29 ± 0.01
Urinary protein, mg/day	10.1 ± 0.8	13.1 ± 1.1^{a}	9.5 ± 0.3^{b}	8.8 ± 0.8^{b}
Ccr, ml/kg body weight/min	8.11 ± 0.73	6.89 ± 0.63	8.87 ± 0.41	9.93 ± 0.78^{b}

Data are expressed as the mean $\pm$ SE. [a] $p < 0.05$ compared with normal rats, [b] $p < 0.05$ compared with diabetic control rats.

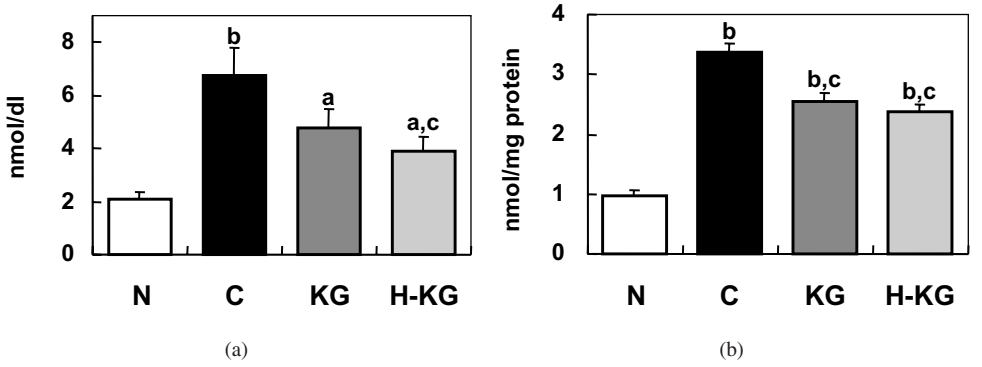


Figure 3. The effects of Korean ginseng and heat-processed Korean ginseng water extracts on (a) serum TBA-reactive substance and (b) renal TBA-reactive substance levels. N, normal rats; C, diabetic control rats; KG, diabetic rats treated with Korean ginseng (100 mg/kg body weight/day); H-KG, diabetic rats treated with heat-processed Korean ginseng (100 mg/kg body weight/day). $^{a}p < 0.05$, $^{b}p < 0.01$ compared with normal rats, $^{c}p < 0.01$ compared with diabetic control rats.

TBA-Reactive Substance Levels of Serum and Kidney

TBA-reactive substance levels of the serum and kidney were significantly increased under diabetes (Fig. 3). The elevated TBA-reactive substance level in the serum was slightly reduced by the administration of KG, but showed a significant decrease by H-KG administration (Fig. 3a). In addition, the elevated TBA-reactive substance levels in the kidney of diabetic rats were significantly reduced by the administrations of KG and H-KG (Fig. 3b).

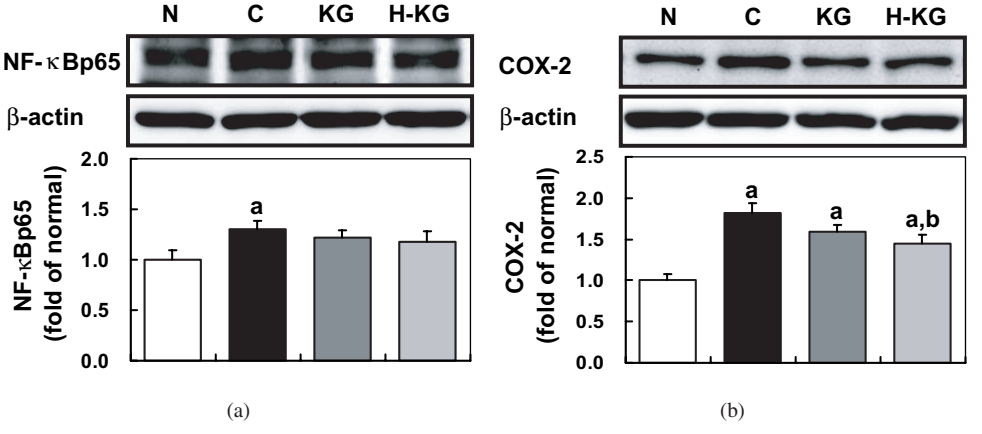
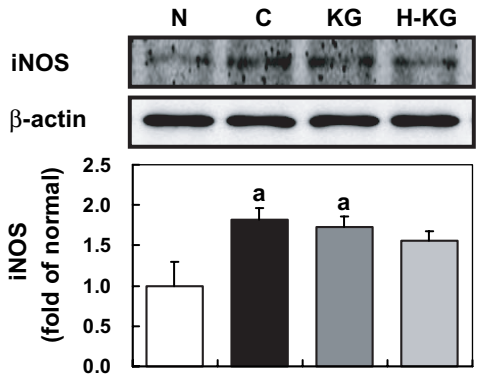


