Korean J. Ginseng Sci.
Vol. 16, No. 3, 228~234(1992)

Chemical and Biochemical Studies on Non-saponin Constituents of Korean Ginseng

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(Received November 14, 1992))

Abstract□There has been general tendency to explain the traditional ginseng efficacy through the pharmacological and biochemical activities of ginsenosides. However, when we analyze the pharmacological and biological data on ginseng reported yet, we can easily arrive at the conclsion that most of the data on pharmacological and biological activities must have been obtained using impure ginsenoside samples, which should contain some non-saponin constituents as impurities. Based on the above background, the non-saponin constituents of ginseng were studied in our laboratory. Phenolic substances including Maltol, Vanillic Acid, Salicylic Acid, Ferrulic Acid and Caffeic acid and impure ginsenoside samples were found to show strong antioxidant and anti-fatigue activities, while pure ginsenosides were devoid of the activities. Maltol, one of antioxidant components in Korean red ginseng drew a special interest due to its very low pro-oxidant acitivity. The antioxidant activity of ginseng may be considered as scientific basis for the anti-ageing activity which was described in traditional medicinal material book as "long-term medication of ginseng will improve bio-efficiency and extend life-span". The lignan components, another non-saponin consitutents, isolated from ginseng extract in our laboratory may eplain the hepato-protective activity of ginseng which has been repeatedly claimed as one of the efficacies of ginsenosides. The β-carboline alkaloids isolated in our laboratory as one of the non-saponin constituents of ginseng may play some pharmacological activites which should also be investigated. Present paper will include chemistry and biochemical aspects of the non-saponin constituents of ginseng with special interests for the explanation of traditional ginseng efficacy on modern scientific basis.

My laboratory engaged for a long time in the studies of chemical and biochemical aspects of saponin constituents of Korean ginseng. The major topics of my studies on Korean ginseng were; 1) Isolation of ginseng saponins as the antiinflammatory components, 2) Synthesis of radio-labelled ginsenosides (^{3}H and ^{14}C),[1,2] 3) Metabolic fate and ADME-studies on ginsenosides,[3] 4) Establishment of RIA-method for ginsenosides,[4] and 5) Chemical studies of acid-catalized transformation products of ginsenosides in gastro-intestinal tract.[5]

During my studies on ginsenosides, I felt from the studies of references published by other scientists that a great number of research papers concerned with pharmacological activities of ginsenosides were worked out using impure ginsenosides as the test samples.

There will be so many experiences in the world of ginseng sciences that the pharmacological activities observed with impure ginsenoside fraction were not reproducible with the purified ginsenoside samples. These facts may imply that the pharmacological activities observed with impure ginsenoside samples would be displayed in part by impurities in the ginsenoside mixture.

We have many experiences that chromatography showed strong UV-absorption when the ginsenosides were not crystallized. These results suggested that the chemical entities of impurities in the apparently pure ginsenoside samples might be phenolic substances. These assumptions led us to assess the anti-oxidant activity of ginseng extract and to isolate the antioxidant components from the extract of Korean Ginseng.

1. Anti-oxidant components of Korean ginseng

In 1969, I. I. Brekhman claimed a new concept "Adaptogenic Activity Theory" for the pharmacological activity of panaxosides (ginsenosides) in his

review article.[6] This theory was so much sensational that general trends of the chemical and pharmacological researches on ginseng thereafter had been for a long time guided to the studies of ginseng saponins, the ginsenosides. In the same review article, I. I. Brekhman also claimed the same adaptogenic activities of the lignan components, phenolic substances in both plants, Eleutherococcus senticosus and Schizandra chinensis. However it would be unlikely from the Brekhman's review article that the molecular species of the adaptogenic substances of both *P. ginseng* and *E. senticosus*, which belong to same taxonomic family, have different chemical natures, one is dammarane triterpene glycoside and the other is lignan compound, i.e., phenolic substance, on the other hand that the adaptogenic substances in *E. senticosus* and *S. chinensis* which belong to different taxonomic families, have the same chemical nature. Based on the above background, we had deep interest on the possible anti-oxidant activity of phenolic substances in Korean ginseng.[7] In order to understand the pharmacological significance of biologically active anti-oxidant substances, a brief summary on the gerontologists' view on the cellular ageing will be discussed.[8]

