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Studies on the Anti-aging Action of Korean Ginseng

(Ⅰ) Comparative Study of Red and White Ginsengs on Anti-aging Action

Jin Ho Choi and Sung Ki Oh*

Dept. of Nutrition and Food Science, National Fisheries University of Pusan,
**Dept. of Food Technology, Kyung Hee University*
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高麗人蔘의 老化抑制作用에 관한 硏究

(第1報) 紅蔘과 白蔘의 老化抑制作用 比較硏究

崔 鎭 浩·吳 成 基*

釜山水産大學 食品營養學科, *慶熙大學校 産業大學 食糧資源開發硏究所
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Abstract

The inhibitory effects of the extract and crude saponin of red and white ginsengs on lipoperoxide formation *in vitro* and *in vivo* were studied and correlated with anti-aging. To this end, antioxidant activity, induction period and lipoperoxide were measured by the methods of EDA, POV and TBA value. And also superoxide dismutase and peroxidase activity were measured by pyrogallol autoxidation method (ΔA 420/min) and initial velocity(ΔA 436/min), respectively.

From HPLC analysis, the PT/PD ratio of red and white ginsengs was found to be 0.561% and 0.401%, respectively, and red ginseng increased the PT/PD ratio in comparison with white ginseng. The EDA activity of red ginseng was higher than that of white ginseng; red ginseng showed stronger antioxidative effect than white ginseng.

The inhibitory effect of red ginseng was lower than that of white ginseng during the induction period. It was proved that high molecular coloring substance was deeply related to the initial stage of lipoperoxidation.

There was no significant difference between red and white ginsengs in both *in vitro* and intraperitoneal administration experiments, and red ginseng was more effective than white ginseng in long-term administration. And also inhibitory effect on lipoperoxide formation was mainly occurred in liver, suggesting that the function of liver played an important role in anti-aging actions.

From the measurement of superoxide dismutase(SOD) activity for both ginseng groups intraperitoneally and orally administered, it was found that red ginseng group administered extract and crude saponin showed remarkable inhibitory effects in comparison with white ginseng. In particular, orally administered group showed more stronger inhibitory effect on lipid peroxidation in comparison with intraperitoneally administered group. It was also found that the continuous oral administration was more effective than temporary administration.

Red ginseng was more notable anti-aging effect in comparison with white ginseng *in vivo*, and this

may be due to the increase of SOD activity in rat-liver. Peroxidase activity also showed similar trend to SOD activity *in vitro* and *in vivo* experiments.

Red ginseng was not only superior to white ginseng for preservation but also for biochemical and pharmaceutical efficacy.

Introduction

Ginseng is one of the most important crude drug which has been used as a traditional orient medicine. Main effective components of ginseng have recently been considered to be saponins according to the biochemical and pharmacological studies.

Fourteen saponins have been isolated and identified from *Panax ginseng* C.A. Meyer using various chromatographic methods such as thin-layer chromatography(TLC)[1~2], gas-liquid chromatography(GLC)[3~4], droplet counter-current chromatography(DCC)[5], and high-performance liquid chromatography(HPLC)[6~7].

In previous papers we found that the application of HPLC technique to the rapid isolation of major and minor components of ginseng saponins[8~14], sugars[15] and vitamin B groups[16] was very effective. And also we studied the stability of ginseng products[17~22] and kinetics on the thermal degradation of ginsenosides[23].

The objects of this study were to demonstrate the inhibitory effect of Korean ginseng (*Panax ginseng* C.A. Meyer) on the lipoperoxide formation *in vitro* and *in vivo*, and to correlate the effect with anti-aging. Numerous theories for the causes and biological changes of aging have been reported; that is, the accumulation theory[24], wear and tear theory[25], mutation theory[26], autoimmune theory[27], cross-linking theory[28], and free radical theory[29].

The free radical formed under various conditions can cause the changes in deoxyribonucleic acid through chromosomal oberrations and/or can initiate lipid peroxidation in subcellular and cellular membrane systems; the accumulation of lipoperoxides in cells and tissues accelerates not only aging action but also causes adult diseases.

Therefore, aging starts with functional and structural damage in cells and tissues which resulted from the side effects of free redicals formed normally in the course of biochemical reaction.

In order to compare the inhibitory effect of red ginseng on lipoperoxide formation *in vitro* and *in vivo* with that of white ginseng, extract and crude saponin separated from red and white ginsengs were added to the substrate of linoleic acid-rat liver, and incubated in a shaking air-bath at 60°C. The inhibitory effect on lipoperoxide formation was examined by measuring the TBA value(thiobarbituric acid value at 532 nm), POV (peroxide value at 500 nm) and EDA value(electron donating ability to α,α-diphenyl-β-picryl-hydrazyl at 525 nm).

On the other hand, extract and crude saponin were administered to Sprague-Dawley rats(150~200*g*) intraperitoneally(i.p.) and/or orally(p.o.), and the inhibitory effects on lipoperoxide formation were examined by measuring the TBA value as described above.

