THE PREVENTIVE EFFECT OF KOREAN GINSENG SAPONINS ON AORTIC ATHEROMA FORMATION IN PROLONGED CHOLESTEROL FED RABBITS

Chung No, Joo
Department of Biochemistry, College of Science, Yonsei University
Seoul, Korea

Introduction

It has been realized from the previous work in this laboratory that the ginseng saponins, one of the major components of Korean ginseng (C.A. Meyer) roots, are surface active and effective solubilizer of non-polar lipids such as cholesterol and triglycerides in aqueous medium. It was also found that moderate amounts of the saponins stimulated not only pancreatic lipase and cholesterol esterase *in vitro*, in which the substrates were water-insoluble triglyceride and cholesterol ester respectively, but also stimulated various dehydrogenases such as mitochondrial dehydrogenases, alcohol dehydrogenase, and aldehyde dehydrogenase so far examined in this laboratory (Joo *et. al.* 1973; Joo and Lee, 1976).

It was realized from the previous work (Joo et. al, 1980) in this laboratory that the ginseng saponin has some effects of preventing the increase of serum cholesterol level of prolonged cholesterol fed animals.

Popov (1975) has demonstrated that the introduction of ginseng extract in addition to usual therapies (such as medication, diet and revitalization) lowered the blood cholesterol level (280–310 mg %) of patients down within a normal level (below 250 mg %).

Naham (1961) examined cytologically the aorta, heart, coronary arteries and atherosclerosis of rabbits which have been suffered from hypercholesterolemia caused by long administration of cholesterol and reported that atheroschlerosis like change and sudanophilic were not formed in ginseng adminstered rabbits.

In connection with the above experimental results, it was attempted in the present study to observe the preventive effects of ginseng saponin against aortic atheroma formation of prolonged cholesterol fed rabbits.

Experimental Materials and Methods

1. Materials

25.05 g of ginseng saponin mixture were obtained from 1.2 kg of powdered Korean white ginseng roots (Keumsan, 4 years, 50 pieces/ 300 g) according to the modified procedure described elsewhere (Joo and Han, 1976).

Cholesterol, Na-cholate, absolute ethanol and vanilin were the products of Merck Co. and cholesterol-4-^{14}C, POPOP [1, 4-bis-(5-phenyl-oxazol-2-yl)-benzene], POP [(2.5-diphenyloxazole) were obtained from New England Nuclear.

Other reagent were obtained from Wako Co. Japan, and organic solvents for extraction were purchased from local market and used after redistillation.

2. Animals

All experimental animals (rabbits) were fed on ordinaly diet (Jeilsalyo Co. Product), which contained crude protein above 19.6%; crude cellulose below 7.0%; crude ash below 9.0%; Ca below 0.6%; P below 0.4% DCP above 16.5%; TDN above 7.3%; antibiotis below 50 ppm until required.

For the observation of the saponin effect on aortic atheroma formation, the ordinary domestic rabbits were fed with 500mg of cholesterol, 3g of corn oil and 100 mg of Na-cholate with 10 mg of the saponin (test group) and without the saponin (control gruop) in bean curd refused daily for suitable the period of time (2,4 and 6 weeks).

3. Chemical analysis

Total lipids were determined according to Joseph (1972). Cholesterol was determined according to Kenny (1952). Direct estimation of serum cholesterol without extraction was made according to pearson et. al, (1953). The amounts of triglycerides were determined by the method of Gottfried and Rosenberg (1973). The total amounts of serum protein were estimated according to Lowry et. al. (1951) and lipid phosphorus was determined according to Joo (1963).

4. Observation of the radioactivities in blood serum of prolonged cholesterol fed with and/ or without the ginseng saponin prior to cholesterol-4-^{14}C administration on time course.

After 4–5 weeks' administration of cholesterol diet (with test group) and without saponin (control), the animals were starved for 24 hr. followed by the administration of cholesterol-4-^{14}C and the radioactivities of the blood serum lipid fraction were traced.

5. Observation of the saponin effect on lipoprotein lipase activity of blood plasma of normal and prolonged cholesterol fed rabbits with and or without the saponin.

As described in section 2 and 4, three groups of rabbits (test, control and normal groups) were fed with the corresponding diet for 5 weeks respectively. To one of each group, 400 units of heparin in saline (0.2 ml) were injected intravenously and the blood was taken from ear vein after 15 min. following the injection.

From the other remained one of each group, the blood was taken without heparin injection in similar way as described above.

The lipoprotein lipase activities were determined by measuring the fatty acids liberated during the incubation of the following reaction mixture in the presence of blood plasma of the experimental animal.

