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Protective Effect of Heat-Processed American Ginseng against Diabetic Renal Damage in Rats

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We investigated the effects of American ginseng (AG) and heat-processed American ginseng (H-AG) on diabetic renal damage using streptozotocin (STZ)-induced diabetic rats in this study. The diabetic rats showed a loss of body weight gain, and increases in kidney weight, food intake, water intake, and urine volume, whereas the oral administration of H-AG at a dose of 100 mg/kg of body weight per day for 20 days attenuated these diabetes-induced physiological abnormalities. Among the renal function parameters, the elevated urinary protein levels in diabetic control rats were significantly decreased by the AG or H-AG administrations, and the decreased creatinine clearance level was significantly increased in H-AG-administered rats. In addition, the markedly high serum levels of glucose and glycosylated protein in diabetic control rats were significantly decreased by the administration of H-AG, implying that H-AG might prevent the pathogenesis of diabetic complications caused by impaired glucose metabolism and glycosylation of serum proteins. Although no significant ameliorations were shown in overexpressed protein expressions related to diabetic oxidative stress by the AG or H-AG administrations, the accumulation of N^{ϵ}-(carboxymethyl)lysine and receptors for advanced glycation endproduct (AGE) expressions were significantly reduced by the administration of H-AG. On the basis of these results, we found that AG and H-AG inhibit AGE accumulation in diabetic rat kidney by their hypoglycemic and renal function ameliorating effects, and this effect was stronger in the H-AG-administered group than in the AG-administered group. These findings indicate that H-AG may have beneficial effect on pathological conditions associated with diabetic nephropathy.

KEYWORDS: American ginseng; heat-processed American ginseng; diabetes; oxidative stress; renal damage; advanced glycation endproduct

INTRODUCTION

Panax quinquefolium L. (Araliaceae), known as American ginseng (AG), grows in the United States and Canada and is one of the 10 most commonly used herbal supplements in the United States. AG has been widely used in herbal medicine for its antioxidant, antilipid peroxidation, antihypoxia, and antifatigue properties (*1, 2*). Bensky & Gamble (*3*) have reported the modulatory effects of AG on the central nervous system in animal experiments. A recent study has demonstrated that extracts from the leaves and stems of AG have an anticonvulsant effect in several animal models of seizures (*4*). In particular, AG is known to have stronger hypoglycemic activity than the other *Panax* species and it directly quenches free radicals, protects low density lipoproteins from oxidation, and inhibits lipid peroxidation (*5–9*).

Another famous ginseng, *Panax ginseng* C.A. Meyer (Araliaceae), mainly cultivated in Korea and Northeast China, has been heat-processed before use on the basis of its long history of ethnopharmacological evidence. Recent studies have reported that the biological activities of *Panax ginseng* are improved by heat processing (*10–12*). Heat-processed *Panax ginseng* has been reported to exhibit more potent pharmacological activities, such as vasorelaxation, antioxidant, and antitumor activities than conventional ginseng products (*10, 11, 13*). These enhanced biological activities of ginsengs were thought to be mediated by the changes in the chemical constituents such as ginsenosides by heat processing because ginsenosides are known as pharmacologically main active components of ginseng (*9, 14, 15*). The efficacies of AG are also thought to be improved by heat processing, but the chemical and biological activity changes of AG in *Panax ginseng* by heat processing are not yet fully elucidated. Therefore, we investigated the changes in contents of ginsenosides and hypoglycemic or antioxidant effects of AG by heat processing using streptozotocin (STZ)-induced diabetic rats.

Diabetes mellitus is characterized by excessive glucose production. An abnormally elevated blood glucose level causes oxidative stress and the formation of advanced glycation

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endproducts (AGEs), which have been closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy (*16*, *17*). Especially, diabetics are at increased risk for several types of kidney disease, and the predominant cause of end stage renal disease in this disorder is diabetic nephropathy (*18*, *19*). However, recent clinical trials suggest that there is no effective treatment for diabetic nephropathy (*20*), and preventions of the occurrence and progression of diabetic nephropathy have become a very important issue.