In addition, the SOD(superoxide dismutase) and peroxidase activity were measured by pyrogallol autoxidation method (ΔA420/min) and initial velocity(ΔA436/min), respectively, in order to examine the inhibitory effect of red and white ginsengs on lipoperoxide formation *in vitro* and *in vivo*.

Materials and Methods

Materials

Six-year-old fresh ginseng(*Panax ginseng* C.A. Meyer) used was purchased from Kangwha, Korea, and it was cultivated in 1981. Red ginseng was prepared by steaming and drying fresh ginseng, and white ginseng by drying fresh gi-

nseng.

The materials were powdered(80 mesh) using Cut-Mill (Arthur, H. Thomas Co., U.S.A.).

Chemicals

DPPH(α, α-diphenyl-β-picrylhydrazyl, Sigma Co., U.S.A.) was used for electron donating ability(EDA), linoleic acid and thiobarbituric acid(Merck Co., Germany) for TBA value, ammonium thiocyanate and ferrous chloride for peroxide value(POV), superoxide dismutase(E.C 1.15.1.1, from horse-radish, Sigma Co., U.S.A.) and peroxidase(E.C 1.11.1.6, from bovine-liver, Sigma Co., U.S.A.), sodium dodecyl sulfate(SDS, Sigma Co., U.S.A.) and dimethyl sulfoxide(DMSO, Sigma Co., U.S.A.) for enzyme activity.

All other reagents used were analytical and/or reagent grade without further purification.

Apparatus

Liquid chromatography was performed on an analytical HPLC ALC/GPC-244(Waters Associates Inc., Milford, Mass., U.S.A.) equipped with carbohydrate analysis column and refractometer R-401(RI detector).

Vortex mixer(HM-77VM, Hwashin Med-lab Co., Korea), Beckman homogenizer(Beckman Co., U.S.A.) and spectrophotometer(UV-110-02, Shimadzu Co., Japan) were used for the measurements of EDA, POV, TBA value and enzyme activity.

Animals

Male rats(150~200g) of the Sprague-Dawley (SD) were maintained in an air-conditioned room by lighting from 6 a.m. to 6 p.m.. Prior to the experiments, rats were locally obtained before one week, and then they were feeded with commercial diet. The diet and water were prepared late in the afternoon and given freely. The rats were weighed every morning (10 a.m.), if possible, for the observation of body condition.

Extraction and Separation

As shown in Fig. 1, the powder of red and white ginsengs was extracted twice with five volumes of 80% ethanol on water bath(75~80°C) for eight hours. The extracts were filtered with filter paper(Toyo No. 5) and concentrated (60°Bx) at 50°C in vacuum.

The extracts were added to five volumes of water, and extracted twice with ethyl ether for the exclusion of fat-soluble components. The

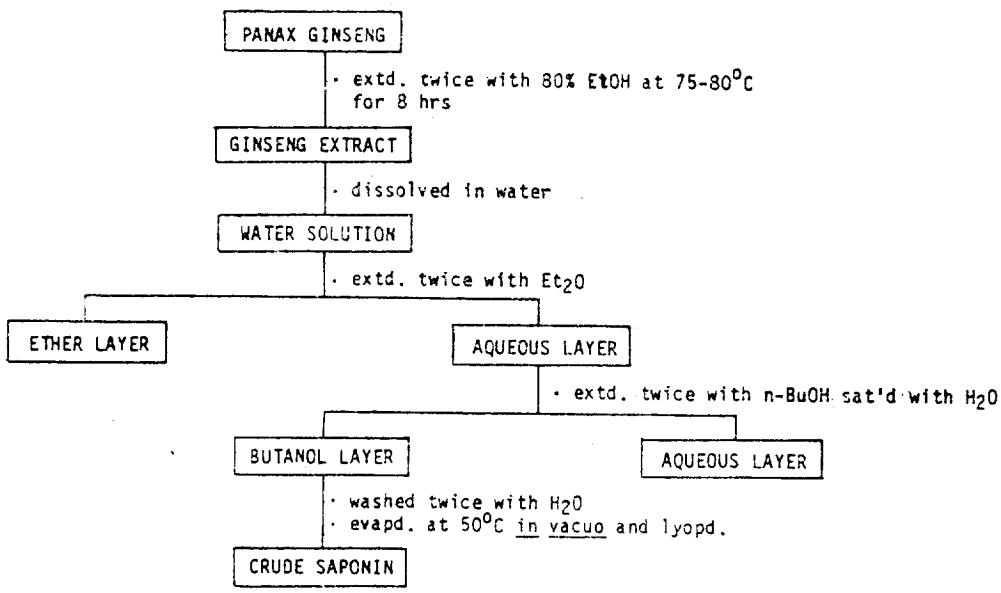

Fig. 1. Extraction and separation of crude saponin from red and white ginsengs.

aqueous layer was extracted twice with n-butanol saturated with water for the separation of crude saponin.

The butanol layer was washed twice with water for the exclusion of water-soluble components, evaporated in vacuum and lyophilized.

