Proceedings of the 5th International Ginseng Symposium, Seoul, 1988

Effect of Polyacetylene Compounds from Korean Ginseng on Lipid Peroxidation

Hyeyoung Kim (Jun), You Hui Lee, Shin Il Kim and Sung-Ha Jin

Korea Ginseng & Tobacco Research Institute, Taejeon, Korea

Abstract

The effect of three polyacetylene compounds, panaxydol, panaxynol and panaxytriol isolated from Korean ginseng on CCl_4-induced lipid peroxidation *in vitro* and *in vivo* hepatic microsomal lipid peroxidation were investigated. Lipid peroxide levels both in serum and liver and serum enzyme (GOT, GPT, LDH) activities of normal or CCl_4-treated mice and rats were also determined after administration of polyacetylenes. Hepatic microsomal cytochrome P-450 content and activities of aniline hydroxylase and aminopyrine demethylase were measured after treatment of polyacetylenes with or without carbon tetrachloride. As results, treatment with polyacetylenes to control mice did not influence the levels of lipid peroxides and serum enzyme activities while panaxynol did. Panaxynol itself inhibited liver lipid peroxidation in normal mice. Polyacetylene compounds protected hepatic lipid peroxidation and lowered serum lipid peroxide levels induced by CCl_4. Polyacetylenes prevented leakage of LDH to serum but elevated GOT and GPT levels caused by CCl_4 were not changed by polyacetylene pretreatment. CCl_4 caused losses in the content of cytochrome P-450 and activities of aniline hydroxylase and aminopyrine demethylase. When polyacetylenes were treated without CCl_4, panaxydol and panaxynol induced aniline hydroxylase and all three polyacetylenes induced aminopyrine demethylase. Cytochrome P-450 contents were not affected by polyacetylenes. *In vitro* hepatic microsomal lipid peroxidation was inhibited by polyacetylenes and DL-α-tocopherol in a concentration-dependent manner.

Introduction

Ginseng has been widely used as one of the most valuable medicines in Oriental countries since ancient times for the prevention of aging, tiredness and illness in biological systems and has so called adaptogenic effect which increases nonspecific resistance to adverse influences on the body[1-4]. The protective effect of *Panax ginseng* and other adaptogens may be elucidated by their antiradical and antioxidant action which might play a significant role in protection from some pathologic states such as stress, irradiation and cancer[5].

For antioxidant fraction of Korean ginseng, non-saponin fraction, ethyl ether, chloroform, methanol, ethanol, petroleum ether and total water extracts were reported[6-10]. As an individual component, maltol, phenolic acid[11] and panaxynes which were crude polyacetylenes[12] were identified from Korean ginseng. Recent investigators isolated polyacetylene compounds which showed cytotoxicities against some tumor cell lines[13-19].

Hepatotoxicity of carbon tetrachloride (CCl_4) is one of the best characterized models of lipid peroxidation. The toxic effect of CCl_4 is due to conversion of the molecule to the highly reactive toxic free radical (CCl_3) in the endoplasmic reticulum by the mixed function (P-450) oxidase system of enzymes involved in the metabolism of lipid-soluble drugs and other compounds[20-23]. The free radicals produced cause autooxidation of the polyenoic fatty acids present within the membrane phospholipids. Oxidative decomposition of the lipid is initiated and organic peroxides are formed after reacting with oxygen (lipid peroxidation)[24].

In the present study, the effect of polyacetylene compounds, panaxydol, panaxynol and panaxytriol on CCl_4-induced lipid peroxidation *in vivo* and *in vitro* hepatic microsomal lipid peroxidation were investigated. Serum enzyme (GOT, GPT, LDH) levels and lipid peroxide levels both in serum and liver were monitored in normal or CCl_4-treated mice and rats after administration of polyacetylenes. To investigate the mechanism of antioxidant effect of polyacetylenes, changes in cytochrome P-450 dependent mixed function oxidase systems were determined in hepatic microsome.