Therefore, this investigation of the changes in hypoglycemic and antioxidant effects of AG by heat processing have significant meaning as a development of novel functional food or agent for diabetics with a simple steaming method.

MATERIALS AND METHODS

Chemicals. Phenylmethylsulfonyl fluoride (PMSF), STZ, and β-actin were purchased from Sigma Chemical Co. (St. Louis, MO). Protease inhibitor mixture DMSO solution was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nuclear factor-kappa Bp65 (NF-κBp65), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), receptors for AGE (RAGE), and goat antirabbit and/or goat antimouse IgG horseradish peroxidase (HRP)-conjugated secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The primary polyclonal antibody against N^{ε}-(carboxymethyl)lysine (CML) was kindly provided by Dr. Nagai (*21*). The other chemicals and reagents used were of high quality and obtained from commercial sources.

Preparation of H-AG Extract. Commercial AG (*Panax quinquefolium*, harvested in Ontario, Canada, 3 years old) water extract was kindly provided by the Iskra Industry Co. (Tokyo, Japan). AG water extract was autoclaved at 120 °C and 0.11 MPa for 3 h, and the product was dried in an oven at 50 °C for 3 days to produce heat-processed AG (H-AG) (*12*, *22*).

Analysis of Ginsenosides in AG and H-AG. Each ginseng extract was 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 × 4 mm I.D.; YMC-Pack Pro) utilizing a solvent gradient system (*22*). 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 used was 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 the comparison of retention times with those of authentic samples, which were previously isolated in our laboratory by the reported method (*23*). Results are expressed as average values (% in each extract) of duplicate analysis. Linearity of the detector responses were tested for all ginsenosides (5–100 μg/mL), and the coefficient of correlation were >0.99. The relative standard deviation value of intraday repeatability was lower than 6%, representing good precision.

Animals and Treatment. 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 of body weight following overnight fasting, except in 5 rats of the normal group. Ten days after the injection, the glucose level of blood from the tail vein was determined and the diabetic rats were divided into 3 groups (each group consisted of 8 rats), avoiding any intergroup differences in blood glucose levels.

Group I: Normal rats

Group II: Diabetic control rats treated with water (vehicle)

Group III: Diabetic rats treated with AG extract (100 mg/kg of body weight) in aqueous solution orally for 20 days

Group IV: Diabetic rats treated with H-AG extract (100 mg/kg of body weight) in aqueous solution orally for 20 days

The dose and schedule used in this study were determined on the basis of previous studies on the effect of ginseng extracts on STZ-induced diabetic rats and oral toxicity assessment (*13*, *24*). 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 glycosylated protein level was measured using a modified thiobarbituric acid (TBA) assay of Fluckiger & Winterhalter (*25*). Urine component levels were determined as follows: protein by the sulfosalicylic acid method (*26*) and Cr using a commercial reagent (CRE-EN Kainos obtained from Kainos Laboratories Inc.). Creatinine clearance (C_{Cr}) was calculated on the basis of urinary Cr, serum Cr, urine volume, and body weight using the following equation: C_{Cr} (mL min^{-1} (kg of body weight)$^{-1}$) = [urinary Cr (mg/dL) × urine volume (mL)/serum Cr (mg/dL)] × [1000/body weight (g)] × [1/1440 (min)].

Electrophoretic Pattern Analysis of Urinary Protein. Samples (8 μg) of protein were loaded on to 10% acrylamide gels and subjected to sodium dodecyl sulfate polyamide gel electrophoresis (SDS-PAGE); the protein bands were stained for 30 min with Coomassie brilliant blue R 250 in a distilled water:MeOH:acetic acid (4.5:5:0.5) mixture and destained overnight in 7% acetic acid. The stained polyamide gels were scanned using a densitometer (AE-6911CX, ATTO Corp., Tokyo, Japan).

