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Influence of Long-term Supplementation with Korean Red Ginseng on *in vivo* Antioxidant Capacities in Rats

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Abstract Effects of ginseng on *in vivo* antioxidant capacities with age were studied in rats. All rats were reared in the conventional system. Ginseng-treated rats were supplied with ginseng water extracts (25 mg/kg/day) continuously from 6 weeks of age to spontaneous death. None of the rats showed any discernible adverse effects of treatment with ginseng-containing water. There was no significant difference in body weight (BW) gains with age between treated and control groups. However, ginseng extracts did cause a decrease in the level of serum low density lipoprotein (LDL)-cholesterol, glucose, and thiobarbituric acid reactive substances (TBARS) in the treated rats. The activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase in liver cytosol decreased with age in the control group. However, these enzyme activities were well maintained in the ginseng-treated rats and, especially, catalase and glutathione peroxidase activities were consistently higher than in control rats. The levels of total sulfhydryl group (T-SH) and glutathione reductase (GR) were unchanged, and glutathione-s-transferase (GST) activity gradually decreased with age in both groups. There were no differences in T-SH, GR, or GST between the control and treatment groups. These results indicate that long-term administration of ginseng retards age-related deterioration in some biochemical parameters such as cholesterol, glucose, and lactate dehydrogenase in serum and it has an enhancing effect on antioxidant capacity in the liver.

Keywords: Korean red ginseng, antioxidant enzyme, long-term supplementation

Introduction

Population aging was one of the most distinctive demographic events of the 20th century. The worldwide prolongation of the mean life expectancy has resulted in a rapid increase in the size of the elderly population, both in numbers and as a proportion of the whole. The free radical theory of aging proposed by Harman (1) hypothesizes that the degenerative changes associated with age might be produced by the accumulation of deleterious side reactions of free radicals produced during cellular metabolism. Organisms evolve enzymatic and nonenzymatic systems for scavenging free radicals and destroying potentially harmful products before further damage can occur. These antioxidant systems appear to be located near sites of generation of peroxidative intermediates, which they are best suited to nullify (2).

Natural medicines have been utilized for the natural healing of disease, and many of them have even enhanced the spirit by their indirect action without any side effects. Korean red ginseng, one of the best known herbal medicines, has been used since AD 190. More than two thousand scientific research papers on red ginseng have reported that various pharmacological efficacies such as antitumor (3), antihypercholesteremia (4), anti-fatigue (5), enhancement of immune function (6), and detoxification effects (7) have been observed in animals and humans. Especially, ginseng has been known to have very high antioxidant effects *in vitro* (8-10). These results suggest strongly that ginseng benefits most degenerative diseases being studied recently. If such efficacies are true, we can easily expect that ginseng may modulate the aging process of organisms.

On the basis of such research and our traditional wisdom as reviewed above, we attempted in this study to study *in vivo* antioxidant capacities by long-term supplementation with Korean red ginseng in rats.

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Materials and Methods

Chemicals Ferricytochrome C, $NADP^+$, NADH, NADPH, reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, xanthine, xanthine oxidase, glucose-6-phosphate dehydrogenase, hydrogen peroxide, 5,5'-dithiobis-2 (2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitro benzene (CDNB), tris[hydroxy methyl] aminomethane, *N*-[2-hydroxyethyl] piperazine-*N*'-[2-ethansulfonic acid] (HEPES), cumene hydroperoxide, potassium ferricyanide, sodium deoxycholate, sodium dodecyl sulfate (SDS), trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the first grade.

Preparation of red ginseng extracts The extract was made from red ginseng powder (from 6-year-old roots, 30-40 mesh). The red ginseng powder was soaked in 5 volume of hot water (70°C) for 4 hr. The temperature of the water was maintained at 70°C to prevent heat destruction of the saponins and other phenolic compounds (11). This procedure was repeated more than twice. The extracts were combined and concentrated to 16% water content.

