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Effect of Ginseng Saponin on LDL Receptor Biosynthesis**

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Abstract

Cholesterol, a component of all eucaryotic plasma membranes, is essential for the growth and viability of cells in higher organisms. However, too much cholesterol can be lethal because of atherosclerosis resulting from the deposition of cholesterol ester plaques. It was attempted in this study to understand the preventive effect of ginseng saponin, one of the major components of the roots of *Panax ginseng* C.A. Meyer, against hypercholesterolemia induced by high cholesterol diet. ^{125}I-LDL was injected intravenously to rabbits and rats, which were fed a high cholesterol diet with and/or without ginseng saponin for 12 days. The disappearance of the radioactivity occurred faster in the test group than the control. The effect of saponin fraction from *Panax ginseng* C.A. Meyer and the purified ginsenosides, Rb_1, Rb_2, Re and Rg_1, on LDL receptor biosynthesis in high cholesterol fed rat has been investigated. Analysis of LDL receptors from various organs such as liver, kidney, adrenal cortex and testis showed that the population of LDL receptors of test group significantly higher than that of the control. It was also found that liver homogenate containing ginsenosides (10^{-3}-10^{-4}%) stimulated the biosynthesis of bile acid form cholesterol. From the above results, it seemed that ginsenosides lower the cholesterol level by stimulating cholesterol metabolism, which result in the suppression of the inhibitory action of cholesterol on LDL receptor biosynthesis.

Introduction

It was demonstrated in this laboratory that the ginseng saponin has some preventive effect against hypercholesterolemia induced by prolonged high cholesterol diet administration in rabbits (Joo, 1980) and rats (Joo et al., 1987).

It has been reported that more than 93% of all cholesterol in the animal body is cell membrans, where it performs vital structural functions, while only about 7% circulates in plasma. It is the plasma cholesterol level, however, which is strongly implicated as a cause of atherosclerosis. Consequently, factors regulating the plasma cholesterol level are the subject of intense study.

Cholesterol is transported in the plasma in macromolecules called lipoproteins, which consist of varying amounts of specific proteins or peptides, cholesterol, cholesterol esters, triglycerides and phospolipids.

The importance of the Low Density Lipoprotein (LDL) receptor is highlighted by studies of familial hypercholesterolemia. The total concentration of cholesterol and LDL in the plasma is markedly elevated in this genetic disorder, which results from a mutation at a autosomal locus. Cholesterol is deposited in various tissues because of the high concentration of LDL-cholesterol in the plasma. Nodules of cholesterol called xanthomas are prominent in skin and tendons. More harmful is the deposition of cholesterol in arterial plaques, which produce atherosclerosis. The molecular defect in most cases of familial hypercholesterolemia is an absense or deficiency of functional receptor for LDL (Brown and Goldstein, 1986).

In present study, we focussed our attention on LDL receptor, upon which how the ginseng saponin might work, to understand the mechanism of hypocholesterolemic action of the saponin.

Experimental

6.5g of ginseng saponin mixture was obtained from 300 g of powdered Korean white ginseng (Keumsan, 4 years, 50pieces) according to the modified procedure described elsewhere (Joo et al, 1976). The chromatogram of the saponin showed that it contained several saponins with Rf values of 0.17, 0.65, 0.49, 0.52, 0.47, 0.43, 0.41, 0.36, 0.34, 0.27, 0.22, 0.20, 0.17 on silica gel pre-made thin plate (Pre-Coated TLC plates, Silica gel 60 F-254) by using chloroform-methanol-H_2O (65 : 40 : 9, v/v/v) as a developing solvent. It appeared that the saponins with Rf values of 0.22, 0.27 were the most abundant, the saponins of Rf values of 0.47, 0.36, 0.34, 0.20, 0.17 were less abundant and the saponins of Rf values of 0.71, 0.65, 0.49, 0.52 were the least.

Purified ginsenosides (Rb_1, Rb_2, Re, Rg_1) were obtained from Korean Ginseng and Tobacco Research Institute.

Albino rats (Sprague Dawley, 150-200g, male) were divided to four groups (three rats each). They were normal group, saponin (1 mg/day/rat) fed rats (Test 1), cholesterol (100 mg/rat/day), corn oil (0.5 g/rat/day), Nacholate (10 mg/rat/day) with (Test 2)/ without(control) ginseng saponin (1mg/rat/day) fed rats for 15 days.

Lipoproteins were fractionated according to Havel (1955) and ^{125}I-LDL were prepared by Lancer (1972) which is a modified Mc Farlane (1958) method.

^{125}I-LDL turnover was determined by measuring the reactive radioactivity(%) of blood serum on time course to that of lomin after the intravenous injection of ^{125}I-LDL (200 cpm/mg) 100μg/rabbit.

In the case of rats, ^{125}I-LDL (100 cpm/mg) 50 μg per rat was injected in travenously and the relative radioactivity(%) on time course to that of 3 min after the ^{125}I-LDL injection.

Total lipids were extracted according to Blye-Dyer (1959). Cholesterol was determined by Kenny (1952)

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method. Triglycerides and phospholipids were assay according to by Biggs et. al. (1979).