Western Blotting. Renal cortical sections (0.2 g) 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 protease inhibitor mixture DMSO. Samples were then centrifuged at 2000 g for 10 min at 4 °C. The protein concentration of tissue was determined using a Bio-Rad protein assay kit, and BSA as a standard, and the immunoblotting was then carried out. For the determination of NF-κBp65, COX-2, iNOS, RAGE, and CML protein levels in the kidney, 30 μg of protein of each sample was electrophoresed through 8 or 12% SDS-PAGE. Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 3 h at 4 °C, and then incubated with primary antibodies overnight at 4 °C. After the blots were washed, they were incubated with goat antirabbit and/or goat antimouse IgG HRP-conjugated secondary antibody for 90 min at room temperature. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents (Amersham, NJ) and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Japan). Band densities were determined by Scion image software (Scion Corporation, Frederick, MD) and quantified as the ratio to β-actin.

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

RESULTS

Changes of Ginsenosides in H-AG. Figure 1 shows the HPLC-ELSD chromatograms of AG and H-AG water extracts. The major components of AG were ginsenoside-Re, -Rb_1, -Rc, -Rb_2, and -Rd. On the other hand, the contents of these polar ginsenosides (peaks 1–5) were decreased and less-polar ginsenosides (peaks 6–9) became major constituents in H-AG (**Figure 1**, **Table 1**).

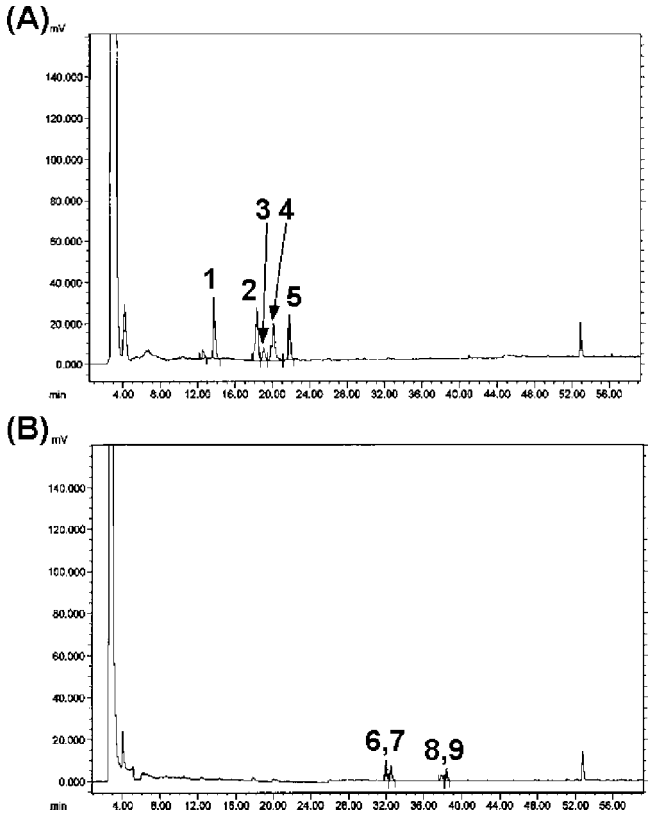

Figure 1. HPLC-ELSD chromatograms of (**A**) American ginseng and (**B**) heat-processed American ginseng water extracts. 1 = Re; 2 = Rb_1; 3 = Rc; 4 = Rb_2; 5 = Rd; 6 = 20(*S*)-Rg_3; 7 = 20(*R*)-Rg_3; 8 = Rk_1; 9 = Rg_5.

Table 1. Changes in Contents of Ginsenosides[a]

	Re	Rb_1	Rc	Rb_2	Rd	20(*S*)-Rg_3	20(*R*)-Rg_3	Rk_1	Rg_5
AG	0.10	0.11	0.03	0.10	0.08				
H-AG						0.03	0.03	0.02	0.02

[a] Data are expressed as % in sample extract.

Changes in Physico-Metabolic Symptoms. Table 2 shows the effects of AG and H-AG on the changes in physico-metabolic symptoms with diabetes over the 20-day experimental period. The body weight gain of STZ-induced diabetic rats was significantly lower than that of normal rats. In addition, the levels of food intake, water intake, and urine excretion were markedly elevated in diabetic control rats. Although there showed no significant ameliorations, the oral administration of H-AG at a dose of 100 mg (kg of body weight)$^{-1}$ day^{-1} for 20 days attenuated these diabetes-induced physiological abnormalities. However, the increased kidney weight under diabetes was significantly reduced by H-AG administration.