Living cells produce very reactive and harmful free radical oxygen species such as singlet oxygen, superoxide anion and hydroxy radicals in parallel with the consumption of oxygen for their respiration. Although the greater part of them are quenched by some self-protective system, a part of them leaks from the protective system and attacks the unsaturated fatty acids in various biomembranes, leading to the production of lipid peroxides and finally resulting in the decreased vital efficiency of the cell.

Once again the greater part of lipid peroxides are reduced to less reactive hydroxy lipIds, but a part of them leaks from the protective system and decomposes to produce highly reactive malondialdehyde.

This binds nonspecifically to nearby biomacromolecules such as enzyme to produce lipofuscin pigment which will be accumulated in living cells in parallel with cellular ageing. The membrance damage caused by those free radical chain reaction products and the protein binding of malondialdehyde are believed to be the cause of various geriatric diseases and ageing of living body. Based on these backgrounds, the gerontologists assume biologically active anti-oxidants to be the anti-ageing agents.

Our first approach to the studies on the anti-ageing drug was started from the in vitro screening on free radical quenching activities of 120 herbal drugs and some ceral extracts, using diphenyl picryl hydrazyl (DPPH) as the free radical reagent. Almost more than 40% of plant extracts showed strong quenching activities for the DPPH free radical.

This wide spread distribution of free radical quenching substances in the herbal drug is very suggestive to speculate the possible role of chinese medicines which sometimes show dramatic therapeutic responses to some geriatric diseases.

It was very difficult to select any specific plants from our in vitro screening data for our phytochemical studies to isolate tha free radical quenching components, since flavonoids and tannins which are widely distributed in the plant kingdom seem to exhibit free radical quenching activity in the in vitro experiments.

In the subsequent approach, we selected 30 herbal drugs as the candidates for anti-oxidant activity screening test by animal feeding experiments, referring to our in vitro screening data and the descriptions in Chinese Material Medical Book in which tonic or anti-ageing activity were strongly suggested.[9]

Pharmacological doses of plant extracts were fed to mice and then acute toxic doses of 50% ethanol were administered orally to induce lipid peroxidation. The animals were killed 12 hours after the enthanol adminstration, and livers were taken for the assay of lipid peroxide content by Masugi's TBA-value procedure.

Some of the plant extracts including *Panax ginseng* showed strong inhibitory activities on the lipid peroxide formation induced by ethanol intoxication. Animals for blank test group showed TBA-values of around 10 units, and the ethanol group 38 units, whereas the animal group treated with ginseng extract and ethanol only 12 units. This dramatic biolo-

Table 1. Anti-oxidant activiteis of ginseng components: Samples were administered orally to mice once daily for 3 days and starved for 8 hrs after last medication. Lipid peroxidation was induced by ethanol intoxication and lipid peroxide contents in the mice liver were assayed by Masugi's TBA method 12 hrs after ethanol intoxication. TBA values were expressed as A_{535}/g wet liver.

	Bland	Ethanol-control	Sample, mg/30 g b. wt. and Ethanol			
			0.001	0.01	0.1	1
Maltol	10.4	31.6	30.7	19.5	12.6	11.8
Salicylic acid	9.7	25.3	21.6	18.2	14.7	13.3
Vanillic acid	10.1	23.4		19.8	15.2	14.4
p-coumaric acid	8.1	15.2	14.2		12.6	
Ginsenoside Rg_1	9	27.5				24
Ginsenoside Re	9	27.5				22.4
Ginsenoside Rb_2+Rc	9	27.5				28.3
Ginsenoside Rb_1	9	27.5				25
α-Tocopherol acetate	10.1	38.5				11

gical data prompted us to isolate the effective components of ginseng by monitoring the anti-oxidant activity. The ether soluble acidic fraction and butanol soluble glycosidic fraction showed the strong activity, whereas the ether-soluble neutral fraction and highly polar water-soluble fraction were devoid of the activity.