Materials and Methods

Materials : The chemicals used in the study were obtained from the following sources: 2-thiobarbituric acid, 1,1,3,1-tetra methoxy propane and ferric chloride, Fluka Chemical Co.; carbon tetrachloride and solvents for HPLC, J. T. Baker Chemical Co.; Silica gel 60 (70-230 mesh, ASTM) and silica gel 60F 254 plate (0.2 mm), Merck Chemical Co. All other reagents were purchased from Sigma Chemical Co. or commercial sources.

Isolation of polyacetylene compounds from Korean ginseng : Polyacetylene compounds from ginseng root were isolated by the method of Ahn & Kim[17-18]. Dried and pulverized red ginseng root were extracted with distilled ethyl ether and concentrated under nitrogen gas. The residue was repeatedly chromatographed over a silica gel column with petroleum / ethyl ether as gradient solvent system. Panaxydol, panaxynol and panaxytriol were isolated by preparative HPLC (Waters Associated Model 244 equipped with Model 6000A solvent delivery system). The structures of polyacetylene compounds are shown in Table 1.

Table 1. Structures of polyacetylene compounds

Compound	Structure
Panaxydol	C_7H_{15}-CH(O)-CH-CH_2-$(C\equiv C)_2$-CH(OH)-CH=CH_2
Panaxynol	C_7H_{15}-CH=CH-CH_2-$(C\equiv C)_2$-CH(OH)-CH=CH_2
Panaxytriol	C_6H_{13}-CH(OH)-CH(OH)-CH_2-$(C\equiv C)_2$-CH(OH)-CH=CH_2

Animal treatment: Male ICR mice (26-28 g) and Wistar rats (200-240 g) were obtained from the animal breeding room of the Korea Ginseng & Tobacco Research Institute (KGTRI) and allowed free access to food and water throughout the experiments. Animals were pretreated i.p. with polyacetylene compounds or DL-α-tocopherol (400μg/100g BW, mouse ; 2mg/100g BW, rat) 1 hr before administration of CCl_4 (12.5μl/100g BW, mouse ; 35μl/100g BW, rat) i.p. dissolved in liquid paraffin. Control animals received equivalent volumes of liquid paraffin. After 20 hr administration of CCl_4, all animals were sacrificed and whole bloods were taken by cardiac puncture and livers were removed for analysis of lipid peroxides and serum enzyme levels. To investigate the effect of polyacetylene compounds only to normal animals, polyacetylenes (400μg/100g BW, mouse ; 2 mg/100g BW, rat) were administered and liver and serum were analyzed 20 hr after treatment.

Chemical and enzymatic determinations: Lipid peroxidation of liver homogenate and microsome and serum lipid peroxide levels were determined by measuring the formation of malondialdehyde with thiobarbituric acid according to the method of Ohkawa et al.[25] and Lee et al.[26]. Activities of serum transaminase (GOT,GPT) and lactic dehydrogenase (LDH) were determined by commercial kits. The content of cytochrome P-450 in microsome was determined spectrophotometrically by the method of Omura and Sato[27]. Activity of aniline hydroxylase was measured according to the colorimetric method described by Riso et al.[28] and intensity of resulting blue color was measured at 630 nm using p-aminophenol sulphate as a standard. For the assay of aminopyrine demethylase, the reaction mixture (2ml) containing 10 mM aminopyrine, the microsomal suspension (1mg protein/ml) and the NADPH-generating system (3mM glucose-6-phosphate, 0.5 IU/ml glucose-6-phosphate dehydrogenase, 2.5 mM $MgCl_2$, 0.32 mM NADP and 8 mM nicotinamide in 0.1M Tris-HCl buffer, pH 8.0) was incubated at 37°C for 20 min. The reaction was stopped by addition of 20% trichloracetic acid (w/v) 0.5 ml and following procedures were same as those described by Wills[29]. Formaldehyde concentration was calculated by using 8000 $mM^{-1}cm^{-1}$ as the molar extinction coefficient for formaldehyde.

Microsomal lipid peroxidation *in vitro*: Liver from CCl_4-treated ICR mouse (26-28g) was homogenized and centrifuged at 1,500xg for 20 min in a refrigerated centrifuge. The supernatant was further centrifuged at 20,000 xg for 10 min and 120,000xg for 60 min to harvest microsomal fraction. The pellet was suspended in 0.15M Tris-HCl buffer (pH 7.4). Protein concentration in microsomal suspension was measured by Lowry method[30] using bovine serum albumin as a standard.