Measurements of Anti-aging Effect

(1) Electron Donating Ability(EDA)

In order to estimate the reducing power of water-soluble antioxidants, the EDA of extract and crude saponin separated from red and white ginsengs to DPPH(α,α-diphenyl-β-picrylhydrazyl) was examined by the modified method of Yamaguchi[31].

To the mixture of 0.8*ml* of 5×10^{-3}M DPPH solution, 1.4*ml* of 0.1M phosphate buffer(pH 6.5) and 1.0*ml* of 99.5% ethanol, was added 0.1 *ml* of the each fractions. And then the mixture (total volume 3.3*ml*; final concentration 0.02%) was shaken in vortex mixer for ten seconds.

The reaction mixture was allowed to stand at room temperature for ten minutes, and the absorbance was measured by spectrophotometer at 525nm.

The EDA was calculated by the absorbance difference from control group.

(2) Inhibitory Effect on Lipoperoxide Formation

(a) Lipoperoxide Assay by TBA Value

In vitro :

The inhibitory effect of extract and crude saponin separated from red and white ginsengs on lipoperoxide formation was estimated by measuring TBA value according to the modified method of Mitsuda[44]. For experiments, linoleic acid and rat-liver homogenate were used as substrate.

Linoleic acid solution(3×10^{-3}M) was prepared with 0.1M phosphate buffer(pH 7.0) and 99.5% ethanol, and rat-liver homogenate(10%) was prepared by homogenizing rat-liver with saline-2% SDS(9:1, V/V) using Beckman homogenizer at 7,500 r.p.m. for 3 minutes.

To the mixture of 0.1M phosphate buffer(19.2 *ml*, pH 7.0) and 0.8*ml* of the each fractions (1%), was added substrate(20*ml*) to give the final concentration of 0.02%. The reaction mixture was slowly shaken in a shaking air-bath(60°C) at 50 r.p.m. The contents of lipoperoxide formed were measured in the course of time. One millilirer of 35% TCA and 2.0ml of 0.75% TBA were added to 2.0ml of the reaction mixture and were shaken for 30 seconds in vortex mixer, and reacted for 40 minutes in water bath(97±2°C) for coloration.

This was then cooled in cold water up to room temperature and added 1.0*ml* of acetic acid and 2.0*ml* of chloroform. The mixture was mixed by using vortex mixer and centrifuged at 3,000 r.p.m. for 5 minutes.

The supernatants were taken into a glass cuvette, and the absorbance was measured by spectrophotometer at 532 nm. TBA value for the formation of lipoperoxide *in vitro* was calculated from absorbance(OD 532)×100.

In vivo Experiment(Ⅰ) :

SD rats(200*g*) were divided into six groups(8 rats/group). Each fraction of extract and crude saponin was diluted with saline to give 1% solution.

One milliliter of each fraction was intraperitoneally administered(50*mg/kg* body weight) to rats. Six hours later, the peroxidation of liver lipid was induced with 1.0*ml* of 40% ethanol according to the intoxication method of Kalish[32].

Blood was collected by heart puncture under ethyl ether anesthesia after 5 hours and allowed to clot overnight in cold room. This was then centrifuged in cold room at 5,000 r.p.m. for 5 minute to separate serum. The TBA value of serum was measured by the same method as *in vitro*.

Liver and kidney were immediately removed after cutting the abdominal aorta under ethyl ether anesthesia, weighed, minced with scissors,

and homogenized with saline-2% SDS(9:1, V/V) at 7,500 r.p.m. for 3 minutes using Beckman homogenizer to give 10% homogenate. The TBA values of liver and kidney were measured by the same method as *in vitro*.

Blank and control groups were treated with an equal volume of saline. However, the lipoperoxidation of liver and kidney were induced by ethanol intoxication only for control group.

In vivo Experiment(Ⅱ):

SD rats(150*g*) were divided into six groups(10 rats/group). Each fraction of extract and crude saponin was diluted with saline to give 0.5% solution.

One milliliter of each fraction was orally administered(33.4*mg/kg* body weight) to rats at 10:00 a.m. every day for two weeks.

As described in experiment(Ⅰ), the peroxidation of liver lipid was induced by the ethanol intoxication method of Kalish[32] after two weeks. The TBA values of blood, liver and kidney were spectrophotometrically determined as the same method as *in vivo* experiment(Ⅰ). Blank and control groups were treated as the same method as experiment(Ⅰ).

(b) Estimation of Induction Period by Peroxide Value

In order to estimate the induction period in the initial stage of lipoperoxide formation, extract and crude saponin were added to substrate to give the final concentration of 0.02%, and the peroxide value(POV) was measured by the modified method of Omura[33]; to the mixture of 75% ethanol (4.7*ml*), 15% ammonium thiocyanate(0.1*ml*) and 2×10^{-2} ferrous chloride(0.1*ml*), were added the reactants, which were used for the measurement of TBA value *in vitro*, in the course of time.

