EFFECTS OF GINSENG SAPONIN ON ENDOTHELIUM-DEPENDENT VASCULAR RELAXATION IN RAT AORTA AND HYPERCHOLESTEROLEMIC RABBIT AORTA

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ABSTRACT

Intravenous administration of saponin extracted from the root of Panax ginseng lowered the blood pressure dose - dependently (10 - 200 mg/kg, B.W.) in anesthetized rats. Therefore, experiments were designed to study the hypothesis that the lowering of blood pressure is associated with the release of endothelium - derived relaxing factor and the accumulation of guanosine 3, 5 - cyclic monophosphate (cGMP). Rings of thoracic aorta with and without endothelium were suspended for the measurement of isometric tension in organ chamber and the tissue content of cGMP was measured by radioimmunoassay. All experiments were performed in the presence of indomethacin(10^{-5}M). Ginseng saponin (10^{-5} - 3×10^{-4}g/ml) relaxed contractions induced by phenylephrine (10^{-6}M) in the aorta with endothelium but not in that without endothelium.

Treatment of aortic rings with N^G monomethyl - L - arginine (L - NMMA, 10^{-4}M for 30 min), a competitive inhibitor of nitric oxide synthase, and methylene blue (MB, 3×10^{-5}M for 30 min), an inhibitor of soluble guanylate cyclase, diminished the relaxation induced by Ginseng saponin. Ginseng saponin (10^{-4} g/ml for 2 min) increased the accumulation of cGMP in rings with endothelium. L - NMMA and MB inhibited the accumulation of cGMP induced by Ginseng saponin. These data suggest that vascular relaxations induced by Ginseng saponin are mediated by release of endothelium - derived relaxing factor and the accumulation of cGMP.

The effect of Ginseng saponin on endothelial function in hypercholesterolemic rabbits was examined. In hypercholesterolemic rabbits fed with 2% cholesterol for 8 weeks, relaxation of aortic rings to acetylcholine was impaired. The impaired relaxations of aortic rings in hypercholesterolemic rabbits were improved by dietary supplementation of Ginseng saponin, probably because of an improved release of endothelium - derived relaxing factor.

INTRODUCTION

In cardiovascular studies, Ginseng saponin has been shown to have an and antihypertensive and antihypercholesterolemic effect(Duke, 1989).

Lee(1980) reported that Ginseng saponin relived hypertension by relaxing the smooth muscle of blood vessel. It was reported that Ginseng saponin reversed norepinephrine and $PGF_{2\alpha}$ - induced contraction of rabbit pulmonary and intrapulmonary arteries (Chen et. al., 1984).

In our study, we found that intravenons administration of Ginseng saponin lowered the blood pressure in dose - dependent manner in anesthetized rats. On the other hand, the endothelium plays an important role in regulating vascular tone by synthesizing and releasing vasodilator mediators(i.e. prostacyclin, Vane et. al., 1990 and nitric oxide, Furchgott et. al., 1980). It is likely that impairment of endothelium is important in the pathogenesis of systemic vascular diseases including atherosclerosis and hypertension. Hypercholesterolemia causes endothelial dysfunction that manifested as an attenuation of endothelium - dependent vasorelaxation(Shimokawa et. al., 1989 and Jayakody et. al., 1985). This abnormality may be due to reduced synthesis and release of endothelium - derived relaxing factor(EDRF) (Verbeuren et. al., 1986 and Streeharen et. al., 1986). EDRF is nitric oxide(NO) or a labile nitroso compound that liberates nitric oxide(Palmer et. al. 1987, Ignarro et. al. 1987) and derived from the metabolism of L - arginine(Palmer et. al. 1988a, Palmer et. al. 1988b).

Therefore, in the present study, we examined 1) whether Ginseng saponin causes vasodilation in vitro, 2) whether this vasodilation is involved in the endothelium - dependent production of EDRF and increase of cGMP, 3) whether dietary supplementation with ginseng saponin would improve endothelium - dependent responses in hypercholesterolemic rabbits.

MATERIALS AND METHOD

Ginseng saponin :

Red Ginseng Saponin (Panaxa ginseng C.A. Meyer), protopanaxatriol and protopanaxadiol were kindly provided By Korea Ginseng And Tobacco Research Institute, Korea.

Measurement of blood pressure :

Male Sprague Dawley rats (300 - 400g) were anesthetized with pentobarbital (65 mg/kg, i.p.). Carotid artery was cannulated with polyethylene catheter and blood pressure was measured using pressure transducer(Model rp1500) connected with strain - gauge coupler on a Narco Biosystems(Narcotrace 80) polygraph. Ginseng saponin (5, 10, 20, 50, 100, and 200 mg/kg body weight) was injected into the femoral vein.

Organ chamber studies :

Male Sprague Dawley rats (300 - 400g) and New Zealand white rabbits(2.0 - 2.3kg) were sacrificed, and their thoracic aortae were removed and placed in a modified Krebs - Ringer - Bicarbonate solution containing (in mM) NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, CaEDTA 0.016 and glucose 11.1. The aortae were cleaned of loose connective tissue and then cut into eight rings (2 - 3 mm wide). In some rings, the endothelium was removed mechanically. The aortic rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 25 ml of control solution (37℃, pH 7.4) and bubbled with 95% O_2 and 5% CO_2. One of the stirrups was anchored to the organ chamber and one was connected to a strain gauge (Narco bio - system) for the recording of isometric tension. The aortic rings were stretched progressively to the optimal tension (2g) before the addition of phenylephrine (10^{-6}M). Once the plateau of the contraction to phenylephrine was obtained, the aortic rings were rinsed three times with warm (37℃) control solution. After a resting period (30 min), the aortic rings were exposed again to phenylephrine (10^{-6} M). When the contraction had stabilized, acetylcholine (10^{-6}M) was added to test the presence or the absence of the endothelium. The organ chambers were rinsed three times with warm (37℃) control solution before the addition of indomethacin (10^{-5} M) to prevent the production of endogenous vasoactive prostanoids. In some experiments, N^{ω} monomethyl - L - arginine (L - NMMA, an inhibitor of nitric oxide synthase ; 10^{-4}M) and methylene blue (MB, an inhibitor of soluble guanylate cyclase activation ; 3×10^{-5}M) were added 30 min before the addition of the phenylephrine(10^{-6}M).