NADPH-dependent microsomal lipid peroxidation reaction mixture contained 0.5 mg of microsomal protein/ml, 1.7 mM ADP, 0.1mM $FeCl_3$, 0.1 mM NADPH and various concentrations of polyacetylene compounds or DL-α-tocopherol in 0.15M Tris-HCl buffer (pH 7.4)[31]. Incubations were carried out at 25°C for 30 min under an air atmosphere in water bath. For nonenzymatic lipid peroxidation, 10 μM $FeSO_4$, 0.1mM sodium ascorbate, liver microsome (1mg protein/ml), 0.15M Tris-HCl buffer, pH 7.4 and various concentrations of polyacetylenes or DL-α-tocopherol dissolved in absolute ethanol were incubated at 37°C for 20 min[32]. Lipid peroxidation was measured by the formation of the TBA-reactive material, malondialdehyde (MDA) using a method of Fairhurst et al[31].

Results

Lipid peroxide and serum enzyme levels of polyacetylene-treated mice: Treatment of polyacetylenes at 400 μg per 100g B.W. i.p. did not influence lipid peroxide levels in serum when compared with those of liquid paraffin treated control. In lipid peroxide levels of liver, panaxynol significantly inhibited the formation of lipid peroxide while panaxydol, panaxytriol and DL-α-tocopherol had no effect on lipid peroxidation in normal mice (Table 2). Blood samples for the determination of serum transaminase and LDH activities were collected 20 hr after treatment. The result showed that treatment of polyacetylene compound by itself had no effect on serum GOT, GPT and LDH levels.

Effect of polyacetylene compounds on lipid peroxide levels in CCl_4-treated animals: Lipid peroxide contents of serum and liver homogenate from CCl_4 treated mice (12.5 μg/100g BW) were increased to 2 times and 4.5 times that of normal group. Pretretment of paxaxydol, panaxynol and panaxytriol (400 μg/100g BW) prevented the increase of lipid peroxides induced by CCl_4. As shown in Fig. 1, the inhibitory effects of panaxydol, panaxynol and panaxytriol against lipid peroxidation were 30%, 34% and 32%, respectively in serum and 32%, 35% and 37% respectively in liver. DL-α-tocopherol inhibited lipid peroxidation 18% in serum and 30% in liver. In comparison with the effect of biological antioxidant, DL-α-tocopherol, polyacetylenes showed similar inhibitory effect against liver lipid peroxidation. With administration of lower dose of CCl_4 (35 μl/100g BW) to rats as compared to mice experiment, on the basis

Table 2. Lipid peroxide and serum enzyme levels of polyacetylene-treated mice.

Treatment	Lipid peroxide		Transaminase		LDH
	Serum	liver	GOT	GPT	
Control	110 ± 25	149 ± 16	25.1 ± 4.6	13.0 ± 3.7	86.1 ± 18.1
Panaxydol	100 ± 22	135 ± 31	23.0 ± 4.9	10.9 ± 3.4	72.8 ± 14.9
Panaxynol	102 ± 22	115 ± 32*	30.1 ± 11.5	10.8 ± 4.7	84.1 ± 15.7
Panaxytriol	98 ± 32	124 ± 28	29.4 ± 7.6	13.0 ± 4.2	73.8 ± 18.3

Polyacetylenes were administered i.p. to mice at a dose of 400 μg/100g.Values represent means ± SD of 6 animals. An asterisk indicates value signigicantly different from control animals. *$P<0.05$
Lipid peroxides are expressed as nmole MDA/ml serum and nmole MDA/g wet wt. of liver.
Serum enzymes are expressed as IU/L for GOT and GPT and μmoles/min/dl for LDH.