Plasma membranes of rat liver were prepared according to Goldstein(1979) and these were used as LDL receptor source. The LDL receptor activity was determined by Schmeider et al. (1980) method. The reaction mixture (total volume : 150 l) for the determination of total binding site activity (EDTA-resistant binding site activity+EDTA-sensitive binding site activity) contained (final concentrations) 60 mM Tris-HCL (pH 8.0). 1 mM $CaCl_2$, 25 mM NaCl, 20% BSA, and liver plasma protein 100g[^{125}I]-LDL (120 CPM/mg (rabbit), 50 CPM/mg (rat)). The reaction mixture for the determination of EDTA-resistant above reaction mixture for total binding site activity.

The effect of ginsenoside on the biosynthesis of bile acids from cholesterol was investigated according to Mostach et al. (1971). Albino rats (Sprague Dawley, 200 g, male) were fasted for 24 hrs, and then anesthetizes using diethyl ether and the livers were removed, homogenized to make 20% homogenate using 12 mM phosphate buffer (pH 7.4). The assay mixture (total volume : 2ml) contained (final concentrations) 137 mM NaCl, 5.2 mM KCl, 0.12 mM $CaCl_2$, 0.9 mM $MgSO_4$, 25 mM $NaHCO_3$, 1% glucose, 0.2 % BSA, 10 mM phosphate butter, 10μg cholesterol, [4-^{14}C-cholesterol] (0.09 μCi), ginsenosides (various concentrations) and 1.7 ml of 20 % liver homogenate. The reaction mixture was incubated at 37°C for 60min added 10% KOH, autoclaved for 2 hrs. The mixture was then extracted four times with petroleum ether and concentrated under reduced pressure. The concentrated extract was acidified with 6N HCl and again extracted four times with petroleum ether. The combined extract was dried in vacus and subjected to silica gel TLC to separate cholesterol and bile acids using chloroform-methanol-glacial acetic acid-H_2O (15:5:1:1) as a developing solvent and the radioactivity of the corresponding fraction was measured.

Radioactive ginseng saponins were prepared as follows. 2 g of sliced raw ginseng root (4 years, Keumsan) was incubated in the reaction mixture(3.3 ml) containing 0.1 M sucrose, 5×10^{-3} M ATP, 3×10^{-4} M NADPH, 5×10^{-4} M Coenzyme A, 8×10^{-4} M NAD, 2.5×10^{-2} M nicotinamide, 1×10^{-2} M glutathione, 1.2×10^{-3} M Na-acetate containing 1.2-^{14}C acetate (150 μCi) at 37°C for 64 hrs.

Following incubation, the reaction mixture was diluted with water and homogenated, followed by centrifugation to remove the insoluble pelletes. The pelletes were washed three times with a little water. The combined soluble extract was lyophilyzed. 5ml of hot methanol was then added to the above extract and mixed sufficiently and the mixture we allowed to stand overnight in a refrigerator to precipitate the insoluble fraction. Following centrifugation to remove the insoluble fraction, the precipitate was extracted three times with a small volume of hot methanol as described above. The combined methanol extract was mixed with 3 volumes of chloroform, vortexed and then centrifuged to remove the insoluble fraction (mainly sugar). The insoluble fraction was washed twice with methanol chloroform mixture (1:3, v/v). The combined extract was then concentrated under reduced pressure and finally dissolved in 2.0 ml of methanol. The above methanolic solution was chromatographed by thin plate of silica gel (Merck nach Stahl) using chloroform : methanol : water mixture (65:40:9, v/v/v) as a developing solvent. The chromatogram of the product showed that the pattern of the radioactive saponin preparation prepared as described above was exactly the same as that of the saponin preparation from ginseng roots (4 years, Keumsan, 50 pieces per 300g).

1 mg of ginseng saponin containing ^{14}C-labelled saponin (5,600,000 DPM) was administered by stomach tubing to Albino rats(Sprague Dawley, male, 180-200g) and then killed on time course after the saponin administration.

The blood was taken directly from the heart and the serum was prepared as usual way. The livers were homogenated in water and made up tp a know volume. Radioactivities of serum and liver homogenates were counted according to Mahin and Lofberg[6]. Blood serum and the liver were then extracted by Bligh Dyer method[1] and the radioactivities of individual fractions were counted as usual way described elsewhere. The radioactive ginseng saponins prepared from ^{14}C-acetate using sliced raw ginseng roots as an enzyme source described above and saponins from blood serum and livers were chromatographed by High Performance Liquid Chromatography using Waters Model 244 (Column : u-Bondapak carbohydrate Analysis. Solvent system : acetonitril-H_2O-Butanol (80 : 20 : 15, v/v/v). Detector : R_I, chart speed : 1 cm/min., flow rate : 1.5 ml/min.).

Chinese hamster ovary (CHO) cells(2×10^5 cells/ml) were cultured in Eagle's minimum essential medium [5%(v/v) Fetal bovine serum, 20 mM HEPES (pH 7.4), 24 mM $NaHCO_3$, streptomycin (100 μg/ml), penicilline G (100 unit /ml)] using CO_2 incubator (5% CO_2, 95% air).

The development of CHO cell's monolayer was observed by inverted microscope.