The reaction mixture contained (total volume: 1 ml); 0.1 ml of 1.25% rabbit chylomicran, 0.28 ml of 0.25 M ammonium buffer (pH 8.5), 0.02 ml of 1M $CaCl_2$, 0.2 ml of 10% bovine serum albumin (pH 8.5), and 0.2 ml of blood plasma in the presence or absence of various concentrations of the ginseng saponins.

Results and Discussion

It has been observed in the previous work in this laboratory that the ginseng saponin has some effects on hypercholesterolemia induced by prolonged cholesterol feeding in rabbits. (Joo et.al, 1980). Present work again clearly showed that the saponin administration lowered the raised total lipid and cholesterol levels of blood sera by continuous cholesterol feeding significantly untill 4 weeks' feeding as shown in table 1. However, the longer feeding of cholesterol diet more than 4 weeks, no appreciable effect of the saponin could be observed. It seemed that when the blood cholesterol level raised up to over certain level, the saponin might not work any longer since we have observed in the previous work that no appreciable effect of the saponin in lowering cholesterol level (733 mg %) of the serum of the rabbits fed with cholesterol for the first three weeks prior to the ginseng saponin administration (Joo, et. al, 1980).

The above experimental results suggested that the saponin might work to delay in the rise of cholesterol level of the blood, subsequently, the saponin was expected to prevent aortic atheroma

Table 1. Variation of blood serum lipid of rabbits administered 500 mg of cholesterol, 3 g of corn oil and 100 mg of Na-cholate per day per rabbit with 10 mg of ginseng saponin (test group) and without the saponin (control) on time course (2, 4 and 6 weeks). The figures are mean values of three rabbits in mg %.

Fed Condition	normal	2 weeks		4 weeks		6 weeks	
Component	diet	Control	test	Control	test	Control	test
Total lipid	174.8	1229.8	458.1	1112.6	599.9	1614.0	1813.2
Tri-glyceride	40.0	65.0	55.0	98.9	77.5	92.4	105.0
Chole-sterol	95.5	782.7	222.9	725.4	388.5	1005.4	1134.5
Phospho-lipid	60.6	215.7	169.5	162.8	190.8	215.4	255.0
TL/P	2.9	5.7	2.8	6.9	3.1	7.5	7.1
TG/P	0.7	0.3	0.3	0.6	0.4	0.4	0.4
CH/P	1.6	3.6	2.8	4.5	2.0	4.8	4.6
Protein	5.8	6.4	6.2	7.2	6.6	6.5	6.6

formation, which used to be associated deeply with hypercholesterolemia occured in prolonged cholesterol fed rabbits.

We examined the atheroma formation in aorta, coronary and renal arteries of prolonged choloesterol administered rabbits (2–6 weeks) with saponin (test group) and/or without (control) microscopically.