Ginseng saponin, protopanaxatriol and protopanaxadiol ($10^{-6} - 3\times10^{-4}$ g/ml) were added to the organ chamber when the 10^{-6}M phenylephrine - induced contraction reached a plateau. The relaxing response was expressed in terms of percent decrease of the maximal contraction developed by phenylephrine (10^{-6}M).

Measurement of cGMP levels :

cGMP was measured in rings that were not under tension. Rings of rat aortae with and without endothelium were incubated for 30 min at 37℃ in flasks containing 10 ml of control solution gassed with 95% O_2 - 5% CO_2. After this equilibration period, the incubation medium was removed and replaced with warmed (37℃) and oxygenated control solution containing indomethacin (10^{-5}M) and 3 - isobutyl - 1 - methylxanthine (IBMX, 10^{-4}M). In some experiments, methylene blue (3×10^{-7} M) or L - NMMA(10^{-4}M) was added to the flasks and was present throughout the remainder of the experiment. The vessels were then allowed to equilibrate for an additional 30 min. Phenylephrine (10^{-6}M) was added to the incubation medium. After 7 min, either acetylcholine (10^{-6}M, 1 min) or Ginseng saponin (10^{-4} g/ml, 2 min) was added. At designated time after exposure to the relaxing agents, the tissues were frozen rapidly with an aluminum clamp cooled in liquid nitrogen. The frozen tissues were pulverized, homogenized in 1 ml of 6% trichloroacetic acid with a glass - glass potter, and centrifuged at 13,600 g for 16 min. The supernatant was extracted four times with four volumes of water - saturated ether, lyophilized and stored at -70℃. On the day of experiments, each sample was resuspended in 0.5 ml of sodium acetate buffer (0.05M, pH 6.2). The content of the cyclic nucleotide was determined using readioimmunoassay kits purchased from Amersham. The amount of protein was determined by the method of Lowry.

Endothelium - dependent responses in hypercholesterolemic rabbits :

New zealand white rabbits, weighing 1.25 - 1.68kg, were housed individually with free access to water. Animals were devided into 3 groups ; Control group (6 animals fed a standard rabbit diet), cholesterol - fed group (6 animals fed the same diet supplemented with 2.0% cholesterol) and cholesterol and Ginseng saponin fed group (6 animals fed the same diet supplemented with 2.0% cholesterol and 50 mg/kg/day Ginseng saponin). All animals were fed 150g of diet daily.

After 8 weeks, the animals were anesthetized with sodium pentobarbital (30 mg/kg, i. v.) and a blood sample was taken for the determination of serum levels of total cholesterol and triglycerides.

The rabbits were then killed and segments of thoracic aorta were carefully removed and immediately immersed in Krebs - Henseleit solution.

Organ Chamber studies were performed with the same method as described above in rat studies.

Relaxants, acetylcholine ($10^{-9} - 10^{-5}$M) was added to the bath medium when the 10^{-6}M norepinephrine - induced contraction reached a plateau.

The relaxing response was expressed in terms of percent decrease of the maximal contraction developed by norepinephrine (10^{-6}M).

Lipid estimation

The serum cholesterol and triglyceride concentrations were estimated using an Automated Analysis Instrument (Abbot, spectrum).

RESULTS

Effects of Ginseng saponin on the blood pressure in rats :

When Ginseng saponin was administered into femoral vein (5 - 200 mg/kg) a dose - dependent decrease of blood pressure was observed. As shown in Fig. 1, at a dose of 10 - 20 mg/kg a transient decrease of blood pressure was observed and soon recovered to normal level. At greater than a dose of 50 mg/kg of Ginseng saponin, a sustained decrease of blood pressure was observed.

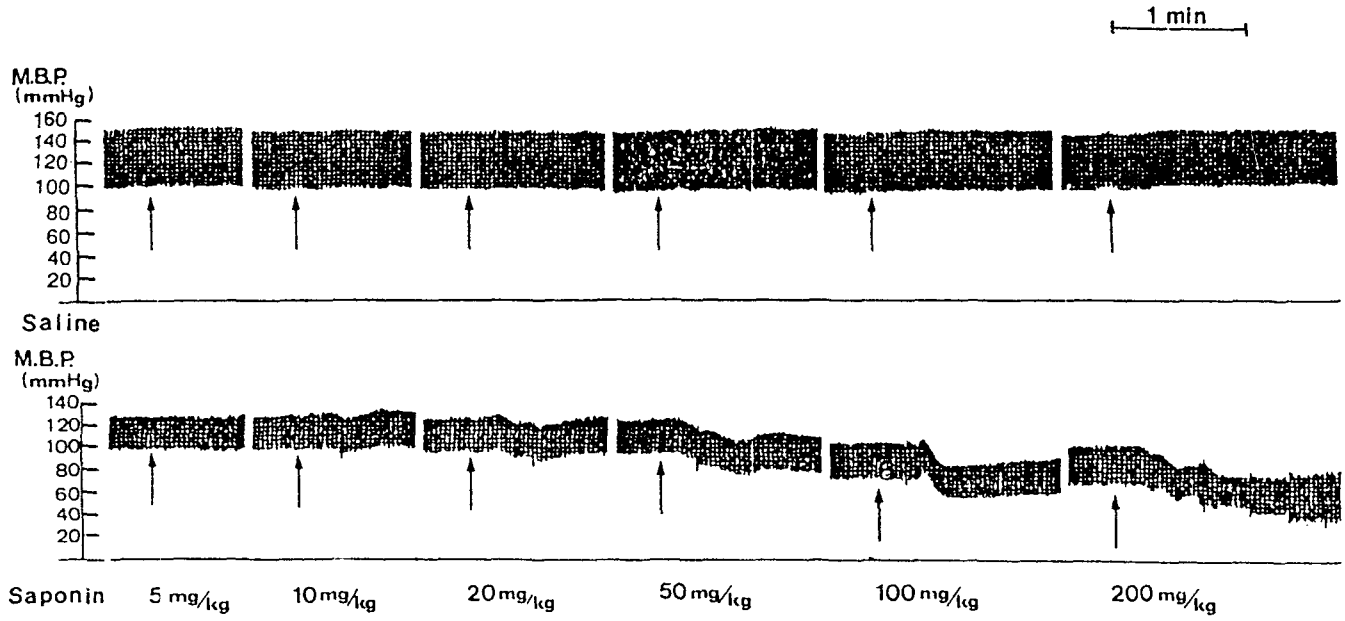

Figure. 1. Effects of Red ginseng saponin (intravenous administration) on the blood pressure in anesthetized rats.