The ether-soluble acidic fraction was further purified to obtain the active substanances in a pure crystalline state by silica gel column chromatography.

They were identified as simple substances, maltol, salicylic acid, vanillic acid and *p*-coumaric acid.[10]

Strong anti-oxidant actitivites of the former three subastances were confirmed by the animal experiments, but *p*-coumaric acid was devoid of the activity, However, maltol was found only in the extracts of red ginseng and the other components were found in the extracts of both red and white ginseng. This probably suggests that maltol is one of the artefact products produced by the heat treatment of ginseng during the manufacturing process of red ginseng. Many other phenolic substances such as ferulic acid and caffeic acid were detected on TLC, from the ether soluble acidic fraction, but there are still many other phenolic substances unidentified yet.

Although further studies are still awaited for the complete elucidation of chemical entities of the anti-oxidant components in ginseng, it will be possible to assume the phenolic substances to be the

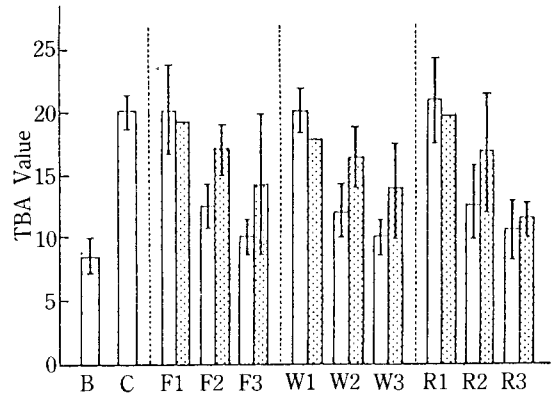

Fig. 1 The effect of ferric ion treatment on the antioxidant activity of ginseng extracts; Blank column: non treated ginseng groups. Shaded column; Ferric ion treated ginsong groups, B: blang, C: control (ethanol intoxication only). To F-1, F-2, F-3, W-1, W-2, W-3, R-1, R-2, and R-3 group animals, the ginseng samples were administered for 3 days before the induction of lipid peroxide by ethanol intoxication was conducted. Each group was consisted of 4~5 mice and the results are the mean value of four repeated experiments. The prefix F, W, and R denote fresh ginseng, white ginseng and red ginseng. The suffix -1, -2, and -3 denote the dosages of 0.2, 2.0, 20 mg ginseng/30 g body wt. mouse.

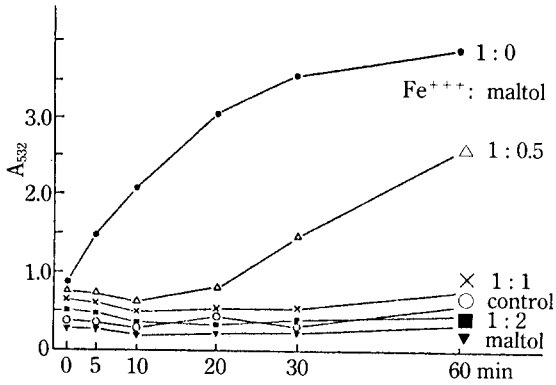

Fig. 2. Inhibitory effect of maltol on the Fe^{+3}- catalyzed oxidation of liver homogenate: 0.2 m*l* of 0.05 M $FeCl_3 \cdot 6H_2O$ and given molar equivalent maltol in 0.175 M KCl and 0.4 m*l* of 9% mouse liver homogenate were incubated at 37℃ for specified time. Lipid peroxide was measured by TBA method (A_{532}).

effective principles of anti-oxidant activity of ginseng. Noteworthy is the fact that none of the purified ginsenosides showed anti-oxidant activity in both in vitro and in vivo animal tests, whereas the semi-purified ginsenoside samples showed strong activity. Therefore it may be inferred that the anti-oxidant activity of impure ginsenoside mixture must be due to the contamination of the impure ginsenoside samples by the phenolic substances.