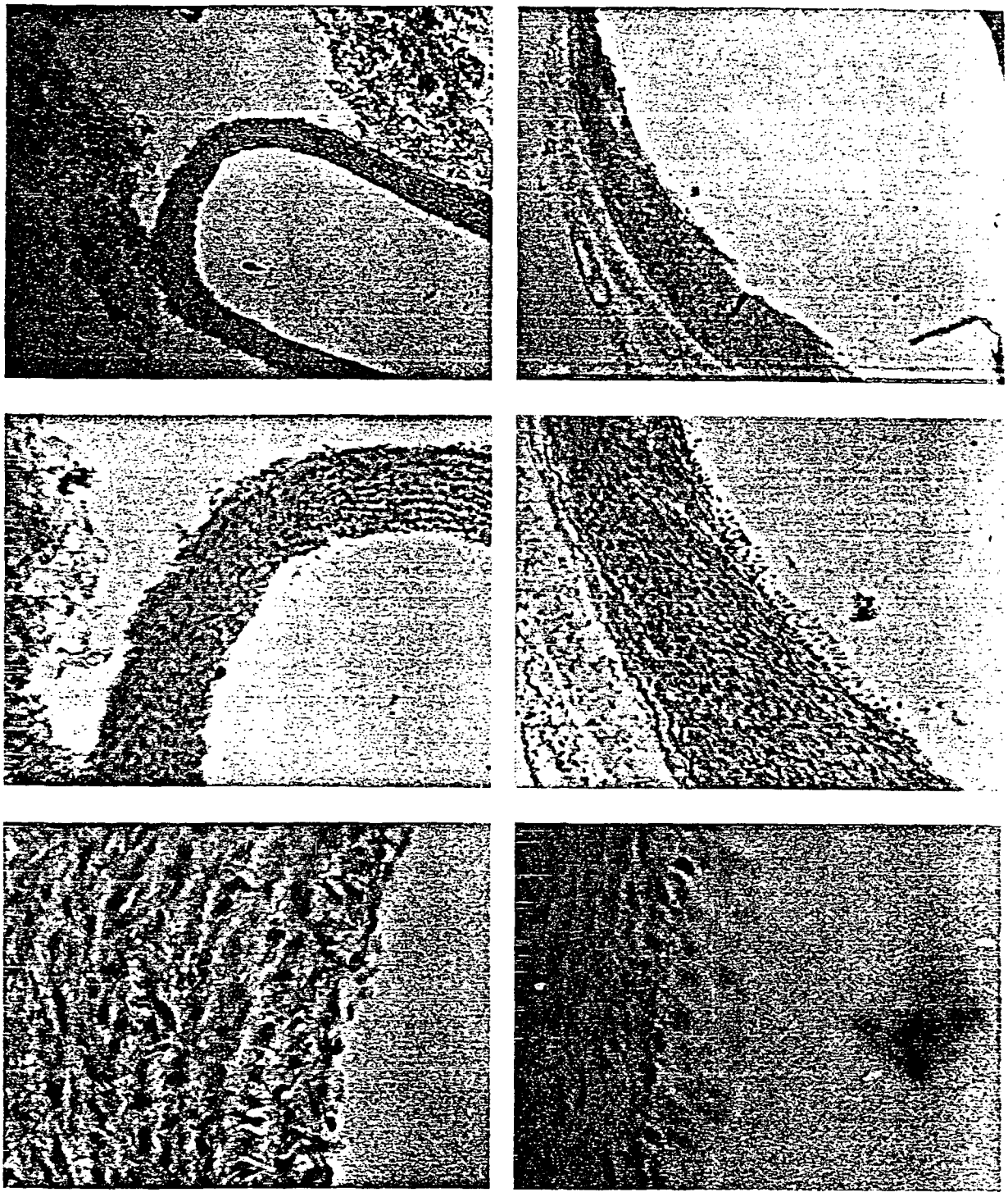
As shown in table 2, a slight macroscopical occurence of atheroma in ascending aorta could be seen even within two weeks administered control-group and the symptom gradually developed as the cholesterol feeding was continued. However, in the rabbits of saponin fed group the occurrence of the atheroma was not observed within 4 weeks' feeding but a slight symptom of atheroma formation occured after 6 weeks' feeding. Observation of histological severity of the atheroma of the control and test groups showed the similar pattern as described above. Figure 1 showed clearly the preventive effect of the saponin on atheroma formation in ascending aorta. Under the present experimental conditions, no atheroma symptoms occured in coronary and renal arteries, and thoracic aorta but a slight symptoms in abdominal aorta of rabbits of 6 weeks' fed control group.

It has been reported that the examination of aorta, heart, coronary arteries and atherosclerosis

Table 2. Macroscopical occurrence and histological severity of atheroma in aorta of prolonged cholesterol fed rabbits with and/or without ginseng saponin administration.

Location	Ascending aorta			Thoracic aorta			abdoninal aorta		
Feeding period (week)	2	4	6	2	4	6	2	4	6
Animal group									
Cholesterol fed group I	+	+	++	−	−	−	−	−	+
II	×	××	×××	−	−	−	−	−	×
Clolesterol and Saponin fed group I	−	−	+	−	−	−	−	−	−
II	−	−	×	−	−	−	−	−	−