The reaction mixture was shaken in vortex mixer for 10 seconds. After 3 minutes, the POV was spectrophotometrically determined by reading absorbance at 500 nm.

Induction period was calculated from the time required to reach the POV 0.4.

(3) Enzyme Activity on the Inhibition of Lipoperoxide Formation

(a) Determination of Superoxide Dismutase Activity

In vitro:

Superoxide dismutase(SOD), which scavenges and inactivates superoxide radical ($O_2\cdot^-$) and singlet oxygen($^1O_2$), catalyzes the dismutation of superoxide radical($O_2\cdot^-$) to hydrogen peroxide (H_2O_2) and oxygen(O_2). Note that in this reaction one $O_2\cdot^-$molecule is oxidized to O_2 while the other molecule is reduced to H_2O_2.

The SOD activity of extract and crude saponin *in vitro* was measured by the pyrogallol autoxidation method of Marklund[34]. Each fraction(1%, 60μl) was added to 2.9*ml* of 0.1M phosphate buffer(pH 7.0) in glass cuvette. The mixture was then preincubated in water bath(37°C) for one minute, added 0.1*ml* of 1M pyrogallol, shaken for 10 seconds in vortex mixer and recorded ΔA 420 nm vs. reference every 20 seconds for 3 minutes.

The autoxidation rate of pyrogallol was calculated from initial velocity(ΔA 420/min) by absorbance changes at 420 nm with respect to H_2O as a reference, and this was expressed as percentage inhibition against control group.

In vivo:

To examine the effect of extract and crude saponin on SOD activity *in vivo*, each fraction was intraperitoneally(50*mg/kg* body weight) and/or orally(33.4*mg/kg* body weight) administered to rats by the same method as the measurment of TBA value *in vivo*. One gram of rat-liver removed by cutting the abdominal aorta was homogenized with 9.0*ml* of 0.1M phosphate buffer (pH 7.0) on ice bath for 3 minutes, and centrifuged in cold room at 5,000 r.p.m. for 5 minutes.

To the liver supernatant(50μl) in glass cuvette, was added 2.9*ml* of 0.1M phosphate buffer(pH 7.0) and preincubated in water bath(37°C).

Pyrogallol(1M, 0.1*ml*) was then added to the mixture, shaken for ten seconds in vortex mixer

and recorded ΔA 420 nm vs. reference every 20 seconds for 3 minutes.

On the basis of this experimental data, the activity of SOD in rat-liver on pyrogallol autoxidation was converted into the values for 50μl of rat-liver homogenate, and the percentage inhibition of SOD against pyrogallol autoxidation was expressed as a ratio of administered group to control group.

(b) Determination of Peroxidase Activity

In vitro :

The effect of extract and crude saponin on peroxidase activity was measured by the modified method of Bergmeyer[35]; the 60μl of each fraction(1%) was added to the mixture of 0.1M phosphate buffer(0.32ml, pH 6.0), 0.147M hydrogen peroxide(0.16ml) and 5% pyrogallol (0.32ml) in a glass cuvette. To the mixture, was added 2.10ml of redistilled water to give the final concentration of 0.02% each fraction. This was then preincubated in water bath(25°C) for one minute. And then 10μl of the standard solution of peroxidase(0.05 unit) was added to the mixture, followed by shaking in vortex mixer for 10 seconds and by recording ΔA 436 nm vs. reference every 20 seconds for 3 minutes.

Peroxidase activity was calculated from initial velocity(ΔA 436/min) by absorbance changes at 436 nm with respect to H_2O as a reference, and expressed in terms of unit per mg sample as the following equation. And also percentage activity was expressed as a ratio of experiment group to control group.

$$\text{unit}/mg \text{ sample} = \frac{\Delta A\ 436/20\text{sec.} \times 3}{12^* \times mg \text{ sample in Rx. mix.}}$$

*12; extinction coefficient

In vivo :

In order to examine the effect of extract and crude saponin on peroxidase activity *in vivo*, each fraction was intraperitoneally and/or orally administered to rats by the same method as the measurement of SOD activity *in vivo*.

Using 100μl of the supernatant of rat-liver homogenate, peroxidase activity *in vivo* was measured by the same method as *in vitro* experiment; the activity was calculated from initial velocity (ΔA 436/min) by absorbance changes, and then expressed in terms of unit per mg wet liver as the following equation.

$$\text{unit}/mg \text{ wet liver} = \frac{\Delta A\ 436/20\text{sec.} \times 3}{12^* \times mg \text{ wet liver in Rx. mix.}}$$

*12; extinction coeffecient

Results and Discussion

Saponin Pattern of Red and White Ginsengs

Ginseng saponins are different from common crude drugs in their basic structure. Ginseng saponins are composed of the neutral glycosides of bisdesmoside constituted dammarane-type triterpene as an aglycone, whereas common crude drug saponins are mainly composed of various glycosides of monodesmoside constituted oleanane-type and steroid-type as an aglycone.