For the determination of LDL receptor activity, ^{125}I-LDL was added to the CHO cells grown under different conditions and the incubation was continued at 37°C for two hours. The monolayer was cooled (4°C) and then washed five times with buffer A [final concentrations Tris-HCl(pH 7.4) : 0.15 M NaCl : 2 mg/ml BSA] and once with buffer B [50 mM Tris-HCl (pH 7.4) : 0.15 M NaCl]. The washed monolayer was dissolved in 0.1 N NaOH and the radioactivity and protein contents were determined.

The amount of cholesterol transported into CHO cells was determined by using the radioactivity, the cells which were cultured (8 hours) in the medium containing cholesterol (2-15 μg/ml) and [4-^{14}C]-cholesterol (0.14 μCi).

[U-^{14}C] leucine and [^{3}H]-uridine were added to CHO cells grown under different conditions and the incubation was continued at 37°C for two hours. Following three time washings with phosphate buffer saline (PBS), the cells were sonicated. Trichloroacetic acid (TCA) was added to precipitate proteins and nucleic acid. After centrifugation, the precipitate was washed twice with 1 M TCA and then added 0.5 N NaOH, heated at 60°C for one hour. The mixture was then neutralized with 2.5 N HCl TCA was added and centrifuged and the radioactivities of the supernatant (^{3}H, for RNA determination) and of the precipitates (^{14}C, for protein determination) were measured.

CHO cells, grown in the essential medium for 3 days, were transferred to freshly made essential medium containing cholesterol (10 μg/ml) and [4-^{14}C] cholesterol

Table. 1. Lipid composition of liver of rats under different feeding conditions.

Group	Cholesterol	Triglyceride (mg/g wet weight of liver)	Phospholipid	Chol./PL	TG/PL
Normal	3.8 ± 0.2	4.9 ± 0.3	18.3 ± 1.2	0.21	0.27
Test I	3.7 ± 0.7	4.4 ± 0.8	16.7 ± 0.9	0.22	0.26
control	13.8 ± 1.7	8.4 ± 0.7	24.0 ± 1.8	0.58	0.35
Test II	7.3 ± 1.2	6.2 ± 0.4	23.8 ± 0.8	0.31	0.26

Normal: Normal diet fed rats.
Test I: Normal diet and ginseng saponin fed rats.
Control: High cholesterol diet fed rats.
Test II: High cholestrol diet and ginseng saponin fed rats.

(0.18 μCi) with (test group)/ without ginseng saponin and the incubation was continued 24 hours. The steroids were then extracted twice with methlyene chloride and twice with ethyl acetate. The combined extract was concentrated and subjected to silica gel thin layer chromatography using cyclohexane-ethylacetate (50 : 50. v/v) as a developing solvent to separate cholesterol. estradiol and progesterone. and the radioactivity of the corresponding fraction was measured.

Results and discussion

Table 1 showed that the level of liver lipids such as cholesterol and triglycerides of ginseng fed group (Test II) was greately lower than that of nonfed group (Control) in high cholesterol administered rats while in ordinary fed rat. no significant difference in the above lipid level occured between ginseng fed group (Test I) and nonfed group (Normal).

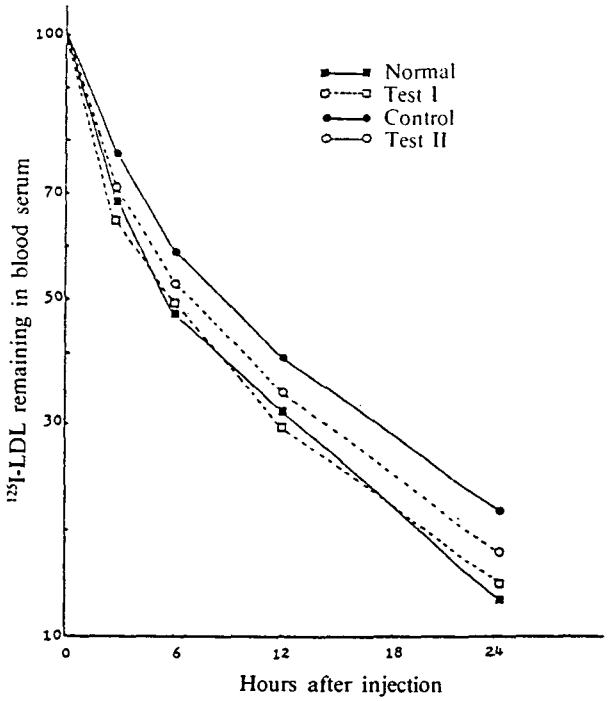

Figure. 1. Effect of ginseng saponin on ^{125}I-LDL removal from the blood serum of normal diet fed rats(normal). normal diet and ginseng saponin fed rats(test I). high cholesterol diet fed rats(control) and high cholesterol diet and ginseng saponin fed rats(test II) for 15 days.

^{125}I-LDL was injected intravenously to rats which had been fed with high cholesterol diet with and/or without ginseng saponin for 15 days and the radioactivity disappearance from test group was faster than from control group as shown in figure 1.

Table 2 showed that the binding activity of ^{125}I-LDL to rat liver plasma membrane. It was found that the activity was higher in test group than control. It was also observed that LDL receptor activities of other organs such as kidney. adrenal cortex and testis of ginseng saponin fed rats are higher than that of control group as shown in Table 3.