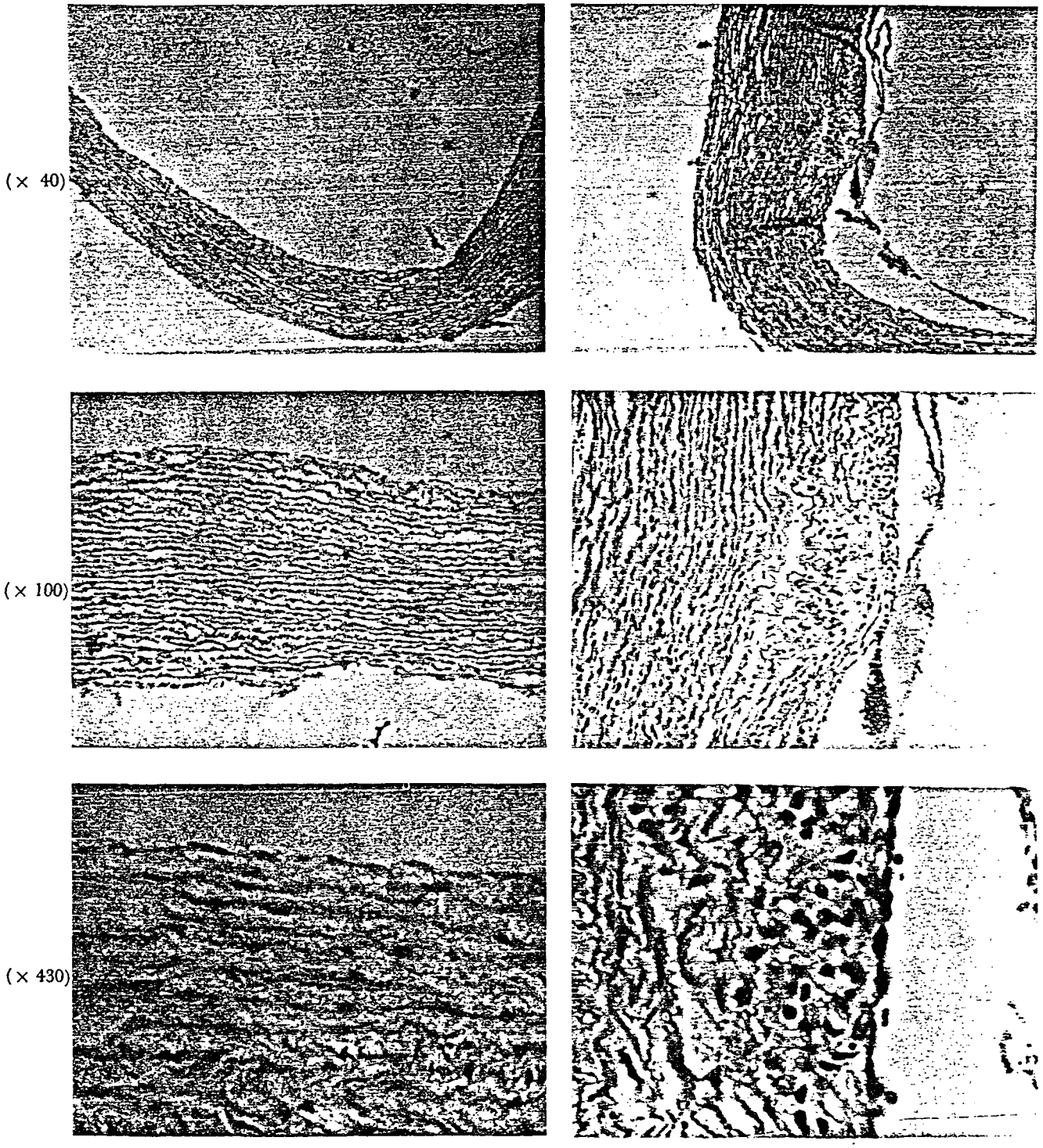
I : Macroscopical occurrence of atheroma
II : Histological severity of atheroma.
− : Absence of atheroma formation
+ : Presence of atheroma, mild, 5–1 %
++ : Presence of atheroma, moderate, 20–40 %
× : Presence of atheroma involving 1 to 2 layers of foam cell intima
×× : Presence of atheroma involving 3 to 5 layers of foam cell intima
××× : Presence of atheroma involving 6 to 9 layers of foam cell intima



(A)
No significant change of the intima occured.

(B)
One or two layers of pavement-like arrangement of foam cells occured focally in the intima.