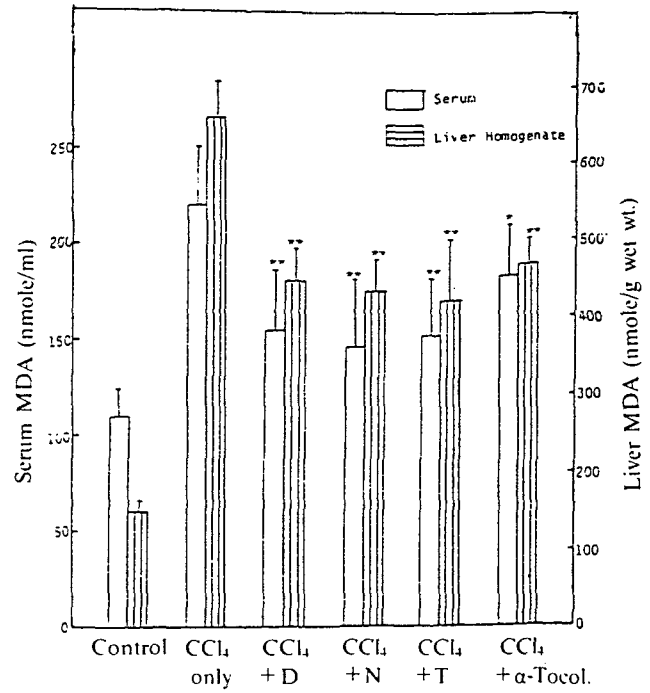

Fig. 1. Effect of polyacetylene compounds on lipid peroxide levels in CCl_4-treated mice. Mice received polyacetylenes (400 μg/100g BW. i.p.) 1 hr before administration of CCl_4. Values are means ± SD of 10 animals. D: panaxydol. N: panaxynol. T: panaxytriol. α-tocol: DL-α-tocohperol. An asterisk indicates value significantly different from CCl_4-treated animals. *p<0.05: **p<0.01.

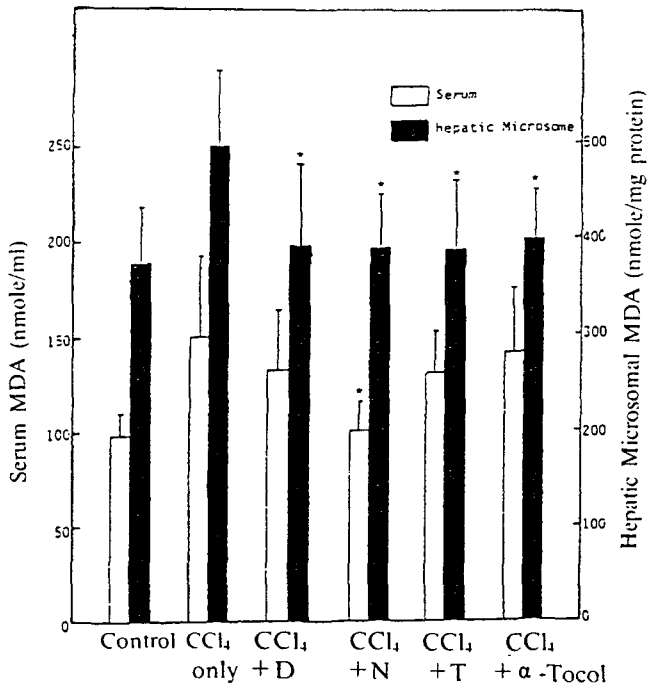

Fig. 2. Effect of polyacetylene compounds on lipid peroxide levels in CCl_4-treated rats. Rats received polyacetylenes (2mg/100g BW. i.p.) 1 hr before administration of CCl_4. Rats were killed 20 hr after treatment of CCl_4. Values are means ± SD of 6 animals. D: panaxydol. N: panaxynol. T: panaxytriol. α-tocol: DL-α-tocohperol. An asterisk indicates value significantly different from CCl_4-treated animals. *p<0.05.

of liver necrosis dose of CCl_4. lipid peroxide levels in serum and liver microsome were increased to 1.5 and 1.3 times (Fig. 2). Pretreatment of all three polyacetylenes and DL-α-tocopherol prevented liver microsomal lipid peroxidation and inhibition range was 20-22%. In serum lipid peroxide levels. only panaxynol sigificantly lowered serum MDA levels which was 66% of CCl_4-treated control group while other polyacetylenes did not.