Endothelium - dependent relaxation in rat aorta :

Fig. 2 shows the representative tracing of the cumulative concentration - response curve to Ginseng saponin in the rat aorta. When the maximal contraction was obtained to 10^{-6}M phenylephrine, Ginseng saponin was added to the organ bath in a cumulative manner. Ginseng saponin significantly relaxed the aortic rings with endothelium in a concentration - dependent manner (from 3×10^{-5} to 3×10^{-4} g/ml), but not in those without endothelium. Pretreatment of aortic rings with N^G - monomethyl - L - arginine(L - NMMA, 100μM for 30 min, an inhibitor of the formation of NO) and methylene blue (MB, 3×10^{-7}M for 30 min, an inhibitor of the guanylate cyclase) reversed the relaxation induced by Ginseng saponin.

Concentration - relaxation curve to acetylcholine(left graph) and Ginseng saponin(right graph) shifted to the right in rings with endothelium in the presence of both inhibitors(Fig. 3) ; acetylcholine(Log EC_{50} changed from -7.20 ± 0.02 to -6.84 ± 0.09($p<0.01$) in the presence of L - NMMA, n=5 and from -7.20 ± 0.02 to -6.75 ± 0.09($p<0.01$), Ginseng saponin(Log EC_{50} changed from -4.37 ± 0.03 to -4.16 ± 0.03 to ($p<0.001$) in the presence of L - NMMA, n=5 and from -4.37 ± 0.03 to 4.15 ± 0.08($P<0.05$) in the presence of methylene blue, n=5). When methylene blue was added after the relaxation by cumulative dose of Ginseng saponin, the relaxation induced by Ginseng saponin was reversed acutely (Data are not shown). Protopanaxatriol (10^{-6} - 10^{-4} g/ml) evoked concentration - dependent relaxation in the phenylephrine - contracted aorta with endothelium but not in those without endothelium (Fig. 4). Treatment of aortic rings with L - NMMA (100μM for 30min) and MB (3×10^{-7}M for 30min) shifted the concentration - relaxation curve to protopanaxatriol to the right in rings with endothelium

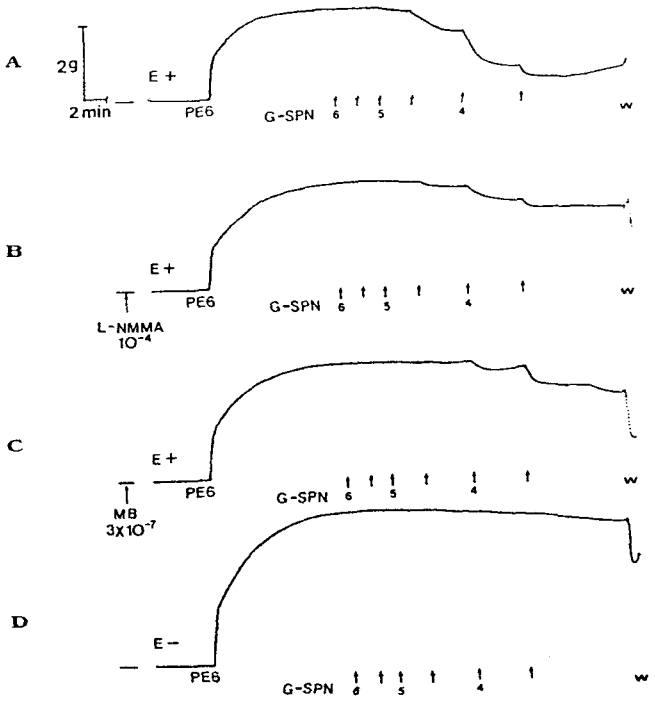

Figure. 2. Representative traces showing that Ginseng saponin (G - SPN) evokes concentration - dependent relaxation of rat aortic rings contracted with phenylephrine (PE) 10^{-6}M. A ; with endothelium, E(+), B ; in the presence of N^G - monomethyl L - arginine (L - NMMA, an inhibitor of nitric oxide synthetase) 10^{-4}M, C ; in the presence of methylene blue (MB, an inhibitor of soluble guanylate cyclase) 3×10^{-6}M, D ; without endothelium, E(-). All experiments were performed in the presence of indomethacin 10^{-5}M.