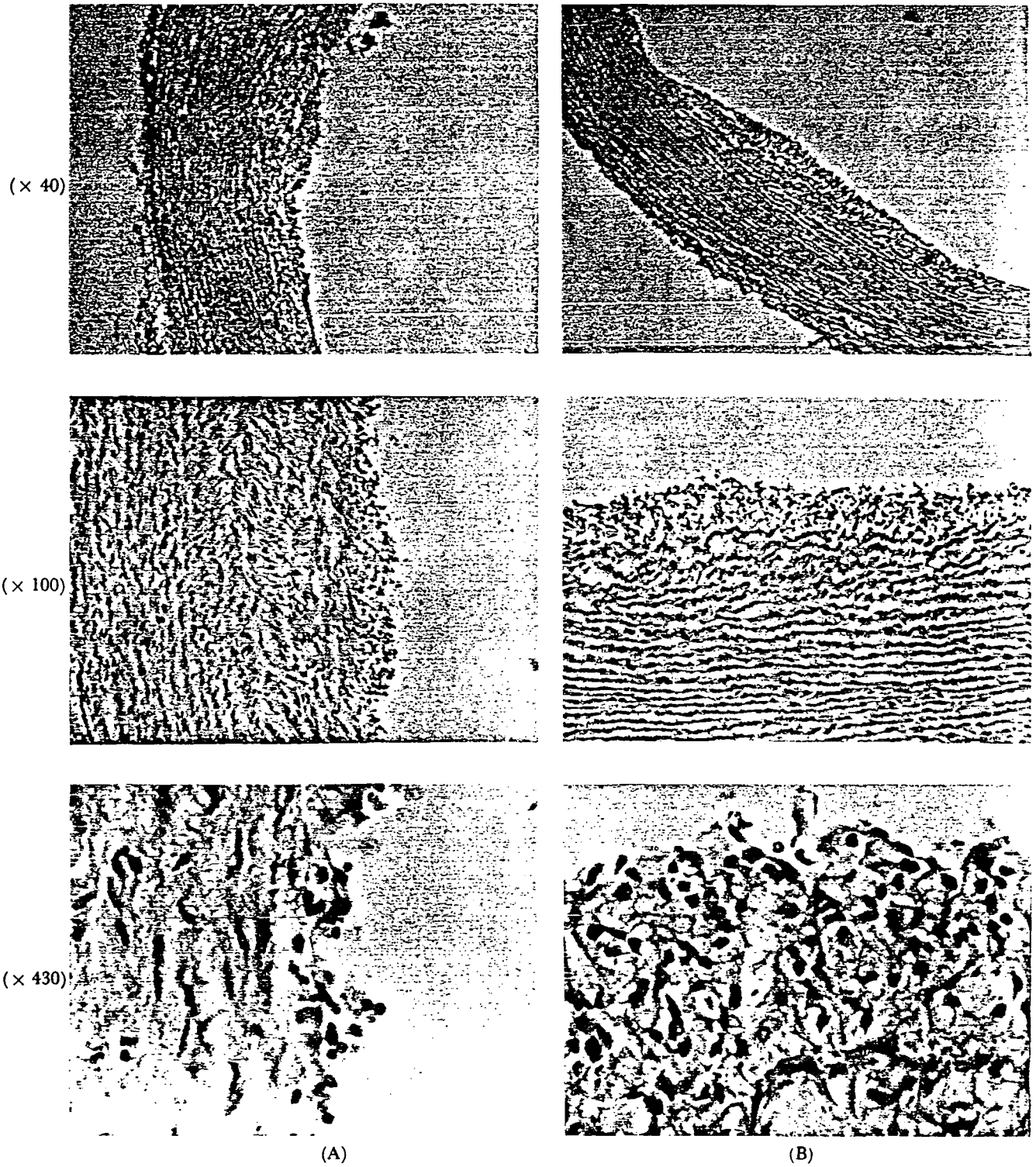
Fig. 1. (a) Microscopic observation (H & E stain) of the intima of ascending aorta of rabbits fed with cholesterol diet for 2 weeks with ginseng saponin (A) and without the saponin (B).



(A)
No significant change of the intima occured.

(B)
Focal collection of foam cells was observed in the intima.

Fig. 1. (b) Microscopic observation (H & E stain) of the intima of ascending aorta of rabbits fed with cholesterol diet for 4 weeks ginseng saponin (A) and without the saponin (B).



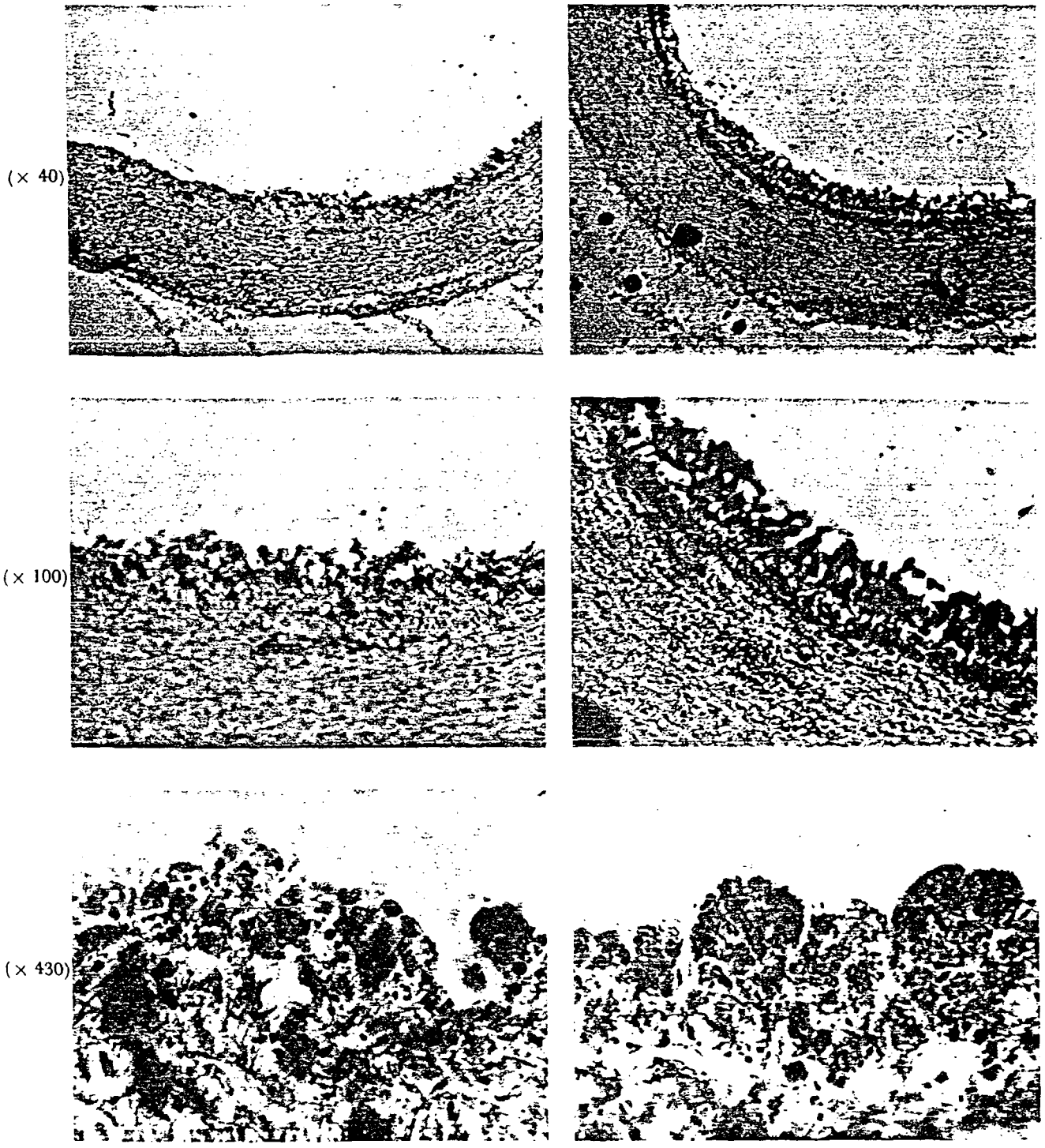
(A)