Effect of polyacetylenes on serum enzyme levels of CCl_4-treated rats: Carbon tetrachloride causes radical-induced membrane damage and leakage of cytosolic enzyme. lactic dehydrogenase (LDH). Pretreament of polyacetylenes or DL-α-tocopherol significantly prevented the leakage of LDH. However. polyacetylenes or DL-α-tocopherol had no effect on the elevation of serum transaminases (GOT.GPT) caused by CCl_4(Table 3).

Changes in Cytochrome P-450-dependent mixed function oxidases in polyacetylene with or without CCl_4-treated rats: Carbon tetrachloride is first metabolized by drug metabolizing enzyme system represented by cytochrome P-450 to highly reactive trichloromethyl radical. Therefore. we measured the content of cytochrome P-450 and activities of aniline hydroxylase and aminopyrine demethylase in liver microsomes. As shown in Table 4

Table 3. Effect of polyacetylene compounds on serum enzyme levels of CCl_4-treated rat.

Treatment	Transaminase		LDH
	GOT	GPT	
Control	53.4± 20.1	22.1± 3.0	67.0± 32.1
CCl_4	139.3± 22.6	83.1± 23.7	118.5± 21.5
CCl_4+ panaxydol	147.7± 20.4	82.2± 6.4	75.9± 39.1*
CCl_4+ panaxynol	120.9± 17.2	67.8± 19.7	68.1± 17.9**
CCl_4+ panaxytriol	137.2± 4.4	69.3± 7.1	81.9± 26.3*
CCl_4+ α-tocopherol	120.8± 16.9	64.9± 21.2	64.0± 20.0**

Polyacetylenes were administered i. p. to rat at dose of 2 mg/100g BW 1hr before administration of CCl_4 (35 μl/100g BW). Rat were killed 20 hr after treatment of CCl_4. Values represent means ± SD of 6 animals. An asterisk indicates value significantly different from CCl_4-treated rats. *:p <0.05. **:p<0.01.
Serum enzymes are expressed as IU/L for transaminases and μmoles/min/dl for LDH.

Table 4. Aniline hydroxylase and aminopyrine demethylase activities in polyacetylene with or without CCl_4-treated rats.

Treatment	Aniline hydroxylase		Aminopyrine demethylase	
	w/o CCl_4	w/CCl_4	w/o CCl_4	w/CCl_4
None	0.98± 0.13	0.43± 0.19	7.18± 0.38	5.11± 0.43
Panaxydol	1.33± 0.13***	0.43± 0.14	9.05± 0.94**	4.88± 0.67
Panaxynol	1.30± 0.13***	0.42± 0.10	8.49± 1.19*	4.95± 0.44
Panaxytriol	1.02± 0.20	0.45± 0.12	8.09± 0.86*	5.11± 0.82

Polyacetylene compounds were treated i.p. at a dose of 2 mg/100g BW with or without administration of CCl_4 (35 μl/100 g BW). Rats were killed 20 hr after treatment of CCl_4 or polyacetylenes only. Values represent means ± SD for 6 animals. An asterisk indicates value significantly different from none group. *: p<0.05. **: p<0.01. ***: p<0.005
Serum enzyme levels are expressed as nmole/mg protein/min

Table 5. Cytochrome P-450 contents in polyacetylene with or without CCl_4-treated rats.

Treatment	Cytochrome P-450	
	w/o CCl_4	w/CCl_4
None	0.59 ± 0.11	0.25 ± 0.10
Panaxydol	0.62 ± 0.11	0.31 ± 0.11
Panaxynol	0.61 ± 0.17	0.33 ± 0.10
Panaxytriol	0.59 ± 0.22	0.30 ± 0.11

Polyacetylenes were administered i.p. to rat at a dose of 2 mg/100g BW with or without administration of CCl_4(35 μl/100g BW). Rats were killed 20 hr after treatment of CCl_4 or polyacetylenes only. Values represent means ± SD for 6 animals. Cytochrome P-450 content is expressed as nmole/mg protein.

and 5. carbon tetrachloride caused marked loss in cytochrome P-450 and activities of aniline hydroxylase and aminopyrine demethylase. Polyacetylene pretreament had no effect in this case. When polyacetylenes were administered to rat without CCl_4 treatment. panaxydol and panaxynol induced aniline hydroxylase and all polyacetylenes induced aminopyrine demethylase. Cytochrome P-450 centents were not changed with 2 mg dose per 100 g body weight for 20 hr administration of polyacetylenes.