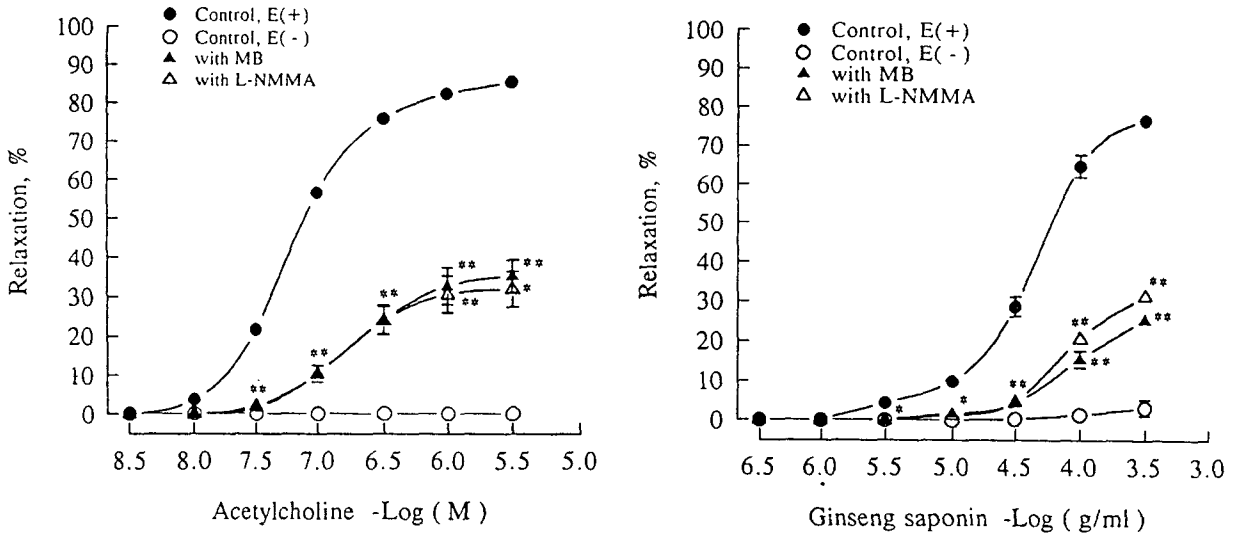

Figure. 3. Effects of treatment of aortic rings with methylene blue(MB) 3×10^{-7}M or N^G - monomethyl L - arginine (L - NMMA) 10^{-4}M on the relaxations evoked by acetylcholine or Ginseng saponin in rat aortic rings. All aortic rings were precontracted with phenylephrine 10^{-6}M. All experiments were performed in the presence of indomethacin 10^{-5}M. Results are shown as mean± SEM of 6 different experiments.

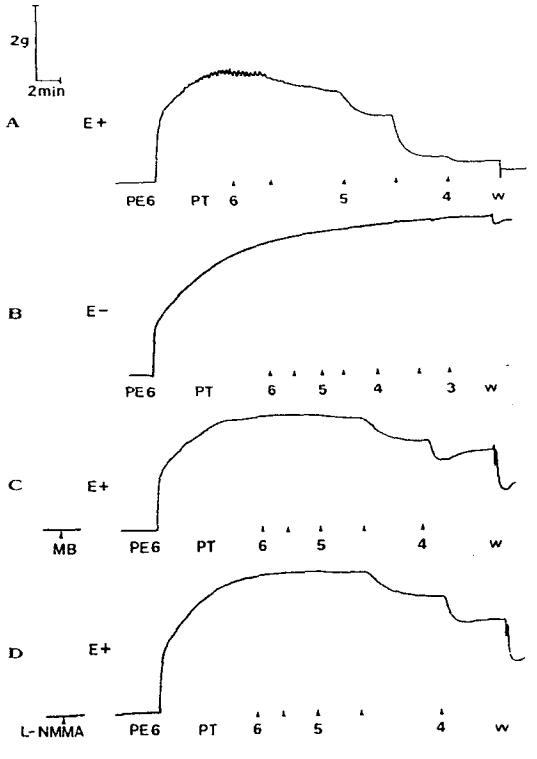

Figure. 4. Representative traces showing that Protopanaxatriol (PT) evokes concentration - dependent relaxation of rat aortic rings contracted with phenylephrine(PE) 10^{-6}M. A ; with endothelium, E(+), B ; without endothelium, E(-), C ; in the presence of methylene blue (MB, an inhibitor of soluble guanylate cyclase) 3×10^{-6}M, D ; in the presence of N^G - monomethyl L - arginine (L - NMMA, an inhibitor of nitric oxide synthetase) 10^{-4}M. All experiments were performed in the presence of indomethacin 10^{-5}M.

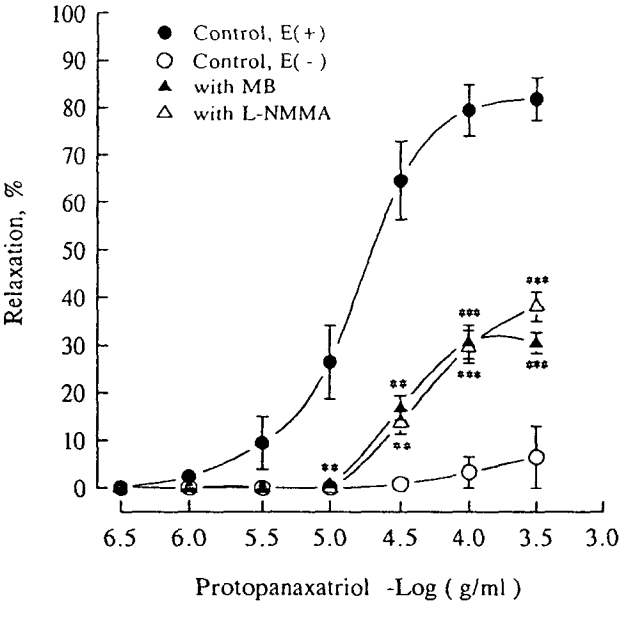

Figure. 5. Effects of treatment of aortic rings with methylene blue(MB) 3×10^{-7}M or N^G - monomethyl L - arginine (L - NMMA) 10^{-4}M on the relaxations evoked by Protopanaxatriol in rat aortic rings. All aortic rings were precontracted with phenylephrine 10^{-6}M. All experiments were performed in the presence of indomethacin 10^{-5}M. Results are shown as mean± SEM of 6 different experiments.

(Log EC_{50} changed from -4.83 ± 0.10 to -4.32 ± 0.07($p<0.01$) in the presence of L-NMMA, and from -4.83 ± 0.10 to -4.50 ± 0.07($p<0.05$) in the presence of MB, respectively, n=6) (Fig. 5).

Protopanaxadiol, however, did not evoked relaxation in the aorta both with and without endothelium (Fig. 6).