A slight focal collection of foam cells in the intima occured.

(B)

Four or five layers of the pavement like arrangement of foam cells were observed focally in the intima.

Fig. 1. (c) *Microscopic obserbation (H & Stain) of the intima of ascending aorta of rabbits fed with cholesterol diet for 6 weeks with ginseng saponin (A) and without the saponin (B).*



(A)
A Slight deposition of fats locally occured.

(B)
A severe deposition of fats was observed in the intima.

Fig. 1. (d) Microscopic observation (oil red 0 stain) of the intima of ascending aorta of rabbits fed with cholesterol diet for 6 weeks with ginseng saponin (A) and without the saponin (B).

of rabbit suffering from hypercholesterolemia due to long administration of cholesterol showed that the atherosclerosis like change and shdanophilic were not found on the administration of ginseng powder (Nahm, 1961).

Analysis of radioactivity of the blood serum lipid fraction of rabbits fed with cholesterol with saponin (test group) and without saponin (control) for 4 weeks prior to cholesterol-4-^{14}C administration on time course (2–20 hrs) showed that the highest radioactivity of test, control and normal group was observed at 6–8 hours, 16–18 hours and around 14 hours after the isotope administration respectivity and the radioactivity seemed to disappear gradually there after in all the above three groups.

The radioactivities of blood serum lipid fractions of rabbits fed with cholesterol with saponin (test), without saponin (control) for 4 weeks followed by two times of cholesterol-4-^{14}C administration at 24 hour's interval of the first two days with a continuous feeding of cholesterol diet with saponin (test) and without saponin (control) for 4 days showed that the former (test) was only two-thirds (617 cpm/ml) that (927 cpm/ml) of the latter (control).

In view of the presence of cholesterol in the atheroselerotic plaques of the aorta, it seems likely that a casual relationship between the disease and an excess of the substance. It is indeed possible to produce atherosclerosis in susceptible animal by prolonged cholesterol feeding and in man the diseases frequently associated with conditions in which the blood cholesterol is elevated, such as diabetes and nephrosis. Although much has been written about the relation between dietary lipids (especially cholesterol) and atherosclerosis, the evidence for a causal connection between diet and atherosclerosclerosis, in man is still indirect and circumstantial. Arterial tissue is perhaps capable of synthesizing cholesterol in situ, and the cholesterol deposits may originate from either endogeneous or dietary cholesterol. In the absence of more conclusive evidence it must be assumed that atherosclerosis is probably due to some abnormality in lipid metabolism, perhaps in the handling of cholesterol. It is generally agreed that hypercholesterolemia favors the appearance atherosclerotic lesions, and factors which decrease the serum cholesterol level, such as estrogens, or interfere with cholesterol absorption, such soybean sterols, have attracted considerable attention.