Biochemical Features of Serum and Urine. Figure 2 shows the effects of AG and H-AG on serum glucose and serum glycosylated protein levels. The diabetic control rats showed a markedly higher blood glucose level than that of normal rats, whereas the elevated glucose level was significantly reduced in diabetic rats given 100 mg of AG or H-AG. In addition, the elevated glycosylated protein level of diabetic control rats was significantly decreased by the H-AG administration.

On the other hand, **Table 3** shows the effects of AG and H-AG on renal function parameters. There were no significant changes in the serum Cr level among the normal, diabetic control, and AG- or H-AG-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. It was significantly reduced by 100 mg (kg of body weight)$^{-1}$ day^{-1} of AG or H-AG administration. In addition, the slightly decreased C_{Cr} level in diabetic control rats was significantly increased in H-AG-administered rats.

Western Blotting. The protein expressions related to oxidative stress-induced damage in renal tissue are shown in **Figure 3**. These protein band intensities were corrected by β-actin and graphed. There were significant increases in NF-κBp65, COX-2, and iNOS expressions in diabetic compared to normal rats. No significant ameliorations were noted in these protein expressions of diabetic control rats by the AG or H-AG-administration, but the NF-κBp65 levels in AG or H-AG-administered groups were not significantly increased from those of normal rats.

Figure 4 shows the protein expressions related to AGE formation in renal tissue. CML accumulation and RAGE expression in diabetic control rats were significantly higher than in normal rats. However, the elevated CML level was significantly reduced by 100 mg (kg of body weight)$^{-1}$ day^{-1} of H-AG administration. Similarly, the elevated RAGE expression was also significantly reduced by 100 mg of H-AG administration to nearly the normal level.

DISCUSSION

Diabetes mellitus is a disorder characterized by hyperglycemia. Hyperglycemia causes protein glycation leading to long-term complications of diabetes. The oxidative stress and AGE formation induced by hyperglycemia are known to influence the diabetic renal changes and nephropathy (*16, 17*). Ginseng has been used as a traditional antidiabetic supplement, and showed marked effects in controlling hyperglycemia and obesity (*27*). In addition, the improvement of some biological activities by heat processing has been well-recognized in *Panax ginseng* (*10–12, 14*). However, the effects of AG and H-AG on diabetic renal damage have not yet been reported, and we investigated it in this study using STZ-induced diabetic rats.

The destruction of β-cells and disorder of insulin secretion in the diabetic state causes physico-metabolic abnormalities such as a decrease in body weight gain and increases in kidney weight, food intake, water intake, and urine volume (*28, 29*). The STZ-induced diabetic rats in this study also showed these changes. However, the administration of AG and H-AG slightly, not significantly, decreased these diabetes-induced physiological changes, but showed significant decrease in kidney weight. These results suggest that the administrations of AG and H-AG may improve physiological abnormalities under diabetes, and these effects were stronger with H-AG than with AG. Further study on concentration dependence is needed to show more convincing evidence for the improvement in these physico-metabolic symptoms.

Over the experimental period, the levels of urinary protein excretion 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. In addition, this rat model showed a slight decrease in C_{Cr}. In patients with diabetes and/or renal failure, C_{Cr}, which is an effective index for expressing the glomerular filtration rate, decreases exponentially and eventually causes nephritic syndrome (*30*). However, the present investigation showed that the administration of AG or H-AG for 20 days significantly reduced the levels of urinary protein excretion, and H-AG also significantly increased the C_{Cr} level (**Table 3**). Therefore, it was considered that the early diabetic renal changes occurred, not