Fourteen ginsenosides have been isolated and identified. The structures of ginsenosides have been established; namely, ginsenoside-Ro in which sapogenin is oleanolic acid, ginsenoside-Ra_1, -Ra_2, -Rb_1, -Rb_2, -Rb_3, -Rc and -Rd in which sapogenin is 20(S)-protopanaxadiol, and ginsenoside -Re, -Rf, -Rg_1, -Rg_2, -Rh_1 and 20-glucoginsenoside-Rf in which sapogenin is 20(S)-protopanaxatriol.

In order to compare the saponin pattern of red ginseng with that of white ginseng, crude saponin separated from red and white ginsengs was analyzed by high-performance liquid chromatography using a carbohydrate analysis column. In the previous paper[14], it was confirmed that acetonitrile/water system(80/20, V/V) and acetonitrile/water/n-butanol system(86/14/10, V/V) were very effective mobile phases for diol and triol saponin analyses, respectively.

The composition of red and white ginseng saponins was shown in Table 1. The diol saponin contents of red and white ginsengs were 3.08% and 3.39%, respectively, and their triol saponin contents were 1.73% and 1.36%, respectively.

The diol saponin content of white ginseng was very close to that of red ginseng, but red ginseng showed greater triol saponin content than white ginseng. The total saponin contents of red and white ginsengs were 4.81% and 4.75%, respectively, and there was no difference between the two saponin contents.

Table 1. Compositions of red and white ginseng saponins

Aglycones	Ginsenosides	White ginseng (%)	Red ginseng (%)
Oleanolic acid	Ro	0.18	0.17
20(S)-protopanaxadiol	Ra_1+Ra_2	0.12	0.11
	Rb_1	1.48	1.35
	Rb_2	0.72	0.65
	Rb_3	—	—
	Rc	0.65	0.60
	Rd	0.42	0.36
	Total	3.39	3.08
20(S)-protopanaxatriol	20-gluco-Rf	—	—
	Re	0.52	0.37
	Rf	0.18	0.16
	Rg_1	0.37	0.24
	Rg_2	0.18	0.32
	Rh_1+Rh_2	0.05	0.09
	Prosapogenin*	0.02	0.18
	Unknown*	0.04	0.37
	Total	1.36	1.73
PT/PD ratio		0.40	0.56

* hydrolyzates obtained during extraction

The PT/PD ratios of red and white ginsengs were 0.561% and 0.401%, respectively. Red ginseng increased the PT/PD ratio in comparison with white ginseng. The great ratio difference between red and white ginsengs may be due to the change of saponin components during the steam-treated processing for red ginseng, and thus the change of PT/PD ratio is believed to be related to the biochemical and pharmaceutical effects of red ginseng.

The Effect on Electron Donating Ability

The electron donating ability(EDA) of extract and crude saponin separated from red and white ginsengs to DPPH was shown in Table 2.

Table 2. Electron donating abilities (EDA) of extract and crude saponin separated from red and white ginsengs to DPPH

Ginseng components (0.02% addition)	Decrease in OD525 during ten minutes	EDA (%)
Control	0.850	—
RG extract	0.716	15.76
WG extract	0.725	14.58
RG saponin	0.702	17.41
WG saponin	0.712	16.23

The extract and crude saponin of red ginseng showed higher % EDA than white ginseng, suggesting that red ginseng was stronger than white ginseng in antioxidative effect by reducing power.

It is believed that some browning pigments[36~40] such as melanoidin and caramel, some polyphenol compounds[41~45] such as maltol and quercetin, and some flavonoids[46] in red ginseng formed during the steam-treated processing were deeply related to antioxidative effect.

Despite of the removal of browning pigments, polyphenols and flavonoids, the crude saponin of red and white ginsengs still showed EDA activity, suggesting that crude saponin itself had EDA activity. And also the EDA activity of red ginseng saponin was higher than that of white ginseng saponin, and this may be attributed to saponin composition; that is, PT/PD ratios of red and white ginsengs were 0.561% and 0.401%, respectively, and red ginseng increased the PT/PD ratio in comparison with white ginseng.

Inhibitory Effect on Lipoperoxide Formation

(1) *In vitro Experiment*

The extract and crude saponin of red and white ginsengs were added to substrates to give the final concentration of 0.02%, and the TBA values were measured in the course of time as shown in Fig. 2.

The TBA value of white ginseng extract was lower than that of red ginseng extract, and this was different from EDA to DPPH *in vitro*. Th-

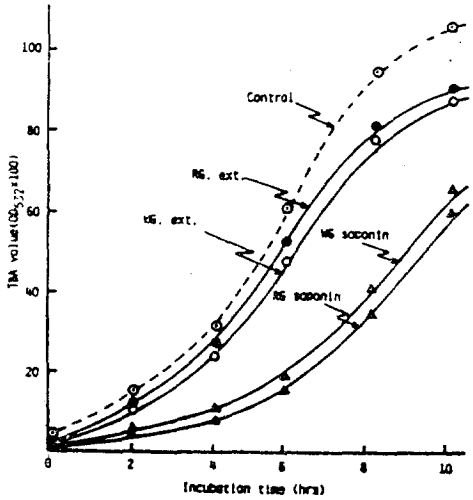

Fig. 2. Antioxidative activities of extract and crued saponin serarated red and white ginsengs at 60°C(0.02% addition).

erefore, it can be said that the inhibitory effect of red ginseng extract on lipoperoxide formation was slightly weaker than that of white ginseng extract.