It seemed that ginseng saponin might stimulate cholesterol transport and the enzyme relating to cholesterol metabolism.

Since it was demonstrated that the ginseng saponin are surface active and good solubilizer of nonpolar lipids such as triglyceride and cholesterol. A significant portion of cholesterol palmitate and olive oil were found hydrolyzer *in vitro* by cholesterol esterase and pancreatic lipase respectively in the presence of the saponin with the enzyme resulting in a better hydrolysis of the lipids. (Joo et. al, 1973)

It has been also observed that moderate amounts of the saponins stimulated the reactions catalyzer by enzymes such as mitochodrial in this laboratory unexceptionally, suggesting that the detergent action of the saponin might give rise to better situation for the reaction being proceeded. (Joo and Lee, 1977)

It was interesting that the ratios of cholesterol to phospholipids of the sera of ginseng administered group (test), was significantly lower than those of control until 4 weeks' cholesterol diet feeding when no atheroma symptoms occured in saponin administered group. This probably suggested that the biosynthesis of phospholipids might be accelerated by the saponin. There is evidence supporting the stimulating effect of ginseng saponin on phospholipid biosynthesis. It was realized from the recent study of this laboratory (Joo and Lee, 1980) that the saponin stimulated the biosynthesis of phospholipids in the liver of rats. Specific radioactivities of lipids fractions of liver of rats, which were administered with saponin for 10 days (5 mg of ginseng saponin/day/rat) prior to intraperitoneal injection of $H_3\,^{32}PO_4$ on time course, showed that in both group, control and ginseng treated group, the highest radioactivity (^{32}P) reached at 7 hours after the isotope administration. At which the specific radioactivity (^{32}P) was found to be as many as

about 4 time in the liver of ginseng administered group that of control rats, suggesting that the saponin stimulated greatly the incorporation of $H_3PO_4$to the hepatic phospholipids of this animal.

From the above considerations, it seemed that the stimulation of phospholipid biosynthesis by the saponin administration might be a significent role to facilitate transport and metabolism of cholesterol in the body.

Furthermore, it appeared that the total serum lipid content of saponin fed group were much lower than that of control group as shown in table 1, suggesting that the saponin seemed to accelerate the hydrolysis of chylomicra probably via lipoprotein lipase action.

Examination of the saponin effect on lipoprotein lipase in the presense of various concentration of the saponin *in vitro* showed that the saponin stimulated the lipoprotein lipase activity and the highest activity (1.6 times that of control) was observed at the final concentration of 10^{-2}% of the saponin in the reaction mixture (Table 3).

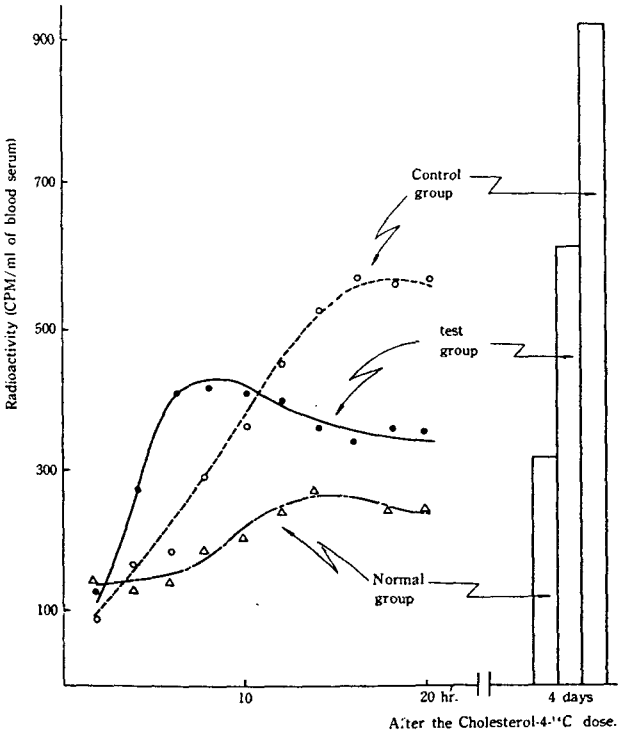


Fig. 2. Variation of radioactivity of the lipid fraction of prolonged cholesteral fed rabbits with and/or without ginseng saponin on time course.

The lipoprotein lipase activities of the blood plasma also showed that ginseng administered group was higher than control in both post-heparin and pre-heparin plasma.

The above results suggested that the saponin might accelerate the intravascular hydrolysis of chylomicra by stimulation of lipoprotein lipase activity.