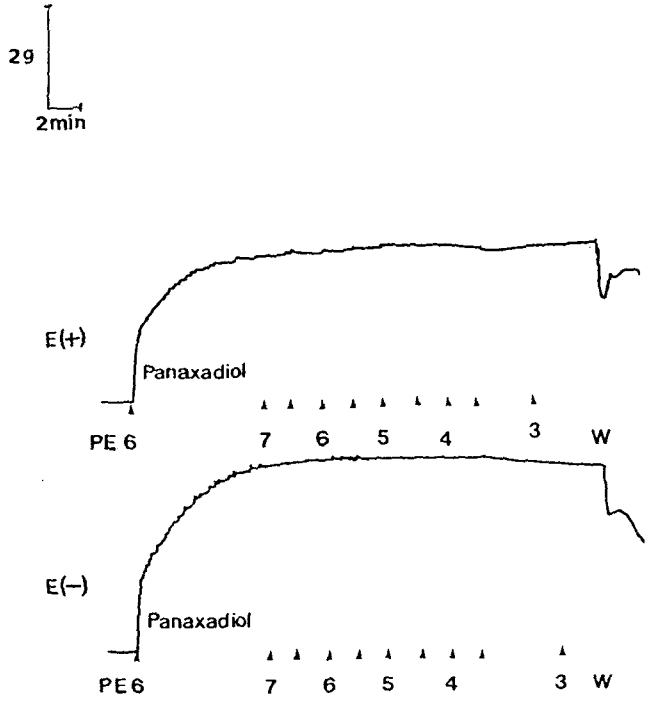

Figure. 6. Representative traces showing that Panaxadiol does not evoke relaxation of rat aortic rings both with endothelium and without endothelium. Rings were contracted with phenylephrine(PE) 10^{-6}M. All experiments were performed in the presence of indomethacin 10^{-5} M. E(+) ; intact endothelium, E(-) ; denuded endothelium.

Endothelium-dependent relaxation in rabbit aorta.

Acetylcholine ($10^{-8}-3\times10^{-6}$M) and Ginseng saponin ($10^{-5}-3\times10^{-4}$M g/ml) evoked concentration-dependent relaxation in the phenylephrine-contracted rabbit aortae with endothelium but not in those without endothelium, which is similar to that observed in the rat aorta.

Maximum relaxation to acetylcholine was 79% and to 3×10^{-4} g/ml Ginseng saponin was 45% of the 10^{-6}M phenylephrine-induced response.

Pretreatment of aortic ring with L-NMMA and methylene blue shifted the concentration-relaxation curve to acetylcholine and Ginseng saponin to the right in rings with endothelium ; acetylcholine(Log EC_{50} changed from -6.94 ± 0.07 to -6.62 ± 0.10($p<0.05$) in the presence of L-NMMA, n=6 and from -6.94 ± 0.07 to -6.63 ± 0.06($p<0.01$) in the presence of MB, n=6), Ginseng saponin (Log EC_{50} changed from -4.22 ± 0.02 to 3.94 ± 0.09($p<0.05$) in the presence of L-NMMA and -4.22 ± 0.02 to -4.14 ± 0.02($p<0.05$) in the presence of MB, respectively, n=6) (Fig. 7).

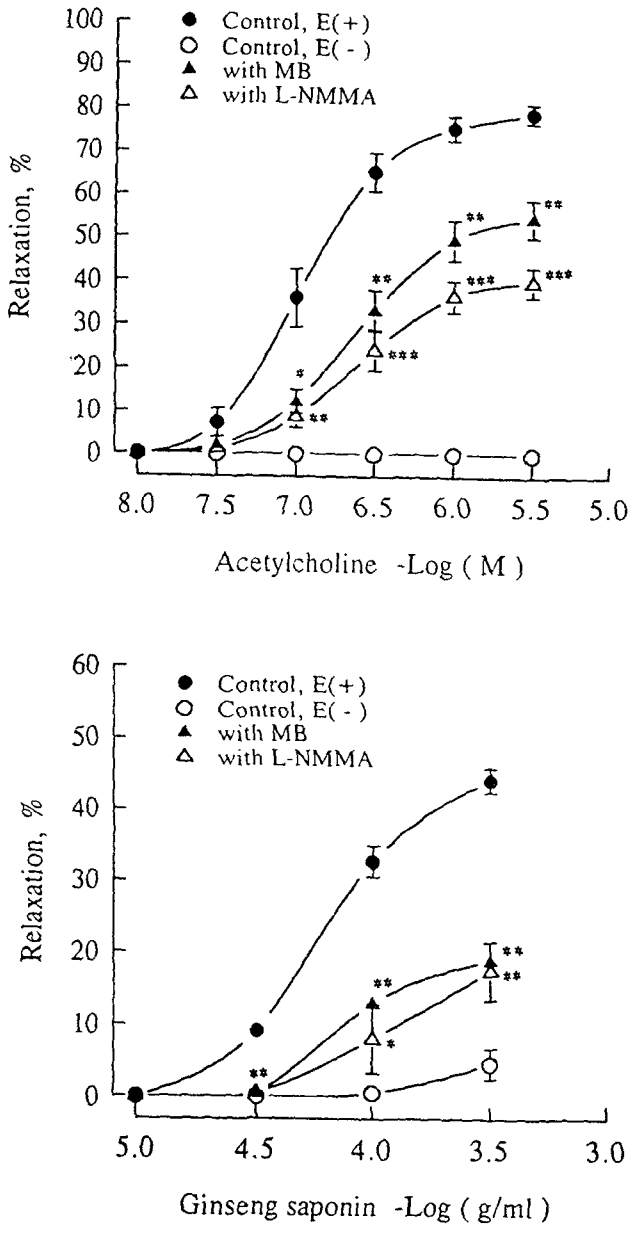

Figure. 7. Effects of treatment of aortic rings with methylene blue(MB) 3×10^{-7}M or N^G-monomethyl L-arginine (L-NMMA) 10^{-4}M on the relaxations evoked by acetylcholine or Ginseng saponin in rabbit aortic rings. All aortic rings were precontracted with norepinephrine 10^{-6}M or phenylephrine 10^{-6}M respectively. All experiments were performed in the presence of indomethacin 10^{-5}M. Results are shown as mean± SEM of 6 different experiments.