We are still trying to isolate some phenolic glycosides from butanol soluble fraction which is highly active in our anti-oxidant activity tests by animal experiments.

In Chinese Material Medical Book we can find a description that ginseng is contraindicated the contact with iron. Actually folkloric customs eliminate all iron-made tools in processing ginseng including decoction and peeling fresh ginseng. These folkloric customs together with the description of Chinese Material Medica Book are very suggestive for our understanding of the real nature of the pharmacologically active principle of ginseng.

In order to see whether the folkloric experiences are reproduced in our modern molecular pharmacological experiments, we added a very small amount of ferric ion to the extraction process of ginseng instead of using iron vessel. It was found in our animal experiments that the anti-oxidant activities of the ginseng samples pretreated with ferric ion were considerably reduced.[11]

2. The anti-oxidant activity mechanism of phenolic substances[12]

When we incubate liver homogenate, the lipid peroxide contents are increased gradually in proportion to incubation time due to the activation of NADPH-dependent microsomal enzyme system for lipid peroxidation. This increase was dramatically enhanced when a small amount of ferric ion was added to the homogenate. This dramatic increase was completely blocked by concomitant addition of two molar equivalent of maltol.

It was also found in our laboratory that two mol. maltol and one mol. feffic ion form a stable chelate.

Thus the formation of a stable maltol-Fe^{+3}-chelate may be considered as the mechanism of anti-oxidant activity of maltol which effectively deprives the ferric ion from ADP-Fe^{3+} complex that plays as the initiation factor in the free radical chain reaction of microsomal enzyme system.

3. Biochemical studies on the antioxidant activity of maltol

Plant extracts contain a wide variety of phenolic compounds, such as chalcones, and the hydroxylated flavonoids. Flavonoids and other phenolics have been demonstrated to possess multiple biological activities including antioxidant activity. However, plant phenolics have often been reported to show pro-oxidant activities as well as anti-oxidant activity. For example, gossypol, isolated from the cotton seeds, has been shown to have prooxidant activity by catalyzing O_2-dependent DNA degradation in vitro[13] and by generating superoxide radical in the presence of liver microsomes and NADPH.[14]

Polyphenolic compounds have an iron chelating activity, and many of them are able to reuce Fe^{3+} to Fe^{2+} ions participate in free-radical reactions by decomposing lipid peroxides to alkoxyl radicals and by reacting with H_2O_2 to give hydroxyl radical as shown by Iron Catalyzed Haber-Weiss Reaction.

(1) $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + \cdot OH + OH^-$

(Fenton reaction)

Table 2. Anti-fatigue activity of ginseng components: Ginseng components were fed to mice (20 mice/group) once daily for 3 days. Mice were subjected to swim 2 hours after last feeding in 24℃ water pool until drowned and recorded the swimming time in minutes.

Exp-1		Exp-2		Exp-3	
Samples 0.22g/kg. wt.	Swimming time (min)	Samples 10 mg/kg. wt.	Swimming time (min)	Samples 10 mg/kg. wt.	Swimming time (min)
Control	88.1± 26.9	Control	108.4± 55.7	Control	122.4± 44.6
Water Ext.	107.6± 41.3	Maltol	162.6± 63.5	Ginsenoside	
Ether Fr	131.9± 43.0	Salicylic	167.5± 69.4	Rg_1	99.3± 26.0
BuOH Fr.	117.2± 42.5	acid		Re	125.5± 41.6
Water Fr.	96.0± 28.7	Vanillic acid	160 ± 73.1	Rb_1	128.4± 46.0

(2) $Fe^{3+} + O_2 \cdot^- \rightarrow Fe^{2+} + O_2$

(3) $H_2O_2 + O_2 \cdot^- \rightarrow \cdot OH + OH^- + O_2$

(Iron-catalyzed Haber-Weiss reaction)

In my laboratory, studies are being carried out to elucidate the biochemical mechanisms of maltol isolated from Korean ginseng as an antioxidant with little proxidant property.