Table 3. The effect of ginseng saponin in lipoprotein lipase of post-heparin blood plasma of normal rabbit *in vitro*.
The reaction mixture (total volume: 3ml) contained (final concentrations: chlyomacron 0.125%, ammonium buffer (pH 8.5) 70 mM, $CaCl_2$ 20 mM, bovine serum albumin 2%, various concentrations of the saponin and 0.2 ml of post-heparin plasma. At the termination of 2 hours, incubation at 37°C, the fatty acid liberated was tirated with 0.02N NaOH and the degree of the hydrolysis was estimated.

Saponin* concentration in reaction mixture (%)	Relative degree** of hydrolysis
1×10^{0}	124.5
1×10^{-1}	130.6
1×10^{-2}	161.4
1×10^{-4}	142.5
1×10^{-6}	130.6
1×10^{-8}	136.0

* Final concentration
** Figures are shown assuming the degree of hydrolusis in the absence of the saponin is 100.

Table 4. The lipoprotein lipase activities of blood plasma of prolonged cholesterol fed rabbits with and/or without ginseng saponin administration. The reaction mixture (total volume: 3ml) contained (final concentration): chylomicron 0.125%, ammonium buffer (pH 8.5) 70mM, $CaCl_2$ 20mM, bovine serum albumium 2%, and 0.2 ml of blood plasma. At the termination of 2 hours' incubation at 37°C, the fatty acid liberated was titrated with 0.02N NaOH and the degree of the hydrosis was estimated.

Plasma	Fatty acid released (μ mole)	
	pre-heparin	post-heparin
normal group (control)	6.73	7.92
Cholesterol fed group	5.40	6.84
Cholesterol and ginseng fed group	6.40	7.04

From the foregoing discussion, it was concluded that the ginseng saponin might stimulate the enzymes relating to the metabolism of lipid including cholesterol, particularly to cholesterol transport, resulting in the delay of cholesterol level rise in blood, consequently, the prevention of atheroma formation in such tissue as aorta.

Chairman: Now the time is open to discussion.

Questioner: I am from Switzerland. I'd like to know what you mean under ginseng saponin. Is that the crude extract or single saponin or mixture of saponins? I think it is very important to know what you use for this test.

Joo: Yes, I agree with you. I should mention about that before I start to discussion. This extract is partially purified saponin mixture.

Song: We did study using a normal Kyoto whisper rat and spontaneous hypertensive rat on lipids. We published already in part and we found that almost same result as you. And in SHR hypertension fell seems to raise cholesterol, triglycerides, and all other lipids. And after ginseng administration following feeding of cholesterol and fat, all this kind of fat, lipid increased responded well to decrease but triglycerides did not respond quickly as cholesterol and other lipids. We did study on the level of serum lipid. Low density lipoprotein seems to elevate before ginseng administration and high density lipoprotein become high but after ginseng extract administration, high density lipoprotein cholesterol seemed to decrease, which means that high density protein is more sensitive to ginseng administration. If you have any comment on this, I'd like to appreciate very much.

Joo: Actually I myself have carry out such type of research, but we have still some difficulties to obtain the low and high lipoprotein fractions. We are now undergoing this type of work.

Okuda: I have some comment or questions. I try to see hydrolysis of sera is regulated upon the LPL content but also upon the character of LPL surface behavior. That's also important factor according to the hydrolysis of lipoprotein. Saponin might incorporate, might modify the surface character of VLDL and accelerated the binding or the reaction between the trigliceride or LPL.

Joo: The surface activity of the saponin might be important to modify the surface character of LPL as well as VLDL. I am now trying to compare the action of the saponin with those of other detergent such as bile salts to find out that there is any similarity between them.

References

1. Gottfried, P. and B. Rosenberg (1973), *Clin. Chem.* **19**, 1077
2. Joo, C.N. (1963), Ph.D. thesis, University of Liverpool, p
3. Joo, C.N., R.S. Choe, S. J. Lee, S. H. Cho and M. H. Son (1973), *Korean Biochem. J.* **6**, 185
4. Joo, C.N. and J. H. Han (1976), ibid **9**, 237
5. Joo, C.N. and S. J. Lee (1977), ibid **10**, 59
6. Joo, C.N, Y.D. Cho and H.Y. Kwon (1978), ibid **11**, 125
7. Joo, C.N. and S. O. Lee (1980), ibid **13**, In preparation
8. Joseph, A (1972), *Clin. Chem,* **18**, 199
9. Kenny, A.P (1952), *Biochem. J.* **52**, 611
10. Lowry, O.H., N. J. Rosenbrough, A.L. Farr and R. J. Randall (1951), *J.Biol. Chem.* **193**, 265
11. Nahm, C.C (1961), *Ch'osin Uihak,* **4**, 51
12. Pearson, S. (1953), *Analytical Chem.* **25**, 813
13. Popov, I.M. (1975), Symp. Gerontology, Lugano, p 121