It was reported (Kita et al.. 1980) that there are EDTA-sensitive binding site and EDTA-resistant binding site for LDL in rabbit liver and the hipocholesterolemic action of cholestryamin and mevinolin is due to increase of the number of EDTA-sensitive binding sites.

As shown in Table 4. the total LDL receptor activity was lowered in control group but the saponin prevented LDL activity loss dueing to high cholesterol administration. However the activity of EDTA-resistant binding site did not altered under different feeding conditions but that of EDTA-senstive binding site did.

Table 5 showed that no significant activity change of both the EDTA-sensitive and resistant binding sites of normal rabbits in the presence of ginseng saponin. This indicates that ginseng saponin might not stimulate binding affinity but the population of hepatic LDL receptor.

It is now known that an excess of cholesterol in the liver reduces transcription of the LDL-receptor gene into messenger RNA. the nucleic acid that is subsequently translated by the cell's protein-synthesizing machinary to make the LDL-receptor.

Table 2. Uptake of ^{125}I-LDL into the liver of rats under different feeding conditions.

Group	cpm/g. liver	cpm/total liver
Normal	3492 ± 181	19316
Test I	3748 ± 74	19672
Control	3069 ± 148	16573
Test II	3649 ± 121	17745

Normal : Normal diet fed rats.
Test I : Normal diet and ginseng saponin fed rats.
Control : High cholesterol diet fed rats.
Test II : High cholesterol and ginseng saponin fed rats.

Table 3. Cholesterol level and LDL receptor activities of kidney, adrenal cortex, and testis of rats fed under various conditions. The values are mean value of five determinations.

Group	Kidney		Adrenal cortex		Testis	
	Cholesterol level (mg/g tissue)	LDL receptor activity μg bound/mg LDL/pro.	Cholesterol level (mg/g tissue)	LDL receptor activity μg bound/mg LDL/pro.	Cholesterol level (mg/g tissue)	LDL receptor activity μg bound/mg LDL/pro.
Normal	3.61± 0.11	3.54± 0.16	15.18± 0.84	4.72± 0.36	0.23± 0.16	2.68± 0.56
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)
Control	3.99± 0.42	2.61± 0.39	25.19± 3.79	3.71± 0.16	0.36± 0.16	0.36± 0.16
	(110.5)	(73.7)	(165.9)	(78.6)	(156.5)	(79.9)
Ginsenoside	3.81± 0.06	3.26± 0.10	23.84± 0.77	4.04± 0.32	0.34± 0.08	2.41± 0.03
mixture	(105.5)	(92.1)	(157.0)	(85.6)	(147.8)	(89.9)
Ginsenoside Rb_1	3.76± 0.05	3.24± 0.02	17.70± 0.54	4.15± 0.74	0.34± 0.06	2.39± 0.13
	(104.2)	(91.5)	(116.6)	(87.9)	(147.8)	(89.2)
-Rb_2	3.80± 0.07	3.16± 0.09	19.90± 1.02	4.42± 0.57	0.34± 0.05	2.44± 0.07
	(105.3)	(89.3)	(131.1)	(93.6)	(147.8)	(91.0)
-Re	3.64± 0.05	3.00± 0.15	20.15± 1.67	4.31± 0.33	0.33± 0.05	4.19± 0.21
	(100.8)	(84.7)	(132.7)	(91.3)	(143.5)	(156.3)
-Rg_1	3.50± 0.10	2.57± 0.14	18.85± 0.97	4.57± 0.47	0.35± 0.05	2.33± 0.38
	(97.0)	(72.6)	(124.2)	(96.8)	(152.2)	(86.9)

Normal group was administered normal diet for 2 weeks. *Control group* was adiministered normal diet with high cholesterol diet (cholesterol 100 mg, olive oil 0.5ml, Na-cholate 10mg/rat/day) for 2 weeks. *Test group* were administered under similar diet conditions of control group but either with ginsenoside mixture (1 mg/rat/day) or with ginsenoside Rb_1 -Rb_2, Re, -Rg_1 fractions (1 mg/diet conditions of control group but either with ginsenoside mixture (1 mg/rat/day) or with ginsenoside Rb_1 -Rb_2, Re, -Rg_1 fractions (1mg/rat/day) for 2 weeks. The figure in brackets are relative percentage assuming that of normal group being 100 *P< 0.05.

Table 4. Binding of ^{125}I-LDL to the liver plasma membrance of rabbits fed under different feeding conditions.

Group	Total ng/mg	EDTA-resistant ng/mg	EDTA-sensitive ng/mg
Normal	52.6	26.1	26.5
Test I	55.3	27.0	28.3
Control	38.4	25.8	12.6
Test II	50.8	26.4	24.4

Normal : Normal diet fed rabbits (12days).
Test I : Normal diet fed and ginseng saponin fed rabbits. (12days).
Control : High cholesterol diet fed rabbits (12days).
Test II : High cholesterol diet and ginseng saponin fed rabbits. (12days).

Table 5. Binding of ^{125}I-LDL to liver membrane of normal rabbit in the presence and/or absence of gineng saponin (*in vitro*).