Animals and ginseng treatment Sprague-Dawley (SD) rats were bred in an animal house; only males were used for experimentation in this study. To keep individual differences at a minimum, healthy 6-week-old SD rats were selected and housed singly in polycarbonate cages. All rats were reared in the conventional system under a cycle of 12-hr light at 200-300 Lux and 12-hr dark. Animals were maintained at a temperature of 22±1°C, 40-60% humidity, with complete air exchange 15 times/hr. Animals were given solid feed purchased from Sam Yang Co. (Seoul, Korea) *ad libitum*. Food intake and the consumption of drinking water were regularly checked every morning. Seventy-two animals each had free access to water extract of ginseng (25 mg/kg BW/day) in drinking water from 6 weeks of age; the same number of rats as given only drinking water for the control group. Water was replaced every day. This treatment was continued throughout their natural life or until the animal was sacrificed for biochemical assays. Cross-sectional studies were carried out on a fraction of 32 rats from the control group and 32 rats from the ginseng-treated group. Eight rats from each of the 2 groups were sacrificed by decapitation at 3, 6, 12, and 24 months of age.

Body weight (BW) and blood biochemistry The BW of each animal was measured every month. Hematocrit value and plasma total protein were determined by a capillary tube method and reflectometry, respectively. Blood was collected by cardiac puncture and serum was separated by centrifugation (150×g for 10 min). Serum was divided into small vials and stored at −70°C until analysis. Levels of serum constituents such as glucose, cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides, and nonfunctional enzymes such as lactate dehydrogenase (LDH), glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), and alkaline phosphatase were measured by using kit reagents (Asan Pharmaceutical Co., Ltd., Seoul, Korea). Thiobarbituric acid (TBA) reactive substance contents in serum were determined by the method of Suematsu *et al.* (12).

Antioxidant capacity in liver Livers were removed, weighed, washed well with saline, and homogenized in 4 volume of 30 mM HEPES buffer (pH 7.4) containing 150 mM KCl. The cytosolic fraction was prepared from homogenates by differential centrifugation (13). In cytosol, superoxide dismutase (SOD) activity was measured according to the procedure of McCord *et al.* (14) by inhibition of cytochrome C oxidation at 550 nm by using xanthine oxidase to generate a superoxide radical. Catalase activity was assayed by the method of Aebi (15), based on the direct measurement of decomposition of hydrogen peroxide at 240 nm spectrophotometically. Glutathione peroxidase activity was measured with the coupled-enzyme system of Flohe and Gunzler (16). The reduction of GSSG was coupled to NADPH oxidation by glutathione peroxidase using cumene hydroperoxide as substrate. Glutathione reductase activity was determined by the method of Racker (17). The oxidation of NADPH was monitored at 340 nm. Glutathione-S-transferase activity was assayed by the method of Habig *et al.* (18) using 1-chloro-2,4-dinitrobenzene as substrate. Total sulfhydryl group level was measured at 412 nm, according to the procedure of Sedlak and Lindsay (19) using 5,5'-dithio-bis-(2-nitrobenzoic acid). Protein concentration was determined by the method of Lowey *et al.* (20) with bovine serum albumin as the standard.

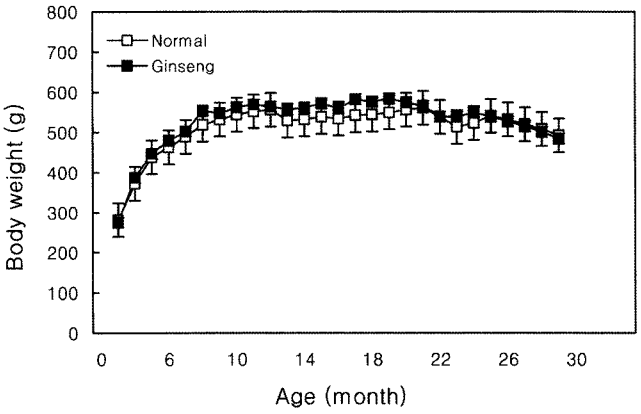

Fig. 1. Age-related changes of body weight (BW) in normal and ginseng administered rats. Male SD rats received drinking water with or without ginseng water (25 mg/kg BW). Values are expressed as mean±SD from 40 rats.