Effect of Ginseng saponin on the production of the tissue cGMP.

Time course study :

We examined whether the relaxation to Ginseng saponin was due to an increase in the tissue cGMP content. At various times after the exposure to the agents indicated, the tissue was frozen in liquid nitrogen and cyclic GMP levels were assayed. Ginseng saponin increased significantly the tissue content of cGMP in rat aortic preparations with endothelium. The accumulation of the cyclic nucleotide reached a maximum in vessel within 2 min of exposure to the Ginseng saponin, approximately 19 pmol/mg protein and declined gradually over a 10 min period, but only slightly in nontreated tissue with endothelium. At the peak accumulation a 2.0 - fold increase in cGMP was observed in aorta with endothelium(Fig. 8).

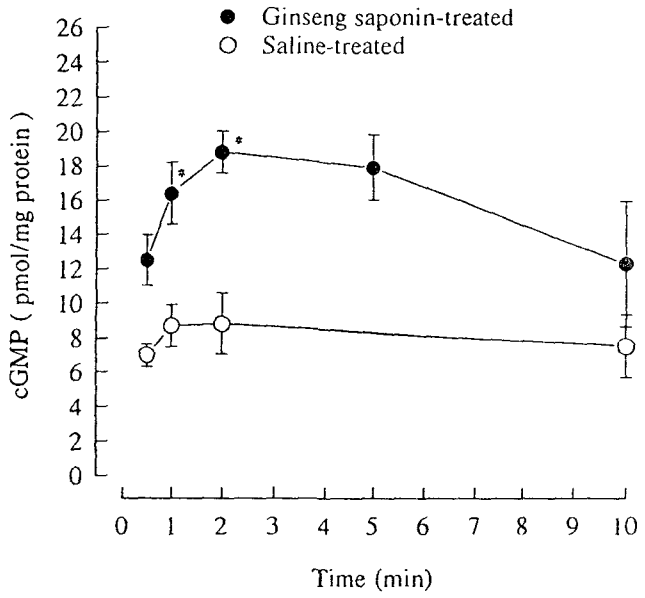

Figure. 8. Time course of accumulation of cGMP induced by Red ginseng sapinin 10^{-4}g/ml and saline in rings of rat thoracic aorta with endothelium. All experiments were performed in the presence of indomethacin 10^{-5} M and IBMX 10^{-4}M. Results are shown as mean± SEM of 5 different experiments.

Production of cyclic GMP :

Times for cyclic GMP determinations were selected that correspond to the period of maximal relaxation.

Treatment of intact aortic rings with acetylcholine, endothelium - dependent vasodilator, increased more than 5 folds of tissue cyclic GMP content. Treatment of aortic rings with 10^{-4} g/ml Ginseng saponin increased about 2 folds in cyclic GMP content of rings with endothelium, compared with nontreated rings with endothelium, but Ginseng saponin did not affect the accumulation of cGMP in rings without endothelium.

Treatment of rat aortic vessels with 3×10^{-7}M methylene blue or 100 μM L - NMMA, a condition that inhibits the effect of endothelium on agonist - induced responses (Miller et. al., 1984), significantly reduced the increase of tissue content of cyclic GMP by Ginseng saponin from vessels with endothelium. (Fig. 9).

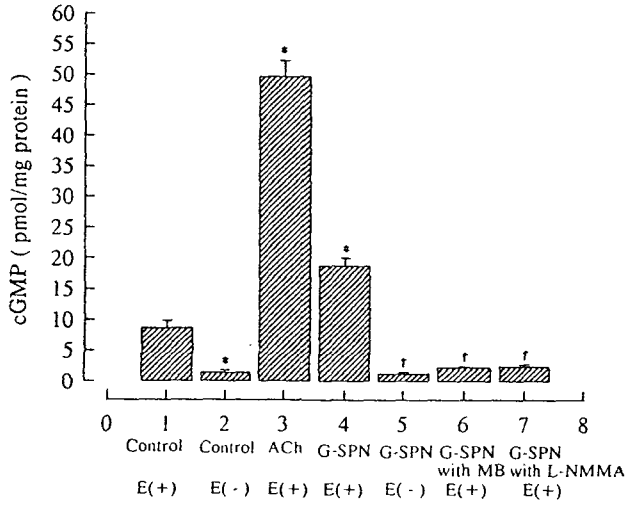

Figure. 9. Effects of acetylcholine(ACh) and Red ginseng saponin(G - SPN) on the accumulation of cGMP and effects of methylene blue(MB) and N^G - monomethyl L - arginine(L - NMMA) on the accumulation of cGMP induced by Ginseng saponin in the rat aorta. All experiments were performed in the presence of indomethacin 10^{-5}M and IBMX 10^{-4}M. Each bar represents mean± SEM of 5 different experiments.

1 ; Control, + endothelium
2 ; Control, - endothelium
3 ; ACh 10^{-6}M, + endothelium
4 ; G - SPN 10^{-4}M, + endothelium
5 ; G - SPN 10^{-4}M, - endothelium
6 ; MB 3×10^{-7}M plus G - SPN 10^{-4}g/ml, + endothelium
7 ; L - NMMA 10^{-4}M plus G - SPN 10^{-4}M, + endothelium

Hypercholesterolemic rabbits :

This investigation was undertaken to determine whether it is possible to restore endothelium - dependent relaxation in cholesterol - fed rabbit model of atherosclerosis after administration of Ginseng saponin.

Endothelium - dependent relaxation :

Response to norepinephrine : The concentration - related effects to norepinephrine and the maximum contractions elicited by norepinephrine(10^{-5}M) were not significantly different in the three groups of animals (Fig. 10).

The EC_{50} in the three groups of animals was not significantly different (Log EC_{50} : - 7.06± 0.13(control), - 7.04± 0.14(cholesterol) and - 6.97± 0.08(cholesterol plus Ginseng saponin), (P=NS), n=4 in each group).