Conc. of saponin(%)	Total ng/mg	EDTA-resistant ng/mg	EDTA-sensitive ng/mg
Control	50.2	24.1	26.1
10^{-5}	49.7	23.3	26.4
10^{-4}	50.8	25.2	25.0
10^{-3}	50.5	24.6	25.9
10^{-2}	52.4	25.4	27.1

Assay mixture (150 μl) contained 100 ug of membrance protein, 5 μg/ml of ^{125}I-LDL (120 cpm/ng) and various concentrations of saponin in the absence or presence of 30 mM EDTA.

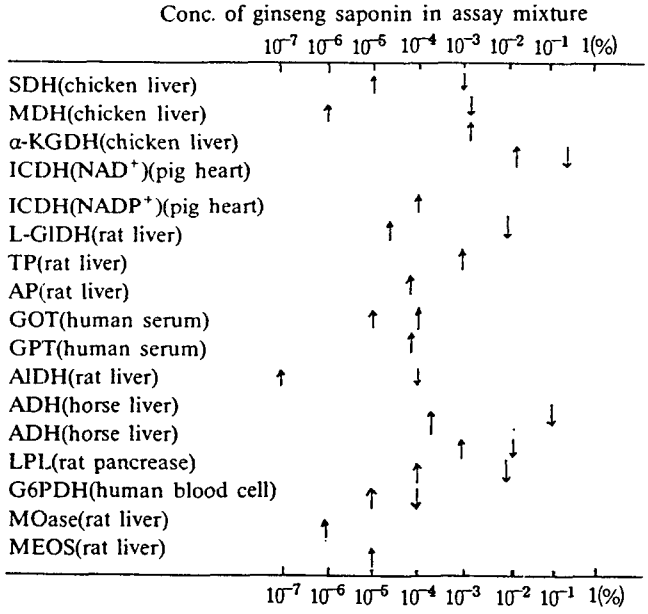

Figure 2. The effect of ginseng saponin on enzyme catalyzed reactions. The corresponding enzyme reaction rate reached maximum at the concentrations shown by mark(↑) and inhibited when the saponin concentration was over those shown by mark (↓).
Abbreviation : Succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-Ketoglutarate dehydrogenase (-KGDH), Isocitrate dehydrogenase (ICDH), L-Glutamate dehydrogenase(GlDH), Glutamate-oxaloacetate transaminase (GOT), Glutamate-pyruvate dehydrogenase (GPT), Aldehyde dehydrogenase (AlDH), Alcohol dehydrogenese (ADH), Lipoprotein lipase (LDL), Alkaline phosphatese (AP), Tryptophan pyrolase (TP), Glucose 6-phosphate dehydrogenase (G6PDH), Monoamine oxidase (MOase), Microsomal ethanol oxidizing system (MEOS).

Table 6. Glycoside levels of blood serum and liver of rats which were administered with 1mg of ginseng saponins containing ^{14}C-labelled saponin (5,600,000 DPM/mg) prepared from ^{14}C-acetate. Individual glycosides were fractionated by HPLC and the amounts were calculated from the radioactivity of the corresponding fraction.

Fractions	Fed Saponins (μg)	Blood serum (1hr.)		Liver (1hr.)		Liver (4hrs.)	
		Recoverd saponins (ng)	saponin level (10^{-5}%)	Recoverd saponins (ng)	saponin level (10^{-5}%)	Recoverd saponins (ng)	saponin level (10^{-5}%)
Rg_2, Rg_1, Rf	110	28 ± 1.55	0.22	160± 9.28	0.29	68.00± 0.11	0.13
Re.Rd	196	720 ± 34.06	5.54	1,270± 37.15	2.37	27.86± 0.84	0.06
Rc	165	1,640 ± 61.91	12.60	2,700± 12.38	5.02	334.86± 4.36	0.65
Rb_2	97	370 ± 18.03	2.84	148± 6.19	0.27	6.19± 0.91	0.01
Rb_1	110	102 ± 6.19	0.78	360± 9.30	0.67	27.86± 0.61	0.06

The bile acids into which most of the cholesterol is converted are secreted into the upper intestine, where they emulsify dietary fats. Having done their work, the bile acids are largely reabsorbed from th intestine, taken up by the liver and again secreted into the upper intestine. This enterohepatic circulation of bile salts ordinary limits the liver's need for cholesterol. Therefore, if the recycling could be interrupted, the liver would be called on to convert more cholesterol into bile acids and this should lead the liver cells to make more LDL receptors.

A class of drugs that interrupt the recycling of bile acid such as cholestyramine was already well known. This drug was found to lower the blood LDL level by an average of 10%. On the other hand, HMGCoA reductase inhibitors such as compactin and mevinolin were also know to lower the blood LDL level in animals. The combination of HMGCoA reductase inhibitor and bile acid binding resin was reported to be more effective.

We have observed the effect of ginseng saponin on various enzymes during the past decade and found that moderate amounts of the saponin stimulates the enzyme catalized reactions unexceptionally so far tested in this laboratory as shown in figure 2.

This nonspecific enzyme stimulation effect of the saponin led us to suggest that the surface activity of the saponin might play a significant role in enzyme catalyzed reactions (Joo, 1984).