Table 2. Physico-Metabolic Symptoms[a]

group	dose (mg (kg of body weight)$^{-1}$day^{-1})	body weight (g) initial	body weight (g) final	body weight (g) gain	food intake (g/day)	water intake (mL/day)	urine volume (mL/day)	kidney weight (g/100 g of body weight)
normal		213.3 ± 7.5	291.2 ± 8.2	71.8 ± 12.8	18.8 ± 2.2	33.4 ± 1.9	14.1 ± 1.6	0.65 ± 0.01
diabetic control		187.8 ± 4.4[b]	210.8 ± 7.9[c]	23.0 ± 4.1[c]	29.3 ± 1.6[c]	156.7 ± 11.0[c]	122.3 ± 6.3[c]	1.09 ± 0.02[c]
AG	100	187.7 ± 4.8[b]	210.0 ± 6.0[c]	23.3 ± 6.7[c]	29.1 ± 0.4[c]	140.8 ± 9.7[c]	111.7 ± 4.3[c]	1.03 ± 0.03[c]
H-AG	100	188.0 ± 5.3[b]	213.0 ± 12.6[c]	26.5 ± 9.5[b]	29.1 ± 3.6	138.3 ± 13.3[c]	100.8 ± 12.8[c]	1.01 ± 0.02[c,d]

[a] Data are expressed as the mean ± S.E. [b] $p < 0.05$ compared with normal rats. [c] $p < 0.01$ compared with normal rats. [d] $p < 0.05$ compared with diabetic control rats.

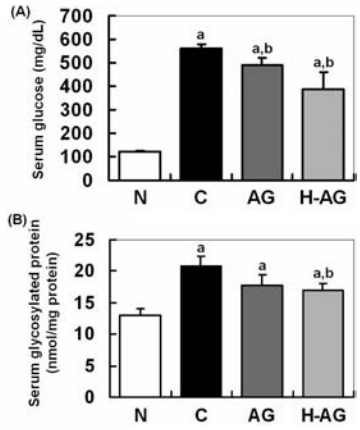

Figure 2. Effects of American ginseng and heat-processed American ginseng water extracts on (**A**) serum glucose and (**B**) serum glycosylated protein levels. N, normal rats; C, diabetic control rats; AG, diabetic rats treated with American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); H-AG, diabetic rats treated with heat-processed American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); [a]$p < 0.01$ compared with normal rats; [b]$p < 0.01$ compared with diabetic control rats.

Table 3. Renal Function Parameters[a]

item	normal	diabetic rats control	diabetic rats AG (100 mg)	diabetic rats H-AG (100 mg)
serum creatinine (mg/dL)	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.02	0.30 ± 0.01
urinary protein (mg/day)	10.1 ± 0.8	13.1 ± 1.1[b]	10.7 ± 0.3[c]	9.4 ± 0.8[c]
C_{Cr} (mL (kg of body weight)$^{-1}$ min^{-1})	8.11 ± 0.73	6.89 ± 0.63	7.68 ± 0.40	8.59 ± 0.40[c]

[a] Data are expressed as the mean ± S.E. [b] $p < 0.05$ compared with normal rats. [c] $p < 0.05$ compared with diabetic control rats.

advanced ones, in this study because of slight decrease in C_{Cr} of diabetic control rats, and the renal functions of this early stage of diabetes were significantly improved by the administration of AG or H-AG.

Hyperglycemia, a primary characteristic of diabetes, is mainly attributed to diabetic oxidative stress brought about by several factors. Hyperglycemia leads to the overproduction of free radicals by the nonenzymatic glycation of proteins through Maillard's reaction, and these free radicals exert deleterious effects on the function of β-cells vulnerable to oxidative stress (*31, 32*). In addition, hyperglycemia can degrade antioxidant enzyme defences, thereby allowing reactive oxygen species to cause cellular and tissue damage. As shown in **Figure 2**, the elevated blood glucose levels in diabetic rats were significantly decreased in those fed AG or H-AG at dose of 100 mg, respectively. The hypoglycemic effect of ginseng has been suggested to be mediated by delayed glucose absorption