Endothelium - dependent relaxation : Endothelium - de-

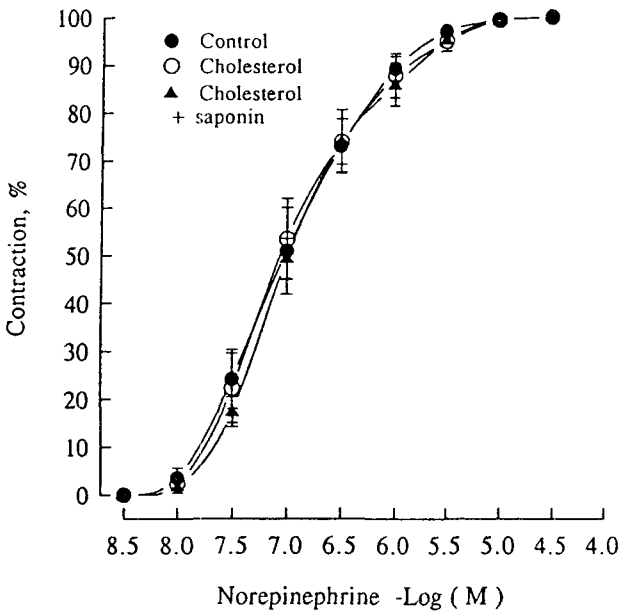

Figure. 10. Cumulative concentration - response curve to norepinephrine in aortic rings from control group, cholesterol group and cholesterol plus Ginseng saponin group at 8 weeks. There were no significant differences in these curves (n=5 for each group). Bars represent SEM.

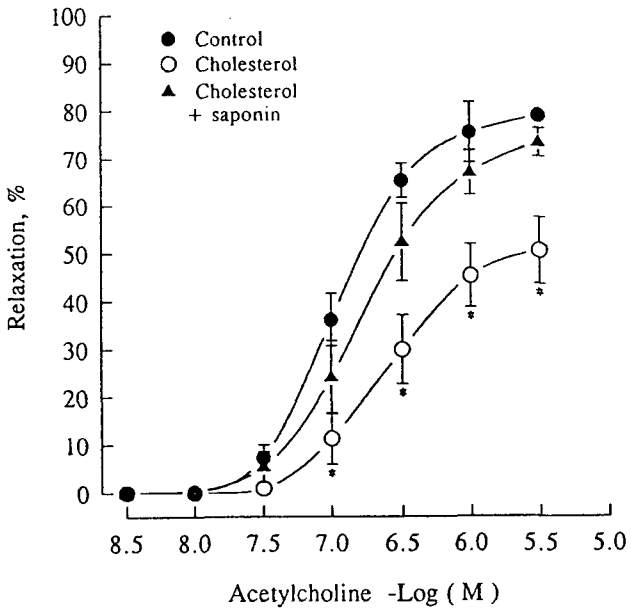

Figure. 11. Endothelium - dependent relaxation induced by acetylcholine (3×10^{-9} - 10^{-5}M) in rabbit aortic rings from control group, cholesterol group and cholesterol plus Ginseng saponin group at 8 weeks. There were significant difference between the control group and cholesterol group ($p<0.05$, n=6). There were no significant difference between the control group and cholesterol plus Ginseng saponin group (p=NS)

pendent relaxation was examined in the control group, cholesterol group and cholesterol and Ginseng saponin group at the end of 8 weeks. Endothelium - dependent relaxations evoked by acetylcholine(3×10^{-6}M) were impaired in aortic rings from 8 weeks hypercholesterolemic aminals (50.4± 6.87%) compared with that in the control group (78.0± 1.84%) ($P<0.05$, n=6, Fig. 11).

In these animals, there was a twofold rightward shift in the sensitivity to acetylcholine compared with control animals ($LogEC_{50}$ changed from - 6.94± 0.07 to - 6.63± 0.13, ($p<0.05$), n=6 in each group).

Ginseng saponin improved endothelium - dependent relaxation in hypercholesterolemic animals. Vascular rings from the 8 weeks hypercholesterolemic animals exposed to Ginseng saponin were 38% more sensitive to acetylcholine than those exposed to cholesterol alone ($LogEC_{50}$ changed from - 6.63± 0.13 to - 6.77± 0.13, $P<0.05$, n=6).

Endothelium - dependent relaxation in aortic rings from hypercholesterolemic animals that received Ginseng saponin were not different from those that observed in control animals (Log EC_{50} changed from - 6.94± 0.07(control) to - 6.77± 0.13 (Ginseng saponin), P=NS, n=6 in each group) (Fig. 11).

Serum cholesterol and triglyceride levels :

After 8 weeks and 4 weeks of experiment, body weight increased significantly but in a similar manner in the control group, cholesterol group and cholesterol plus Ginseng saponin group (Data are not shown).

The serum cholesterol concentrations significantly increased in all fractions in cholesterol group and cholesterol plus Ginseng saponin group. There was no difference in each fraction between cholesterol group and cholesterol plus Ginseng saponin group in the 8 week - cholesterol feeding group. The serum triglyceride

Table 1. Effect of Ginseng saponin on the serum cholesterol and triglyceride levels in hypercholesterolemic rabbits.

Experiment	Serum lipids		Control (n=6)	Cholesterol (n=6)	Cholesterol and Ginseng saponin (n=6)
8 weeks	Cholesterol	(mg/dl)	55.0± 9.51	1355.0± 178.1[a]	1318.0± 133.9[b]
	Triglyceride	(mg/dl)	81.0± 8.72	123.0± 52.15[c]	111.6± 25.4[d]

The control group was fed the standard diet for 8 weeks, cholesterol group fed the standard diet supplemented with 2.0% cholesterol for 8 weeks and cholesterol and Ginseng saponin group fed the standard diet supplemented with 2.0% cholesterol and 50 mg/kg/day Ginseng saponin for 8 weeks. Data shown are mean± SEM of 6 animals. a : indicates a significant difference from corresponding control animals ($p<0.05$) (n=6). b : indicates no significant difference from cholesterol animals (n=6). c : indicates no significant difference from corresponding control animals (n=6). d : indicates no significant difference from cholesterol animals (n=6).

concentrations were not significantly altered in both cholesterol and cholesterol plus Ginseng saponin group in 8 week experiments (Table 1).