Km of various enzyme for their substrates were lowered in the presence of moderate amounts of ginseng saponins as well as other surfactants such as Triton X-100, SDS, deoxycholate, Lubrol-WX. UV difference spectra, CD spectra, electrophoretic mobility, DTNB titration and substrate binding data demonstrated that moderate amounts of the saponin might bring about a slight change of the enzyme conformations, which would be in favour of the enzyme reactions being accelated (Kim, 1986).

Our saponin absorption experiment in rats using radioactive saponins prepared from ^{14}C-acetate using ginseng root slices showed that ginsenosides were absorbed partly in the undissociated form and saponin level of the liver might be maintained at 10^{-6}%-10^{-5}%, which is good for enzyme stimulation, for a considerable period of time in ginseng administered rats as shown in table 6.

From the above considerations, it was expected that the saponin might stimulate bile acid biosynthesis from cholesterol, and *in vitro* experiment showed the case as shown in Table 7. Furthemore, the secretion of bile acids by ginseng saponin fed group was faster than non-saponin fed group (Fig. 3). We don't know yet whether the recycling of bile acids is interrupted by the saponin or not which remains to be solved.

Table 7. The effect of ginsenosides on the bile acid biosynthesis from (4-^{14}C)-cholesterol by rat liver. The values are mean value of three determinations.

Concentration of ginsenosides in the reaction mixture (%)		0	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Ginseng saponin mixture		115,509	126,558	147,889	136,856	128,977
		(100)	(110)	(128)	(118)	(112)
Ginsenoside	Rb_1	115,509	150,869	152,448	145,796	131,368
		(100)	(131)	(132)	(126)	(114)
	-Rb_2	115,509	115,323	135,233	153,808	151,571
		(100)	(100)	(117)	(133)	(131)
	-Re	115,509	142,564	143,201	156,015	137,205
		(100)	(123)	(124)	(135)	(119)
	-Rg_1	115,509	144,016	150,837	126,744	136,024
		(100)	(125)	(131)	(110)	(118)

Reaction mixture(2ml) contained 137 mM NaCl, 5.2 mM $NaHCO_3$, 1% glucose, 0.2% bovine serum albumin, 10 mM phosphate buffer (pH 7.4), 10 μg cholesterol containing (4-^{14}C) -cholesterol (0.09μCi), ginseng saponin mixture and ginsenoside Rb_1, -Rb_2, -Re, -Rg_1 fractions (10^{-2}%-10^{-5}%), and 20 % rat liver homogenate.The figure in brackets are relative percentage assuming that of control group (0 % saponin) being 100.

Effect of total saponin extract and some purified ginsenoside -Rb_1 and -Rb_2 on LDL receptor biosynthesis of chinese hamster ovary (CHO) cells cultured in a high cholesterol medium was investigated.

Cholesterol uptake by CHO cell cultured in a medium containing various amounts of cholesterol was traced and found that the cholesterol uptake was proportional to the concentration of cholesterol in the medium (Fig 4). and the population of LDL receptors were gradually decreased as the increasing cholesterol concentration in the medium (Table 8).

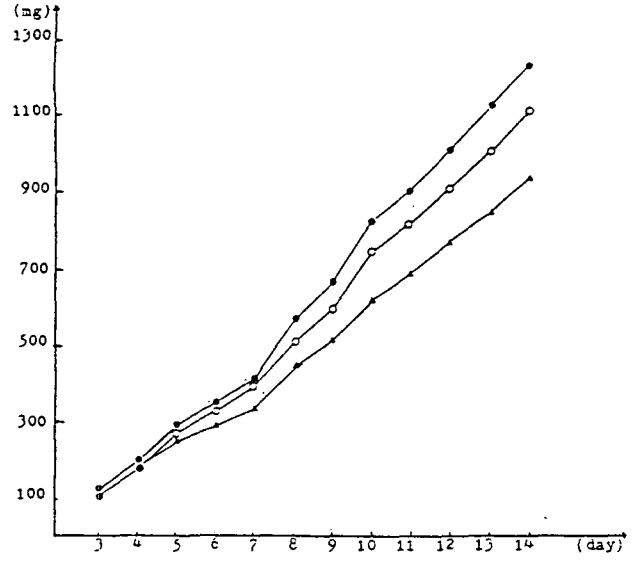

Fig. 3. The accmulative amount of bile acids from feces of rats fed under various conditions two weeks course. The values are mean value of five determinations.
Control group(▲-▲) were administered normal diet with high cholesterol diet (cholesterol 100 mg. olive oil 0.5 ml. Na-cholate 10mg/rat/day). Test I group (○-○) were administered under similar diet conditions of control group but either with ginsenoside mixture (1mg/rat/day). Test II group (●-●) were administered under similar diet conditions of control group but either with ginsenoside Rb_1 (1 mg/rat/day).