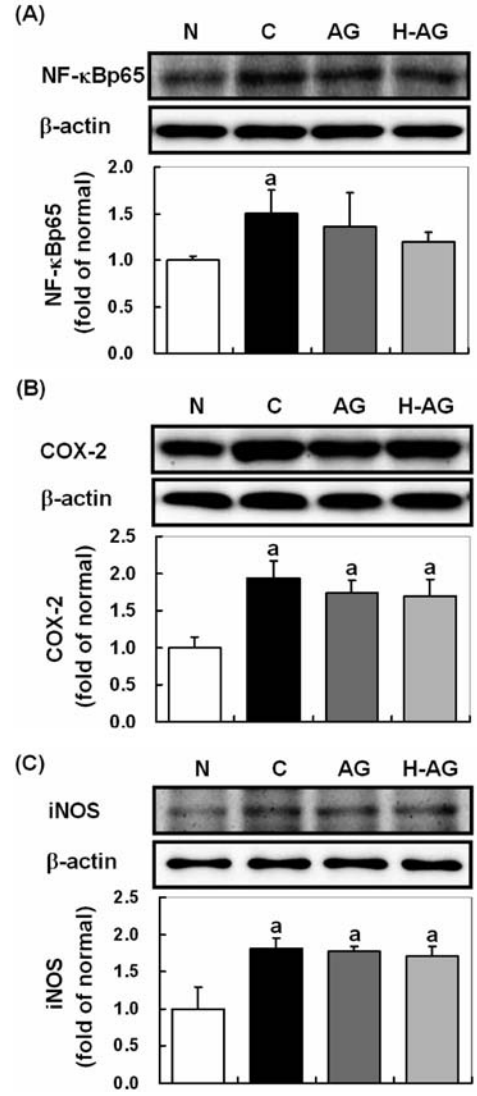

Figure 3. Effects of American ginseng and heat-processed American ginseng water extracts on (**A**) NF-κBp65, (**B**) COX-2, and (**C**) iNOS protein expressions. N, normal rats; C, diabetic control rats; AG, diabetic rats treated with American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); H-AG, diabetic rats treated with heat-processed American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); [a]$p < 0.05$ compared with normal rats.

in the gut, increased glucose uptake/elimination, and glucose-stimulated insulin secretion (*33, 34*), and the improved hypoglycemic effect of AG by heat processing was also thought to be mediated by these. On the other hand, the hyperglycemic condition, a chronic metabolic disorder of glucose, results in irreversible tissue damage via the protein glycation reaction that leads to the formations of glycosylated protein and AGEs (*35, 36*). Glycosylated serum protein was increased in the present diabetic rat model, which implies that it stimulates the oxidation of

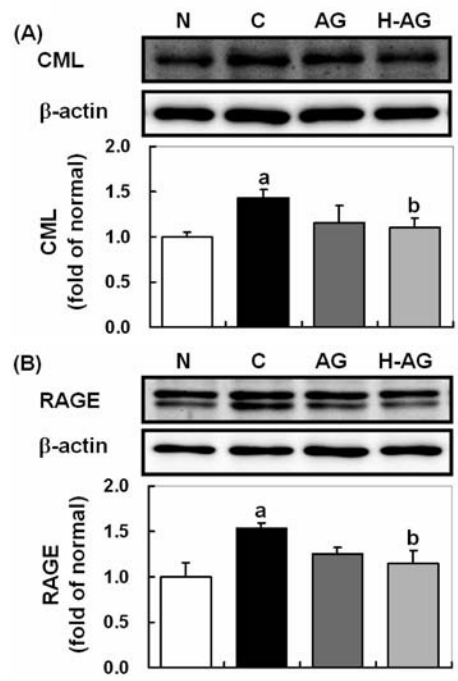

Figure 4. Effects of American ginseng and heat-processed American ginseng water extracts on (**A**) CML and (**B**) RAGE protein expressions. N, normal rats; C, diabetic control rats; AG, diabetic rats treated with American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); H-AG, diabetic rats treated with heat-processed American ginseng (100 mg (kg of body weight)$^{-1}$ day^{-1}); $^{a}p < 0.05$ compared with normal rats; $^{b}p < 0.05$ compared with diabetic control rats.

sugars, enhancing damage to both sugars and proteins in the circulation and vascular wall, continuing and reinforcing the cycle of oxidative stress and damage. H-AG significantly decreased the levels of glycosylated serum protein, suggesting that it would inhibit oxidative damage caused by the protein glycation reaction under diabetes. These results on glucose and glycosylated protein levels indicate that the administration of AG, especially H-AG, might prevent the pathogenesis of diabetic complications caused by impaired glucose metabolism and the glycosylation of serum proteins, eventually resulting in improvement of the diabetic pathological conditions.