Figure 4. The effects of Korean ginseng and heat-processed Korean ginseng water extracts on (a) NF-κBp65, (b) COX-2, and (c) iNOS protein expressions. N, normal rats; C, diabetic control rats; KG, diabetic rats treated with Korean ginseng (100 mg/kg body weight/day); H-KG, diabetic rats treated with heat-processed Korean ginseng (100 mg/kg body weight/day). $^{a}p < 0.01$ compared with normal rats, $^{b}p < 0.01$ compared with diabetic control rats.



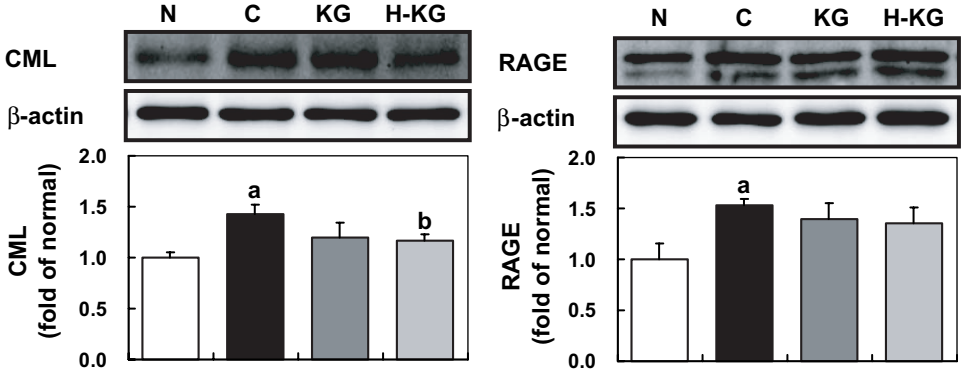
(c)

Figure 4. (*Continued*).

Western Blotting

The expressions of proteins related to oxidative stress-induced damage in renal tissue are shown in Fig. 4. These protein band intensities were graphed and corrected by β-actin. There were significant increases in NF-κBp65, COX-2 and iNOS protein expressions in diabetic rats compared to normal rats. The elevated COX-2 protein expression was significantly decreased by H-KG administration. There were no significant reductions in NF-κBp65 and iNOS levels by KG or H-KG administration.

Figure 5 shows the protein expressions related to AGE formation in the renal cortex. CML accumulation and RAGE expression in diabetic control rats were significantly higher



(a) (b)

Figure 5. The effects of Korean ginseng and heat-processed Korean ginseng water extracts on (a) CML and (b) RAGE protein expressions. N, normal rats; C, diabetic control rats; KG, diabetic rats treated with Korean ginseng (100 mg/kg body weight/day); H-KG, diabetic rats treated with heat-processed Korean ginseng (100 mg/kg body weight/day). $^{a}p < 0.01$ compared with normal rats, $^{b}p < 0.01$ compared with diabetic control rats.

than those in normal rats. The elevated CML level was significantly reduced by H-KG administration; however, there was no significant decrease in the elevated RAGE expression of diabetic rats by the ginseng administrations.

Discussion

When glucose and other reactive carbonyl compounds react non-enzymatically with proteins, lipids, nucleic acids, Schiff bases, and Amadori products are formed. Additional rearrangement and modification leads to the generation of diverse AGEs, and these AGEs can alter the structure and function of intra- and extra-cellular molecules, increase oxidative stress, and modulate cell activation, signal transduction, and the expression of cytokines and growth factors through receptor-dependent and receptor-independent pathways. Reciprocally, oxidative stress is known to induce AGEs (Cooper, 2001; Wendt *et al.*, 2003; Williams, 2003; Schrijvers *et al.*, 2004). Therefore, the inhibitors of AGEs and oxidative stress have received considerable interest because of their close relation to the prevention of diabetic complications.