DISCUSSION

Our results show that Ginseng saponin decreased blood pressure of anesthetized rats in a dose - dependent manner and induced endothelium - dependent vascular relaxation in rat aortic rings. From this data, it can be suggested that Ginseng - induced decrease of the blood pressure is caused by a relaxation of the blood vessels. In the present study, Ginseng saponin markedly relaxated phenylephrine - induced contration in the aorta with endothelium, but had no effect on that without endothelium. In addition, like the relaxations induced by acetylcholine, the responses to Ginseng saponin were inhibited by incubation with methylene blue (an inhibitor of guanylate cyclase) demonstrating the involvement of NO.

The effect of EDRF is mediated by increase in smooth muscle cyclic GMP content (Rapoport R.M. and Murad F., 1983, Ignarro et. al, 1986) by stimulating guanylate cyclase and relaxed the norepinephrine induced contraction.

The hypothesis that Ginseng saponin relaxes the smooth muscle through EDRF production is supported by the present data that Ginseng saponin increases the tissue contents of cGMP and markedly relaxed the phenylephrine - induced contraction. In several mammalian cell types there exists an oxidative L - arginine pathway, which leads to the formation of L - citurulline and NO (Palmer et. al., 1988a). No in turn participates in endothelium - dependent relaxation, and evidence has been presented that EDRF is identical with NO (Palmer et al., 1987). Recently L - NMMA was found to inhibit NO biosynthesis in vascular endothelial cells (Rees et. al., 1989).

In order to clarify whether the EDRF produced by Ginseng saponin - induced endothelium - dependent relaxation is truly identical with NO, we examined the effect of L - NMMA in isolated rat aorta. Ginseng saponin - induced relaxation was blocked by pretreatment with L - NMMA. These results suggest that the EDRF released or synthesized from endothelial cells in the relaxation caused by Ginseng saponin is identical with NO.

Protopanaxatriol, a partially purified fraction of total Ginseng saponin, but not protopanaxadiol, also induced endothelium - dependent vascular relaxation in rat aortic rings. The relaxing effect of protopanaxatriol was about 3 folds greater than that of Ginseng saponin.

On the other hand, it is known that the vascular endothelial cells release prostaglandin I_2(PGI_2), as a relaxing substance (Forstermann and Neufang, 1984), and that synthesis of PGI_2 is inhibited by indomethacin, an inhibitor of cyclooxygenase (Skidgel & Printz 1987).

Ginseng saponin - induced relaxation was not inhibited by treatment with indomethacin. From our present data, it seems that Ginseng saponin probably relaxes the vascular smooth muscle involving the synthesis or release of EDRF, but not of PGI_2. In our studies, all experiments were performed in the presence of indomethacin to avoid the production of endogeneous vasoactive prostanoids. An endothelium - dependent vasodilator response to Ginsenoside has also been demonstrated in the perfused pulmonary and intrapulmonary arteries of rabbit (Kim et. al., 1992). The present observations indicate that the stimulated production of NO following activation of endothelial cells by Ginseng saponin participate in the relaxation response.

However, the present results do not explain how Ginseng saponin releases or synthesizes EDRF in the endothelial cells. Thus, further experiments are required to elucidate the precise mechanism of the Ginsenoside - induced endothelium - dependent relaxation.

This study also demonstrates that dietary supplementation with Ginseng saponin involves endothelium - dependent responses in hypercholesterolemic rabbit aorta.

In the present rabbit model, hypercholesterolemia impairs endothelium - dependent relaxation to acetylcholine. Compared with the responses of normal aorta from rabbits fed a normal diet, the present results indicate that the dietary Ginseng saponin delays the process of impairment of endothelium - dependent relaxations by normalizing the responses in hypercholesterolemia and by improving the impaired responses in atherosclelosis to the levels obtained with hypercholesterolemia alone. Our results regarding serum lipids are in agreement with the previous study by Koo(1983), in which dietary supplementation with Ginseng saponin in cholesterol - fed rabbit for 6 weeks had no effect on the increases in plasma lipids.

A series of recent investigations have shown that atherosclerosis profoundly alters endothelium - dependent relaxations. In isolated arteries of hypercholesterolemic rabbits, pigs and monkeys, the endothelium - mediated relaxations caused by acetylcholine, bradykinin and thrombin was significantly suppressed (Jayakody et. al., 1985, Verbeuren et. al., 1986). It was observed that bioassay rings relax less when exposed to perfusate from hypercholesterolemic rabbit thoracic aorta (Verbeuren et. al., 1986). Therefore the present results indicate that dietary supplementation with Ginseng saponin improve endothelium - dependent relaxation in hypercholesterolemic rabbit aorta by augmenting the release of EDRF.

CONCLUSION

1. Intravenous administration of ginseng saponin lowered the blood pressure in a dose - dependent manner in anesthetized rats.
2. Ginseng saponin and protopanaxatriol but not protopanaxadiol relaxed contractions induced by phenylephrine in the rat and rabbit aorta with endothelium but not that without endothelium.
3. Methylene blue and N^G - monomethyl - L - arginine(L - NMMA) reversed the relaxing effect of Ginseng saponin on contractions evoked by phenylephrine. L - NMMA and meth-

ylene blue inhibited the accumulation of cGMP induced by Ginseng saponin. These observations indicate that the mode of vasorelaxing effect of Ginseng saponin is mediated through the release of NO and subsequent accumulation of cGMP.

4. Dietary supplementation of Ginseng saponin restores endothelium - dependent relaxation to acetylcholine in hypercholesterolemic rabbits, probably because of an improved release of endothelium - derived relaxing factor.

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