The protein expressions related to oxidative stress and AGE formations, such as NF-κBp65, COX-2, iNOS, RAGE, and CML protein levels in the kidney, 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 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 (*17, 37*). 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 enhanced in the kidney of STZ-induced diabetic rats or mice (*20*). Our results also showed significant increases in NF-κBp65, COX-2, and iNOS expression levels of the diabetic rat kidney. However, AG or H-AG administration did not reduce these overexpressed NF-κBp65, COX-2 and iNOS levels significantly (**Figure 3**), but the NF-κBp65 levels in AG or H-AG-administered groups were not significantly increased from those of normal rats. These results imply that AG and H-AG may alleviates oxidative stress by preventing the NF-κB activation, but the 20 days of experimental diabetes and administration period was thought to be too short to increase or inhibit COX-2 and iNOS protein expressions with more significant manner.

On the other hand, CML, one of major AGEs in human tissues, is known to be a marker of cumulative oxidative stress and be involved in the development of diabetic nephropathy (*38*). Moreover, activation of RAGE by CML results in the activation of NF-κB and production of proinflammatory cytokines (*17, 39*). The CML accumulation and RAGE expression in diabetic rats were markedly higher than normal, but they were significantly ameliorated in H-AG-administered groups (**Figure 4**). These findings imply that H-AG can prevent diabetic nephropathy via inhibiting RAGE activation by AGE formation.

Ginseng saponins, referred to as ginsenosides, are believed to play a pharmacologically important role (*15*). The HPLC chart in **Figure 1** shows that the amounts of polar ginsenosides such as Re, Rg_1, and Rb_1 were decreased, but those of less-polar ginsenosides such as Rg_3, Rk_1, and Rg_5 were newly formed in H-AG. The ginsenosides (such as Rb_1 and Rb_2) contained in AG are known to change into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat processing through glycosyl elimination and the epimerization of carbon-20 by the SN1 reaction (*14, 40*). The elimination of glycosyl moieties at carbon-20 is responsible for the loss of about 30% of the weight of ginsenosides. In addition, there are unidentified less-polar ginsenosides such as F_4, Rh_4, Rs_3, and Rs_5 because of their very low contents. These factors were thought to be related to the reduced total ginsenoside contents by heat processing, and the total ginsenoside content of steamed American ginseng berry was also decreased to less than 30% of the original weight by heat processing at 120 °C for 3 h (*41*). Although the total ginsenoside content was reduced by heat processing, less-polar ginsenosides such as Rg_3 and Rg_5 are known to have stronger •OH-scavenging, antioxidant, and anti-inflammatory effects than the other ginsenosides contained in ginseng (*42–45*). Therefore, the fortified effects of H-AG compared to AG in diabetic rats may be explained with the chemical transformation of ginsenosides by heat processing. On the other hand, ginsenoside is known as a prodrug that is activated in the body by intestinal bacterial deglycosylation and fatty acid esterification in the liver, and this process is crucial for its pharmaceutical expression (*46*). Metabolites in plasma or tissue were not measured in the present study, but 20(*S*)-protopanaxadiol, a metabolite of 20(*S*)-Rg_3, is known to have an anti-inflammatory effect by its inactivation of NF-κB and suppression of iNOS in LPS-induced RAW 264.7 cells (*47*).

This study demonstrated that AG and H-AG ameliorate diabetes-induced physiological abnormalities and inhibit AGE generation by reducing the blood glucose level and improving renal function under diabetes. Therefore, AG and H-AG may improve diabetic pathological conditions and prevent renal damage associated with diabetic nephropathy, and these preventive effects of AG can be improved by heat processing. Further studies about the effects of H-AG and less-polar ginsenosides at several concentrations on diabetic renal damage are needed for the identification of active compounds and the concentration dependence, which were not considered in the present study.

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