Statistical analysis Data are expressed as mean±standard deviation (SD). Data differences in the control and ginseng-administered rats were determined using analysis of variance (Stat View version 4.0; Abacus Concepts, Inc., Berkeley, CA, USA). If the differences between 2 groups were statistically significant ($p<0.05$), Fisher's protected least significant difference test or Scheffe's F test was used to distinguish between pairs of groups.

Results and Discussion

Changes in BW Adult rats in both groups consumed about 25 g of food/day; animals in the ginseng-treated group did not exhibit signs of aversion to the red ginseng solution. Figure 1 shows the change in BW of rats with age. There was no significant difference in BW gain between the 2 groups. BW of ginseng-treated rats was slightly higher than that of the control group at maturity. BW of rats in both groups decreased slightly before death. The actual amounts of ginseng administered to the rats in this study correspond to 1.5 g of dry red ginseng powder/60 kg BW/day. These data indicate that red ginseng water extract of this concentration is safe for long-term supplementation. Aphale *et al.* (21) reported that ginseng did not reveal any toxicity in their sub-acute toxicity study in rats with 90 days oral administration. However, Coon and Ernst (22) explained that ginseng monopreparations are rarely associated with adverse effects and drug interactions.

Serum constituents Hematocrit values were 44.0±0.7 and 46.2±3.3% in 3- and 24-month-old control rats, respectively. The content of serum total protein was also slightly increased with age, but there was no significant difference between the 2 groups (Table 1). In both groups, the levels of serum albumin and blood urea nitrogen were virtually unchanged with age. Creatine and triglyceride

Table 1. Age-related changes of serum constituents in control and ginseng-administered rats[1)]

Serum constituent[2)]		Age (month)			
		3	6	12	24
HDL-Cholesterol (mg/dL)	N	11±4	13±3	13±3	19±3
	G	14±2	15±1	13±2	30±7*
LDL-Cholesterol (mg/dL)	N	78±13	73±9	68±8	95±15
	G	76±12	70±6	49±7*	66±13*
Triglyceride (mg/dL)	N	77±18	83±13	99±17	49±7
	G	79±15	99±12	116±13	59±6
Albumin (mg/dL)	N	3.3±0.2	3.1±0.6	3.3±0.5	3.1±0.2
	G	3.4±0.1	3.5±0.2	3.3±0.3	3.0±0.3
Uric acid (g/dL)	N	0.7±0.4	1.8±0.4	1.8±0.47	2.4±1.4
	G	0.9±0.2	2.3±1.0	1.5±0.5	2.1±0.8
Creatine (g/dL)	N	0.50±0.06	0.62±0.03	0.68±0.04	0.43±0.04
	G	0.48±0.03	0.63±0.03	0.52±0.05	0.54±0.02
Blood urea nitrogen (mg/dL)	N	23±3	20±2	18±1	20±3
	G	18±2	21±2	22±3	17±1
Glucose (mg/dL)	N	167±11	184±16	190±18	177±27
	G	158±13	172±15	165±14*	155±32

[1)]Male SD rats received drinking water with or without ginseng water extract (25 mg/kg BW). Values are expressed as mean±SD from 8 male rats; *Significantly different from control rats of the same age ($p<0.05$).
[2)]N, normal rats; G, ginseng-administered rats.

contents were slightly increased in only adult rats and it was decreased in 24-month-old rats. Uric acid content increased with age in both groups. The levels of HDL-cholesterol and low density lipoprotein (LDL)-cholesterol were increased in 24-month-old rats. Serum HDL-cholesterol content in ginseng-supplemented rats was higher than in control groups; on the contrary, LDL-cholesterol content in ginseng-supplemented rats was lower than in control rats ($p<0.05$). Glucose content gradually increased until 12 months; thereafter, it remained at a constant level. In the rats supplemented with red ginseng extract, interestingly, glucose level in 12-month-old rats was lower than in the control group ($p<0.05$). Some researchers have reported that some ginseng fractions stimulated insulin release, especially glucose-induced insulin release from pancreatic islets and thereby lowered the blood glucose level (23,24). Yokozawa and Oura (25) have posited that ginsenoside-Rb_2 of ginseng components might play a key role in reducing the level of blood glucose, but it is not clear yet whether the effect results from saponins such as Rb_2. Several researchers used a large amount of saponin to elucidate its effect on cholesterol metabolism, and found that saponins decrease cholesterol and triglyceride level, but increased HDL-cholesterol. In our study, the level of cholesterol and HDL-cholesterol showed a similar tendency to the results of others (26,27), but we observed a reverse pattern for triglycerides. These data clearly indicate that long-term red ginseng administration has a modulating effect on lipid metabolism.