Accordingly, it seems likely that prooxidants are possible to exist in red ginseng extract.

There were no singificant difference between reducing power and antioxidant activity[38], and this is in accord with earlier report that prooxidants exist in high molecular coloring substance[47].

In case of saponin, however, the inhibitory effect of red ginseng on lipoperoxide formation was stronger than that of white ginseng, and this seems to be resulted from PT/PD ratio.

As shown in Table 3, on the other hand, the induction period on lipoperoxide formation of extract and crude saponin separated from red and white ginsengs was measured by peroxide value; in the initial stage of lipoperoxide formation, the inhibitory effect of red ginseng was

Table 3. Induction period of extract and crude saponin measured by peroxide value

Ginseng components (0.02% addition)	Induction period* (minutes)
Control	30.0
RG extract	42.5
WG extract	80.0
RG saponin	135.2
WG saponin	187.5

* time required to reach the peroxide value 0.4 (OD500)

lower than that of white ginseng. This suggests that high molecular coloring substance is deeply related to the initial stage of lipid peroxidation.

(2) *In vivo Experiment*

The extract and crude saponin of red and wh-

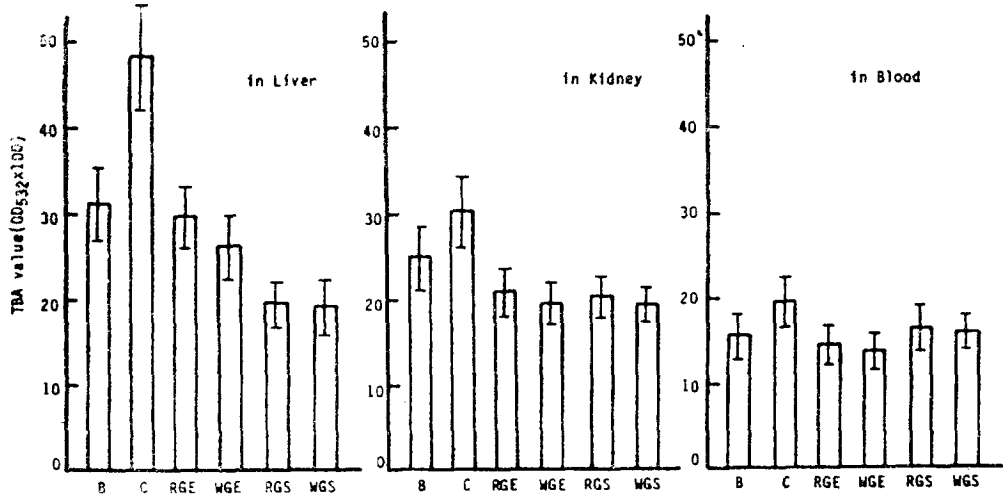

Fig. 3. Indibitory effect of extract and crude saponin interaperitoneally administered to rats on the formation of lipoperoxide($p<0.02$): B, blank; C, control; RGE, red ginseng extract WGE, white ginseng extract; RGS, red ginseng saponin; WGS, white ginseng saponin.

ite ginsengs were intraperitoneally(50*mg/kg* body weight) administered to rats, and the TBA values of blood, liver and kidney were measured, and the results are shown in Fig. 3.

In the inhibitory effect on lipoperoxide formation, the groups which administered the extract and crude saponin of red and white ginsengs, were greater than blank and control groups. In particular, the inhibitory effect on lipoperoxide formation was remarkable in liver, suggesting that the induction of lipid peroxidation by ethanol was mainly occured in liver.

Significant difference was recognized in liver of saponin group, in the kidney of extract and saponin group, and in the blood of extract group ($p<0.02$). The groups administered the crude saponin of red and white ginsengs showed higher inhibitory effect on lipoperoxide formation than the groups administered the extract. In case of intraperitoneal administration, however, signficant difference between red and white ginsengs was not found, and the inhibitory effect showed similar trend to the results obtained from *in vitro* experiment.

On the other hand, extract and crude saponin were orally administered(33.4*mg/kg* body weight) to rats for two weeks, and the TBA values of blood, liver and kidney were measured. The results obtained are shown in Fig. 4.