As indicated by the above Fenton reaction and iron catalyzed Haber-Weiss reaction, the capability of reducing Fe^{3+} to Fe^{2+} is a prerequisite for a phenolic compound to have prooxidant activity, although the magnitude of pro-oxidant activity is determined by various factors such as affinity for Fe ions, solubility and metabolic pathway, etc. Several test systems have been utilized to assess antioxidant or prooxidant activity in our laboratory.

When the antioxidant activity of maltol was assayed by non-enzymic lipid peroxidation induced with rat liver microsomal fraction, Fe^{3+} and ascorbate, maltol gave a relatively high IC_{50} value of 4.9×10^{-4} M as compared to other known antioxidants such as BHT and α-tocopherol, indicating that maltol is not a strong antioxidant.

Prooxidant activity of a compound is being measured by the tendency of the compund to generate hydroxyl radicals in a given system. Hydroxyl radicals thus generated can be measured by deoxyribose method[15] and DNA-bleomycin assay.[16]

Using these methods, prooxidant activity of maltol was compared to other known antioxidants. It was demonstrated that maltol possessed little prooxidant activity (unpublished results). The lack of prooxidant activity of maltol was reinforced by the findings that maltol at 2 mM did not reduce NBT (nitroblue tetrazolium) and showed a diminished Fe^{3+}-reducting ability compared to pyrogallol when reduction of Fe^{3+} ions was measured as the bathophenanthroline sulfonate-Fe^{2+} complex. Therefore, in spite of the relatively weak antioxidant activity, maltol with little prooxidant activity should be excpected to protect the biological system against oxidative stress. Recently the possible use of Fe^{3+}-maltol complex to cure iron deficlency is being discussed. This fact reflects the safety and efficacy of maltol as an antioxidant.

4. Anti-fatigue activity of ginseng[17]

The anti-oxidant activity of ginseng may be considered as an underlying mechanism for other pharmacological activities which have been repeatedly reported as the ginseng efficacy by others. Such activities are as the followings: 1) protection from radiation injury, 2) protection of liver from drug intoxication, 3) prevention of hangover symtoms, 4) anti-fatigue activity, 5) anti-artherosclerosis and 6) anti-thrombosis, etc. Here we have a question whether those activities are arising from ginsenosides or from phenolic substances included in ginseng saponin fraction as impurity. It is very difficult to give a decisive conclusion at present, but I will give very suggestive data on this problem.

Twenty years ago I. I. Brekhman reported the anti-fatigue activity of ginseng and panaxosides by the increase of swimming time of mice. In our laboratory we reexamined the anti-fatigue activity of ginseng and ginsenosides by the same swimming

test as Brekhman did. The distribution of anti-fatigue activity in various fractions of ginseng extract was very similar to that of antioxidant activity in the ginseng fractions. Brekhman's anti-fatigue activity was reproduced in our experiment on the ginseng extract and impure ginsenoside samples, but not on any purified ginsenosides which were isolated in our laboratory by repeated crystallization.

On the other hand, maltol and phenolic acids isolated as the anti-oxidant components of ginseng were strongly active in the prologation of swimming time.

5. Lignan components

Recently two lignan components were isolated from the ether soluble neutral fraction of ginseng in our laboratory. They were identified spectrometrically as the known lignan components, Gomisin A and N which had already been described by others as the hepatoprotective and adaptogenic substances of *S. chinensis*. Thus it will be possible to deduce that a part of adaptogenic and hepatoprotective activity of ginseng may be played by the lignan components and phenolic substances in ginseng.