H-KG is different from KG by virtue of its increased contents of less-polar ginsenosides such as Rg_3, Rk_1, and Rg_5, as shown in Fig. 1. The antioxidant and anti-tumor-promoting activities of KG were improved by heat-processing, and these improved biological activities were suggested to be mediated by the roles of less-polar ginsenosides (Park *et al.*, 1998; Keum *et al.*, 2000; Kim *et al.*, 2000). However, a comparison of the effects of KG and H-KG on diabetic oxidative stress has not been conducted to date. Therefore, we conduct this study using STZ-induced diabetic rats. The destruction of pancreatic β-cells and disorder of insulin secretion induced by STZ injection causes physico-metabolic abnormalities such as a decrease in body weight gain and increases in kidney weight, food intake, water intake, and urine volume (Yokozawa *et al.*, 2004; Yamabe *et al.*, 2006). The STZ-induced diabetic rats in this study also showed these changes. Although there were no significant ameliorations in these physico-metabolic abnormalities by KG administration, the ameliorating tendencies were stronger in H-KG-administered groups and they showed a significant reduction in kidney weight (Table 2). The increase in kidney weight in proportion to body weight indicates renal hypertrophy, which is one of the features of early-stage diabetic renal change and is related to increased urinary protein due to diabetes (Schrijvers *et al.*, 2004). Therefore, H-KG was thought to have beneficial effects on attenuating these diabetes-induced physiological abnormalities, and this effect can be improved by heat-processing of KG.

Intensive therapy of the blood glucose level in patients with type 1 diabetes has been emphasized to delay the onset and slow the progression of diabetic complications (The Diabetes Control and Complications Trial Research Group, 1993). As shown in Fig. 2, the elevated blood glucose levels in diabetic rats were significantly decreased in those fed with KG or H-KG at a dose of 100 mg. The hypoglycemic effect of ginseng has been suggested to be mediated by the delayed glucose absorption in the gut, increased glucose uptake/disposal, and glucose-stimulated insulin secretion. Among the various pharmacological active components of ginseng, the standardization of the ginsenoside profile has received much attention to achieve a reproducible hypoglycemic effect (Sievenpiper *et al.*,

2004; Vuksan and Sievenpiper, 2005). Therefore, the improved hypoglycemic effect of KG by heat-processing was thought to be related to the increased contents of less-polar ginsenosides such as Rg_3, Rk_1, and Rg_5.

Over the experimental period, the levels of urinary protein were significantly elevated in diabetic rats, indicating the changes in the capillary filtration barrier that result in the increased permeability of the glomerular basement membrane. However, there was a slight, but not significant, decrease in CCr of diabetic rats compared to normal rats. In patients with diabetes and/or renal failure, CCr, which is an effective index for glomerular filtration rate, decreases exponentially, and it eventually causes nephritic syndrome (Bell, 1991). Based on these results and notions, it was thought that the early-stage diabetic renal changes occurred (not advanced-stage changes) in this study because of the slight decrease of CCr in diabetic control rats. However, the administration of KG or H-KG significantly reduced the elevated levels of urinary protein caused by diabetes. Moreover, H-KG significantly increased the lowered CCr level in diabetic rats (Table 3). Therefore, the renal dysfunctions in this early-stage diabetic rats were ameliorated by administrations of KG and H-KG, and more significantly by H-KG.

Free radical reactions lead to lipid peroxidation that is mainly responsible for cell and tissue damages. A significant increase in TBA-reactive substances as an index of endogenous lipid peroxidation has been noted in diabetic conditions. In addition, the measurement of TBA-reactive substances is frequently used to determine the oxidative stress level in diabetic patients (Altomare *et al.*, 1992; Gallou *et al.*, 1993; Turk *et al.*, 2002). As shown by present study, the levels of TBA-reactive substance in serum and kidney of diabetic rats were significantly increased, whereas the administration of KG or H-KG water extract significantly decreased these TBA-reactive substance levels compared to diabetic control rats (Fig. 3). Therefore, the administration of KG or H-KG was suggested to alleviate oxidative stress of diabetic pathological conditions through the inhibition of lipid peroxidation.