Serum enzymes The change of nonfunctional enzyme activities in serum has been utilized as an index of cellular damage, because the increased activity reflects leakage of

Table 2. Age-related changes in serum enzymes activities of control and ginseng-administered rats[1)]

Serum enzyme[2)]		Age (month)			
		3	6	12	24
Alkaline phosphatase (unit/L)	N	32±5	24±3	23±7	27±6
	G	30±3	28±2	20±2	29±6
Glutamate-oxaloacetate transaminase (unit/L)	N	55±9	47±4	45±7	66±13
	G	54±5	55±6	54±10	60±15
Glutamate-pyruvate transaminase (unit/L)	N	17±4	37±5	28±5	43±4
	G	18±2	33±11	22±4	36±4*
Amylase (unit/dL)	N	761±5	760±6	760±6	680±22
	G	760±5	750±3	756±6	734±39
Lactate dehydrogenase (unit/L)	N	1,172±177	1,215±153	798±212	636±159
	G	1,288±198	1,494±165	1,053±169	854±162*

[1)]Male SD rats received drinking water with or without ginseng water extract (25 mg/kg BW). Values are expressed as mean±SD from 8 male rats; *Significantly different from rats in 24-month-old control group ($p<0.05$).
[2)]N, normal rats; G, ginseng-administered rats.

intracellular enzymes. Table 2 shows the change in activities of such enzymes in the rats with age. GPT and GOT were slightly increased only at 24 months in both groups, but GPT activity was consistently lower in ginseng-treated rats than in control rats. Alkaline phosphatase, amylase, and urease activities were also unchanged with age in both groups. The activity of LDH in control rats was remarkably decreased from 1,172 units at 3 months old to 636 units at 24 months old. The decrease of LDH activity was, however, effectively attenuated by red ginseng supplement. Kang *et al.* (28) reported that ginseng has an effect to retard the decline of serum LDH activity induced by the treatment of doxorubicin intraperitoneally. The age-related changes in serum components such as cholesterol, glucose, and LDH were effectively modulated by red ginseng supplement.

Antioxidant capacity The content of TBA reactive substances in serum as an index of oxidative damage were determined (Fig. 2). Because TBA reactive substances in serum reflect the status of oxidative stress in the body, it is used as a sensitive marker of oxidative stress *in vivo*. Level of serum TBA was increased in adult rats, and then decreased with age, showing a similar pattern with age in both groups. But, interestingly, the levels in ginseng-treated rats showed lower tendency than in control rats at the corresponding age throughout their whole life. To compare the antioxidant status in the livers of the 2 groups, we measured the levels of major antioxidant enzymes, related enzymes, and total sulfhydryl groups with age; these are presented in Table 3. There was a significant age-dependent decrease in cytosolic SOD activity in both groups. This enzyme activity in the control group was much higher until 12 months of age than in the ginseng-treated group, but it was maintained at a high level at 24 months of age in the ginseng-treated group ($p<0.05$). Catalase activity was also decreased age-dependently and was higher in the ginseng-treated group than in the control group at a corresponding age ($p<0.05$). Glutathione peroxidase activity was decreased