6. Alkaloid components

The presence of choline in ginseng extract has already been described by others. Recently we detected twelve Dragendorff-positive spots on TLC, and designated them tentatively GA-1, 2, ···, 12 according to the increasing polarity (Hexane: EtOAc/2 : 1). The major alkaloid components, GA-4, GA-11, and GA-12 were isolated by us from the alkaloidal fraction of Korean ginseng and the structures were established by spectrometry as being N_9-formylharmance, ethyl-β-carboline-1-carboxylate and perlolyrine. The remaining minor alkaloid spots were isolated by other Korean scientists and the structure of them were establisbed by spectrometry as being the β-carboline derivatives. The same β-carboline alkaloids had been isolated in our laboratory from *Codonopsis lanceolata, Polygala tenuifolia* and *Lycium chinensis*. It is very interesting that these four plants are used as tonic agent in oriental medicine.

7. Other non-saponin constituents

We isolated iso-maltol-α-D-glucoside, ketopropyl-α-D-glucoside and adenosine from the saponin fraction of Korean ginseng. The pharmacological properties of isomaltol-α-D-glucoside and ketopropyl-α-D-glucoside were not investigated yet. It is well known fact that adenosine is strongly bioactive substance which modify various biological responses in the in vitro systems. However it is also very clear that no pharmacological significances would be expected from adenosine due to its short biological half-life in biological system.

8. Conclusion

We isolated many non-saponin constituents including anti-oxidant phenolic substances, Maltol, lignan components, alkaloidal components and some other non-saponin constituents. As far as anti-oxidant components are concerned, we are trying to explain the ginseng efficacy by the clearing effects on the biological sludges, which are produced by abnormal oxidation of unsaturated fatty acids in various biological membrane, through their anti-oxidant activity. This property of anti-oxidative phenolic substances may explain a singificantly great portion of adaptogenic activity of Korean ginseng.

Chinese description 久服輕身延年 found in old Chinese Material Medica Book which means "repeated medication of ginseng for a long time will increase the vitality and life-span" may be explained by our finding of strong antioxidant activity of Korean ginseng.

References

1. Woo, L.K.: *J. Pharm. Soc. Korea*, **17**, 123 (1973).
2. Han, B.H. and Woo, L.K.: *Arch. Pharm. Res.* **1**, 27 (1978).
3. Han, B.H., Park, M.H. *et al.*: *Kor. Biochem. J.*, **19**(3), 213 (1986).
4. Sankawa, U., Han, B.H. *et al.*: *Chem. Pharm. Bull.* **30**(5), 1907 (1982).
5. Han, B.H., Sankawa, U., Tanaka, O. *et al.*: *Planta Medica*, **44**(3), 144 (1982).
6. Brekhman, I.I. and Dardymov, I.V.: *Ann. Rev. Pharmacol.*, **9**, 419 (1969).
7. Han, B.H., Woo, L.K. and Park, M.H.: *Kor. Biochem. J.*, **12**, 33 (1979).
8. Pryor, W.A.: *Free Radical in Biology*, Academic Press, 1976.

9. Han, B.H., Yoo, S.Y., Park, M.H. and Lee, H.J.: *Korean J. Pharmacol.*, **10**, 108 (1979).
10. Han, B.H., Park, M.H. *et al.*: *Arch. Pharm. Res.*, **4**(1), 53 (1981).
11. Han, B.H. and Park, M.H.: *Kor. J. Pharmacog.*, **9**, 169 (1978).
12. Han, B.H., Park, M.H. and Han, Y.N.: *Kor. Biochem. J.*, **18**(4), 337 (1985).
13. Srivastava, A.K. and Padmanaban, G.: *Biochem Biophys Res Commum*, **118**, 1515 (1984).
14. Peyster, A.de, Quintanilha, A., Packer, L. and Smith, M.T.: *Biochem Biophys Res Commum*, **118**, 573 (1984).
15. Harriwell, B. and Gutteridge, J.M.C.: *Anal Biochem*, **165**, 215 (1987).
16. Gutteridge *et al.*: *FEBS Lett*, **123**, 71 (1981).
17. Han, B.H., Park, M.H. and Han, Y.N.: *Yakhak Hoeji*, **28**(4), 231 (1984).