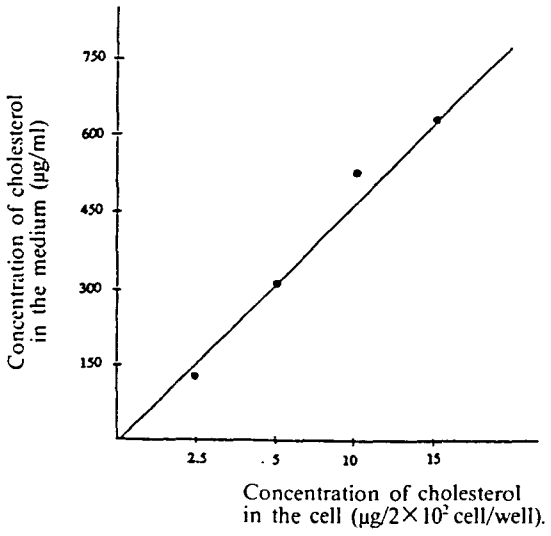

Fig. 4. Uptake of cholesterol by CHO cell cultured in medium containing both cholesterol and[4-^{14}C] cholesterol.

However. when the CHO cells were cultured in the medium containing ginsenosides. cholesterol concentration was lowered resulting in less loss of LDL receptor activity (Table 9 & 10).

The biosynthesis of protein and RNA of the above cells was high than that of CHO cells cultured in the absence of the ginsenosides (Table 11). suggesting that the ginsenosides might stimulate LDL receptor biosynthesis. It was also observed that the ginsenosides stimulated the biosynthesis of estradiol and progesterone from cholesterol in the CHO cells. as shown in Table 12.

From the above results. it seemed that the ginsenosides lower the cholesterol level by stimulating the cholesterol metabolism including bile acids (in liver) and steroid hormon biosynthesis (in ovary). resulting in the lowering of inhibitory action of cholesterol on LDL receptor biosynthesis.

Reference

1. Brown. M.S. and J.L. Goldstein (1984) Scientific America. 251, 52
2. Brown. M.S. and J.L. Goldstein (1986) Science. 232, 34
3. Joo. C.N. (1963) Ph. D. Thesis. University of Liverpool.
4. Joo. C.N. and S.J. Lee (1977) Korean Biochem. J.. 10, 59
5. Joo. C.N. and D.S. Kim and J.H. Koo (1980) Korean Biochem. J.. 13, 51

Table 8. LDL receptor activity of CHO cells cultured in medium containing various amounts of Cholesterol. The values are mean value of three determination.

Conc. of Cholesterol μg/ml	LDL receptor activity ng bound LDL/mg protein	Relative activity (%)
0 (countrol)	1.158± 113	100
2.5	1.026± 186	87
5.0	977± 106	84
10.0	816± 81	70
15.0	793± 106	68

The relativity activity of LDL receptor was expressed assuming that of control being 100.

Table 9. Cholestrol concentration of CHO cells cultured under various conditions. The values are mean value of three detrminitations.

Group	Cholesterol concentration (ug)	Relative (%)
Normal	37± 1.51	100
Control	56± 2.16	150
Ginseng saponin mixture (100-5%)	34± 3.92	91
Ginsenoside Rb_1(10^{-6}%)	32± 2.39	86
Ginsenoside Rb_2(10^{-4}%)	23± 1.41	61
Ginsenoside Re(10^{-7}%)	40± 7.32	107
Ginsenoside Rg_1(10^{-6}%)	36± 0.82	96

Table 10. LDL receptor activities of CHO cells cultured under various conditions*. The values are mean value of three determination.

Group	Concentration of ginseng saponin (%)						
	0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Normal	1,326± 500 (100)						
Control	797± 90 (60)						
Ginseng saponin				911± 70 (69)	924± 40 (70)	881± 80 (66)	
Ginsenoside Rb_1		1,024± 75 (77)	1,325± 380 (100)	1,498± 670 (113)	978± 540 (74)		
-Rb_2				812± 40 (61)	876± 190 (66)	1,390± 390 (105)	1,000± 400 (75)
-Re		701± 20 (53)	1,062± 200 (80)	1,025± 120 (77)	697± 220 (53)	672± 220 (51)	
-Rg_1		832± 130 (63)	850± 40 (64)	875± 70 (66)			

Normal group was cultured in standard medium.
Control group was cultured in standard medium containing cholesterol (10μg/ml)
Test groups were cultured under similar conditions of control group but either with ginseng saponin mixture or with ginsenoside Rb_1, -Rb_2, -Re and -Rg_1 fractions.
The figure in brackets are relative percentage assuming that of normal group being 100.
*LDL receptor activities were defined as ng LDL bound in 2 hours at 37°C by mg protein.

Table 11. The biosynthesis of RNA and protein of CHO cells cultured under various contions.*
The values are mean value of three determinations.

	Radioactivity of ^{3}H-Uridine incroporated into RNA (cpm)	Radioactivity of ^{14}C-Leucine incorporated into protein(cpm)
Normal	8,239 (302)	18,383(183)
Control	2,730 (100)	10,473(100)
Ginseng saponin	4,103 (150)	12,774(122)
Ginsenoside Rb_1	4,099 (154)	11,438(109)
-Rb_2	4,095 (154)	11,274(108)
-Re	2,821 (103)	11,391(109)
-Rg_1	3,799 (139)	11,439(109)

Normal group was cultured in standard medium.
Control group was cultured in standard medium containing cholesterol (10ug/ml).
Test groups were cultured under similar conditions of control group but either with ginseng saponin mixture (10^{-5}%) or with ginsenoside Rb^1(10^{-6}%), -Rb_2(10^{-4}%), -Re (10^{-7}%), -Rg_1 (10^{-6}%) fractions.