Subsequently, protein expressions of NF-κBp65, COX-2, iNOS, CML, and RAGE, which were related to oxidative stress, and AGE formation in the renal cortex were investigated using Western blot analyses. NF-κB is normally present in the cytoplasm of eukaryotic cells as an inactive complex with the inhibitory protein, IκB. When cells are exposed to various external stimuli, such as reactive oxygen species or AGEs, IκB undergoes rapid phosphorylation with subsequent ubiquitination, leading to the proteosome-mediated degradation of this inhibitor. The functionally active NF-κB exists mainly as a heterodimer consisting of subunits of the Rel family (e.g., Rel A or p65, p50, p52, c-Rel, v-Rel, and Rel B) and translocates to the nucleus, where it binds to specific consensus sequences in the promoter or enhancer regions of target genes, thereby altering their expression (Surh *et al.*, 2001; Ahmed, 2005). In addition, NF-κB is involved in the regulation of COX-2 and iNOS expressions, which are known to be involved in the pathogenesis of many chronic diseases associated with oxidative stress. These protein expressions are known to be significantly increased in the kidneys of STZ-induced diabetic rats or mice (The Diabetes Control and Complications Trial Research Group, 1993). Our results also showed a significant increase in NF-κBp65, COX-2, and iNOS protein expressions in diabetic kidneys. Although there were no significant reductions

in NF-κBp65 and iNOS protein expressions by KG administration, H-KG administration reduced over-expressed COX-2 levels significantly (Fig. 4). However, NF-κBp65 protein expression was not significantly increased in KG- and H-KG-administered groups when compared to the normal rats. Meanwhile, iNOS level in H-KG-administered diabetic rats was not significantly increased compared to the normal rats. These results imply that H-KG may attenuate oxidative stress by preventing the increases of iNOS and COX-2 protein expressions through the deactivation of NF-κB during diabetes.

CML, one of the major AGEs in human tissues, is known to be a marker of cumulative oxidative stress and to be involved in the development of diabetic nephropathy (Horie *et al.*, 1997; Nagai *et al.*, 2002). Moreover, activation of RAGE by CML results in the activation of NF-κB and production of proinflammatory cytokines (Yan *et al.*, 1994; Ahmed, 2005). In the present study, CML accumulation and RAGE expression in diabetic rats were markedly higher than normal, but the elevated CML level was significantly ameliorated in H-KG-administered groups (Fig. 5). In addition, the CML and RAGE expressions of KG- or H-KG-administered diabetic rats were not significantly different from the normal rats. These findings imply that H-KG can prevent diabetic nephropathy *via* inhibiting AGE generation in the diabetic kidney.

Ginseng saponins, referred to as ginsenosides, are believed to play an important pharmacological role (Attele *et al.*, 1999). The HPLC chart in Fig. 1 shows that the amounts of polar ginsenosides such as Re, Rb_1, Rc and Rb_2 were decreased, but those of less-polar ginsenosides such as Rg_3, Rk_1, and Rg_5 were newly formed in H-KG. Among the ginsenosides, ginsenoside Rg_3 is known to be as the strongest $\bullet$OH-scavenging compound in heat-processed *Panax ginseng* and its content was significantly increased by heat processing (Kang *et al.*, 2006b; Kang *et al.*, 2007b). In addition, the potential antioxidant and anti-inflammatory effects of Rg_3 have been reported (Keum *et al.*, 2003; Tian *et al.*, 2005). Therefore, the fortified effects of H-KG compared to KG in diabetic rats may be explained by the chemical transformation of ginsenosides by heat-processing.

In summary, this study demonstrated that KG and H-KG ameliorate early-stage diabetes-induced physiological abnormalities through reducing the blood glucose level and improving renal dysfunction. The oxidative stress-induced increases of TBA-reactive substance levels in serum and kidneys were significantly reduced by KG or H-KG administrations. Moreover, the expressions of proteins related to oxidative stress and AGEs were significantly reduced in diabetic rats and/or not significantly increased from the normal rats by KG or H-KG administrations. All these beneficial effects of H-KG in diabetic rats were stronger than those of KG. These improved effects by heat-processing were thought to be related to the increased contents of less-polar ginsenosides such as Rg_3, Rk_1, and Rg_5. Therefore, KG and H-KG may improve diabetic pathological conditions and prevent renal damage associated with diabetic nephropathy, and these protective effects of KG can be improved by heat-processing. This study provides scientific evidence to support the use of H-KG as a potential therapeutic agent for pathological conditions associated with diabetic complications including diabetic nephropathy. Further studies on the effects of less-polar ginsenosides on diabetic renal damage are underway for the elucidation of their roles in H-KG.

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