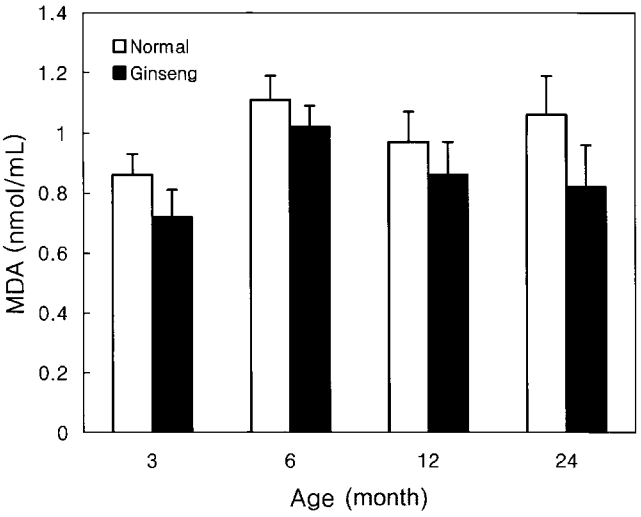

Fig. 2. Age-related changes in the content of serum TBA reactive substances in normal and ginseng administered rats. Male SD rats received drinking water with or without ginseng water (25 mg/kg BW). Values are expressed as mean±SD from 8 male rats.

in older rats of both groups, but it was maintained at a higher level in the aged rats receiving the red ginseng supplement ($p<0.05$). The preservation of glutathione peroxidase activity in old age might contribute more to eliminate the toxic lipid peroxides. This phenomenon seems to be closely related to the decrease of TBA reactive substances. Cytosolic concentration of the total sulfhydryl group varied a little with age but there was no clear difference between the 2 groups. Glutathione reductase activity showed a decreasing tendency with age, with no clear difference between the 2 groups. Cytosolic glutathione-S-transferase activity was decreased in aged rats, but was maintained at a high level in the 24-month-old ginseng-treated group.

This is the first report that the activities of antioxidant

Table 3. Age-related changes of major antioxidant enzymes, related enzymes, and total sulfhydryl groups in the livers of control and ginseng-administered rats[1)]

Enzymes[2)]		Age (month)			
		3	6	12	24
Catalase (μmol/min/mg protein)	N	201.6±12.4	190.4±23.4	170.4±21.7	112.1±9.4
	G	224.5±17.4	210.8±21.7	195.5±23.1	145.4±19.3*
Superoxide dismutase (unit/min/mg protein)	N	25.3±3.1	22.2±3.1	20.6±2.0	14.1±2.2
	G	21.8±5.1	20.2±3.7	19.4±3.3	18.0±2.0*
Glutathione peroxidase (μmol/min/mg protein)	N	2.46±0.13	2.15±0.18	2.26±0.21	1.44±0.15
	G	2.10±0.17	2.23±0.28	2.41±0.42	1.96±0.32*
Glutathione reductase (μmol/min/mg protein)	N	30.9±2.7	31.7±6.8	26.1±5.3	25.1±6.3
	G	32.7±2.7	31.6±4.1	28.1±4.2	24.4±6.9
Glutathione s-transferase (μmol/min/mg protein)	N	1.16±0.02	1.00±0.10	0.96±0.07	0.69±0.07
	G	1.15±0.02	0.99±0.07	1.06±0.07	0.81±0.06
Total sulfhydryl group (μmol/g tissue of liver)	N	11.4±0.7	14.9±1.6	15.2±1.9	10.2±0.8
	G	11.9±1.0	16.1±1.3	13.5±2.4	12.9±1.4

[1)]Male SD rats received drinking water with or without ginseng water extract (25 mg/kg BW). Values are expressed as mean±SD from 8 male rats; *Significantly different from rats in 24-month-old control group ($p<0.05$).
[2)]N, normal rats; G, ginseng-administered rats.

enzymes are well preserved in elderly rats when ginseng extract is administered chronically. Maintenance of these enzyme activities in old age leads us to understand a connection with *in vivo* free radical metabolism. These data suggest that long-term red ginseng administration may have major antioxidant enzyme activities in the livers of old rats *in vivo*.

In conclusion, our study demonstrates that long-term supplementation of red ginseng is safe, it offers a modulating effect especially on lipid metabolism, it serves to maintain the antioxidant capacity in old age *in vivo*, and it seems to have some preventive effect against degenerative changes of the organism rather than a curative effect.

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