As shown in Fig. 4, inhibitory effects on the

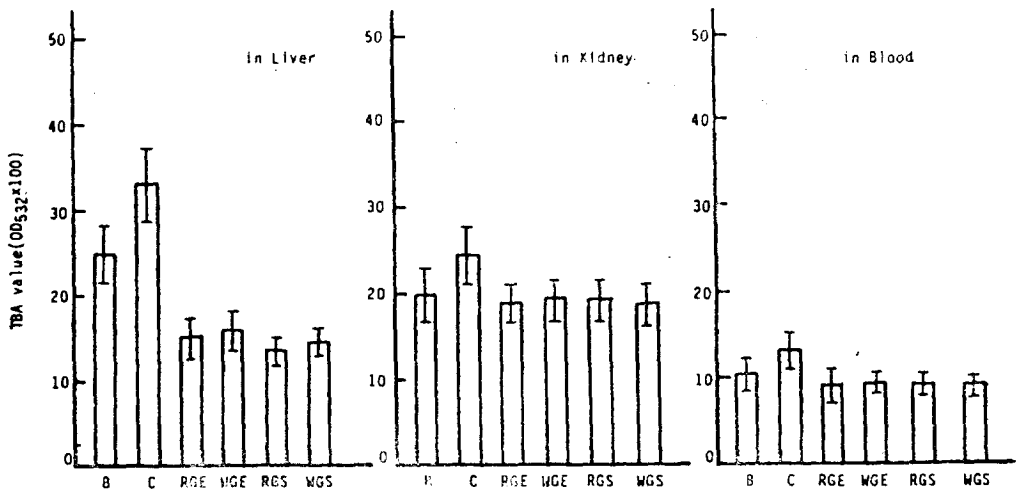

Fig. 4. Inhibitory effect of extract and crude saponin orally administered to rats on the formation of lipoperoxide($p<0.03$): B, blank; C, control; RGE, red gioseng extract; WGE, white ginseng extract; RGS, red giinseng saponin; WGS, white ginseng saponin.

lipoperoxide formation of extract and crude saponin separated from red and white ginsengs were remarkable in comparison with blank and control groups in liver when administered intraperitoneally, but there was no significant difference between the extract and crude saponin of red and white ginsengs. And also there was no significant difference between extract and crude saponin, red and white ginsengs in both kidney and blood.

Thus, inhibitory effect on lipoperoxide formation by ginseng administration mainly occurred in liver, indicating that the function of liver played an important role in anti-aging actions.

In particular, it is noteworthy to find that there was no significant difference between red and white ginsengs *in vitro* as well as in the experiment of intraperitoneal administration and that red ginseng was more effective than white ginseng in case of long-term oral administration.

It seems likely that inhibitory effect on lipoperoxide formation was related to the antioxidtive components which were formed during the steaming, drying and extraction of fresh ginseng.

The Effect on Enzyme Activity

(1) *Superoxide Dismutase Activity*

The extract and crude saponin of red and white ginsengs were added to substrate to give the final concentration of 0.02%, and the percent inhibition of superoxide dismutase on pyrogallol autoxidation was determined by measuring the initial velocity(ΔA 420/min).

Table 4. Effect of extract and crude saponin on pyrogallol autoxidation *in vitro*

Ginseng conponents (0.02% addition)	Rate of autoxidation* (ΔA 420/min)×10³	Inhibition /Control (%)
Control	33.0	—
RG extract	28.5	13.6
WG extract	31.0	6.1
RG saponin	29.0	12.1
WG saponin	29.5	10.6

As shown in Table 4, it was observed from both extract and crude saponin that red ginseng was greater than white ginseng in the inhibitory effect on pyrogallol autoxidation. The extract of red ginseng showed the inhibitory effect of 13.6%, and red ginseng was two times greater than white ginseng(6.1%). And it was also observed from crude saponin that in the inhibitory effect on pyrogallol autoxidation red ginseng was slightly higher than white ginseng.

These facts indicate that superoxide dismutase was able to decompose superoxide radical($O_2\cdot^-$) at the inital stage of lipid peroxidation, but this was not always consistent with the inhibitory pattern of lipoperoxide formation obtained from TBA values.

On the other hand, the extract and crude saponin of red and white ginsengs were intraperitoneally(50*mg/kg* body weight) and/or orally(33.4 *mg/kg* body weight) administered to rats by the same method as the measurement of TBA values *in vivo*, and SOD activity in rat-liver was compared with percent inhibition against pyrogallol autoxidation. The results obtained are shown in Table 5.

It was found from both intraperitoneal and oral administration, that the groups administered

Table 5. Effect of extract and crude saponin on superoxide dismutase(SOD) *in vivo*

Ginseng components (0.02% addition)	SOD activity(×10³) (50μl of 10% liver homogenate)		Inhibition/Control (%)	
	i.p.	p.o.	i.p.	p.o.
Control	29.25±0.42	29.75±1.82	—	—
RG extract	25.54±0.23	25.20±2.04	12.7	15.3
WG extract	28.02±0.80	28.12±2.56	4.2	5.5
RG saponin	26.04±0.65	24.60±2.17	10.9	17.3
WG saponin	27.50±1.30	25.65±2.92	5.9	13.8

the extract and crude saponin of red ginseng showed remarkable inhibitory effect in comparison with that of white ginseng.

The extract and crude saponin of red ginseng were 2.0 to 2.5 times higher than that of white ginseng in case of intraperitoneal administration (i.p).