*The figure in brackets are relative percentage assuming that of cholesterol being 100.

6. Joo, C.N. and J.H. Koo and T.H. Baik (1980) Korean Biochem. J., 13, 63
7. Kim, J.W.;(1986) Ph.D. Thesis, Yonsei University
8. Kita, T., M.S. Brown, Y. Watanabe and J.L. Goldstein (1980) Proc. Natl. Acad. Sci. USA, 78, 2268
9. Naham, C.C. (1961) Choesin Uihak, 4, 51
10. Popov, I.M. (1975) Sym. Gerontology, Lugano, 121

H. Saito : Does ginsenoside decrease the absorption of cholesterol?

C.N. Joo : Absorption is O.K. Ginsenoside affect the convertion of cholosterol in to bile salt and more bile salts are coming out.

F. Soldati : Did you use ^{14}C-ginsenoside?

C. N. Joo : We prepared ^{14}C-ginsenoside and used as a enzyme source. We fed natural one and ^{14}C-ginsenoside and calculate how ginsenoside absorbed into liver cell.

B. H. Han: Enzyme activity was reversed with concentration. How can you reasonably explain?

C. N. Joo: We found that enzyme reaction are catalyzed at 10-5 %. Enzyme confirmation is changed by saponin. Too much saponin results in too much confirmational changes of enzyme and enzyme did not work well due to denaturation.

Table 12. The effect of ginsenoside on the biosynthesis of progesterone and estradial from[4-^{14}C] cholesterol in cultured CHO cell

	Radioactivity (DPM)					
	Cell			Medium		
	Prog.	Est.	Chol.	Prog.	Est.	Chol.
Control	1.168± 421	885± 231	75.472± 25.128	412± 212	885± 340	109.355± 21.269
	(1.5)	(1.2)	(100)	(0.4)	(0.8)	(100)
Ginseng saponin mixture	2.691± 968	962± 57	67.659± 19.457	414± 98	855± 32	64.818± 4.659
	(4)	(1.4)	(100)	(0.6)	(1.3)	(100)
Ginsenoside Rb_1	1.690± 279	1.054± 144	47.033± 15.854	1.016± 203	2.349± 48	64.866± 15.717
	(3.6)	(2.2)	(100)	(1.6)	(3.6)	(100)
-Rb_2	1.546± 211	1.242± 1.089	70.892± 7.398	1.115± 585	1.612± 1.361	84.396± 19.145
	(2.2)	(1.8)	(100)	(1.3)	(1.9)	(100)
-Re	2.265± 719	915± 147	68.704± 14.487	1.005± 722	1.083± 561	64.052± 12.635
	(3.3)	(1.3)	(100)	(1.6)	(1.7)	(100)
-Rg_1	1.813± 113	938± 81	57.544± 6.244	514± 121	2.472± 1.510	88.197± 21.216
	(3.2)	(1.6)	(100)	(0.6)	(2.8)	(100)

The figure in blackets are relative percentage assuming that of cholesterol being 100.

인삼사포닌의 저밀도지질단백질(LDL)수용체에 미치는 영향

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세포막의 주요 구성성분인 콜레스테롤은 고등생명체의 성장 및 생존에 필수적인 물질이다. 그러나 혈중 콜레스테롤의 농도가 너무 높으면 동맥경화증을 유발하여 매우 치명적이다. 본연구는 고려인삼(*Panax ginseng* C.A. Meyer) 뿌리의 주요성분의 하나인 사포닌류의 고콜레스테롤 혈중 억제효과를 규명하기 위해 시도되었다. 고콜레스테롤 식이를 인삼사포닌과 함께 또는 단독으로 투여한 토끼나 쥐에 ^{125}I-LDL을 주입한 후 혈액으로부터 ^{125}I-LDL의 제거속도를 추적한 결과 고콜레스테롤 식이만을 투여한 대조군보다 인삼사포닌을 함께 투여한 시험군이 ^{125}I-LDL의 제거속도가 훨씬 빨랐다. 고콜레스테롤식이를 투여한 쥐의 LDL수용체 생합성에 미치는 ginsenoside 혼합물과 정제된 ginsenoside Rb_1, Rb_2, Re, Rg_1의 영향을 조사하였다. 쥐 간의 LDL 수용체 수준을 분석한 결과 시험군이 대조군에 비해 크게 높았으며 다른 장기(신장, 정소, 부신피질)의 경우에도 간에서의 결과와 유사하였다. 또한 간파쇄액을 효소원으로 사용하여 콜레스테롤로 부터의 담즙산 합성에 미치는 ginsenoside의 영향을 시험관내에서 관찰한 결과 반응 혼합물에서의 ginsenoside의 농도가 10^{-3}-10^{-4}%일 때 담즙산합성이 현저히 증가되었다. 위와 같은 실험결과로 미루어 보아 ginsenoside는 세포내부에서의 콜레스테롤 대사를 촉진하여 세포내의 콜레스테롤 농도를 저하시킴으로써 콜레스테롤의 LDL 수용체 합성 억제를 완화시켜주는 것이라고 생각된다.