In oral administration(p.o.) the extract of red and white ginsengs showed 15.2 and 5.5% inhibition, respectively, whereas the crude saponin showed 17.3 and 13.8% inhibition, respectively. It was observed that the extract and crude saponin of red ginseng were respectively 3.0 and 1.3 times greater than those of white ginseng in the percent inhibition.

In particular, oral administration showed more stronger inhibitory effect on lipid peroxidation than intraperitoneal administration. This suggests that it is desirable to administer ginseng continuously for a long period of time.

Thus, it was confirmed that red ginseng showed more stronger anti-aging effect than white ginseng *in vivo*. This may be due to the activity of antioxidation increased with the increase of

SOD activity in rat-liver; SOD activity was highest in liver, and those enzymes were present, for the most part, in liver[48]. Such a trend is in accord with the TBA values obtained from oral administration(p. o.) *in vivo*.

(2) *Peroxidase Activity*

The extract and crude saponin of red and white ginsengs were added to substrate to give the final concentration of 0.02%, and the effect of extract and crude saponin on peroxidase activity *in vitro* was determined by initial velocity (ΔA 436/min).

As shown in Table 6, there was no significant difference between red and white ginsengs. But the peroxidase activity of red ginseng saponin was slightly higher than that of white ginseng saponin.

Table 6. Effect of extract and crude saponin on peroxidase *in vitro*

Ginseng components (0.02% addition)	Peroxidase activity($\times 10^2$) (unit/*mg*)	Activity /Control (%)
Control	5.000±0.42	100
RG extract	5.264±0.34	105.3
WG extract	5.375±0.46	107.5
RG saponin	5.472±0.35	109.4
WG saponin	5.347±0.52	106.9

On the other hand, the extract and crude saponin of red and white ginsengs were administered to rats by the same method as the measurement of SOD activity *in vivo*, and peroxidase activity was measured by the same method as the measurement of peroxidase activity *in vitro*.

Table 7. Effect of extract and crude saponin on peroxidase *in vivo*

Ginseng components (0.02% addition)	Peroxidase activity($\times 10^5$) (unit/*mg* wet liver)		Activity/Control (%)	
	i. p.	p. o.	i. p.	p. o.
Control	4.20±0.32	6.08±0.42	100	100
RG extract	4.82±0.24	8.13±0.34	114.8	133.7
WG extract	5.12±0.30	7.85±0.26	121.9	128.9
RG saponin	5.60±0.26	8.38±0.42	133.4	137.8
WG saponin	5.35±0.34	8.08±0.50	127.4	132.9

As shown in Table 7, in intraperitoneal administration(i. p.) peroxidase activity was increased by similar trend to *in vitro*; that is, the peroxidase activity of red ginseng showed a tendency to be lower than that of white ginseng in extract, whereas the peroxidase activity of red ginseng was inclined to be higher than that of white ginseng in crude saponin.

It was also observed that the percent activity of the administered group to control group was in the range of 114.8 to 133.4%.

In the continuous oral administration(p. o.), it is noteworthy to state that the peroxidase activity of red ginseng showed a tendency to be higher than that of white ginseng in both extract and crude saponin administration. The percent activity of the orally administered group was higher than that of the intraperitoneally administered group, and the percent activity was in the range of 128.9 to 137.8%.

Thus, it was confirmed that the continuous oral administration for a long period of time was more effective compared with temporary administration.

As shown in Table 7, the peroxidase activity of oral administration(p. o.) was 1.5 times greater than that of intraperitoneal administration(i. p.) in the activity, and this showed a similar trend to the inhibitory effect on lipoperoxide formation obtained from TBA values *in vivo*.

This may be due to the fact that some saponins and its hydrolyzates formed by the various enzymes and acids in stomach during the digestion process were deeply related to the inhibitory effect on lipoperoxide formation.

約 要

紅蔘과 白蔘의 抗酸化作用에 의한 老化抑制作用을 究明하기 위하여 80% ethanol extract 및 水飽和부탄올로 移行한 saponin을 試料로 하여 *in vitro* 및 *in vivo* 實驗을 통하여 紅蔘과 白蔘의 老化抑制作用을 比較하였다.

DPPH에 대한 電子供與能(EDA), TBA value 및 peroxide value에 의한 過酸化脂質生成抑制作用, superoxide dismutase 및 peroxidase의 活性測定등으로 紅蔘과 白蔘의 老化抑制作用을 比較한 結果, 紅蔘은 長期間에 걸친 連續投與에서 白蔘보다 過酸化脂質生成抑制作用. superoxide dismutase 및 peroxidase의 活性을 有意性있게 增加하였다. 生體內實驗에서 紅蔘이 白蔘보다 老化抑制作用이 현저한 것은 蒸蔘 및 加工處理中 saponin pattern의 變化에 起因하며 白蔘의 PT/PD ratio가 0.401인데 反해 紅蔘의 PT/PD ratio가 0.561로서 triol saponin이 增加되었기 때문이다.

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