Inhibition of microsomal lipid peroxidation *in vitro* : Polyacetylene compounds, panaxydol, panaxynol and panaxytriol inhibited lipid peroxidation induced by CCl_4 not only *in vivo* but also *in vitro* microsomal lipid peroxidation induced by enzymatic (ADP-Fe^{+3}/NADPH) and nonenzymatic (Fe^{+2}/Ascorbate) systems. When microsomal suspension obtained from CCl_4-treated mouse was incubated with various concentrations of polyacetylenes or DL-α-tocopherol to reaction mixtrue *in vitro*. lipid peroxide formed during the incubation period was decreased in a concentration-dependent manner. In enzymatic lipid peroxidation system. there seems no differences in inhibition rate among panaxydol. panaxynol. panaxytriol and DL-α-tocopherol up to 50 μM concentration (Fig. 3). However. panaxynol showed the lowest rate of lipid peroxide formation among others at 100μM ; the inhibition rates of lipid peroxidation with treatment of panaxydol. panaxynol. panaxytriol and DL-α-tocopherol were 65%. 50%. 68% and 70%. respectively. In nonenzymatic system. panaxydol and panaxynol were more effective than panaxytriol and DL-α-tocopherol in inhibition of lipid peroxidation from 50 μM concentration (Fig. 4).

Discussion

Polyacetylene compound from ginseng root was first isolated by Takahashi et al.[33] as panaxynol. Recent investigators identified other C_{17} polyacetylenes from ginseng[13-18]. Even though some polyacetylenes such as panaxydol, panaxynol and panaxytriol showed cytotoxicities against L1210 leukematic lymphocyte. Sarcoma 180. HRT-18 and HT-29 cells *in vitro*[16-19] and crude polyacetylenes named as panaxyne had antioxidant activity[12], the biological significance of polyacetylene compounds from ginseng has been rarely known.

Liver injury caused by carbon tetrachloride is generally accepted model for the study of lipid peroxidation. The

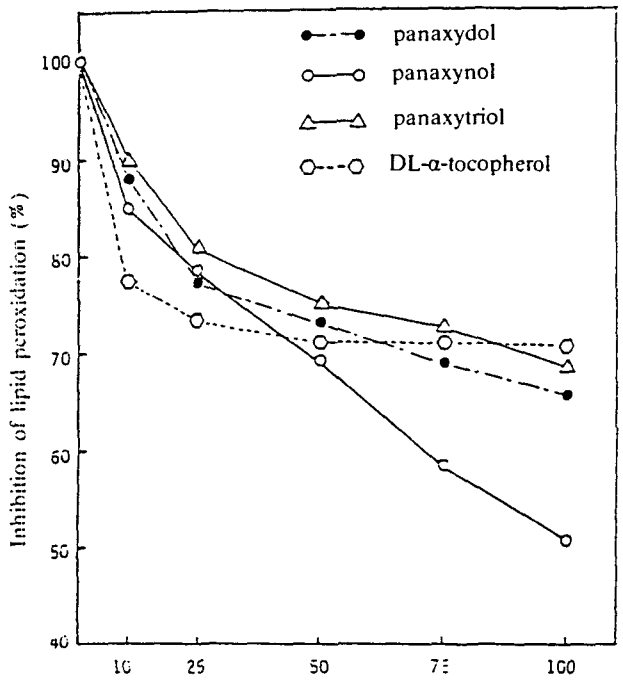

Fig. 3. Inhibition of various concentrations of polyacetylene compounds against microsomal lipid peroxidation *in vivo*. The microsomes obtained from CCl_4-treated mice were incubated with various concentrations of polyacetylenes or DL-α-tocopherol and incubation mixtures were subjected to TBA reaction for measuring lipid peroxide contents. Lipid peroxidation was induced by NADPH/Fe^{+3} -ADP and NADPH-dependent cytochrome P-450 reductase.

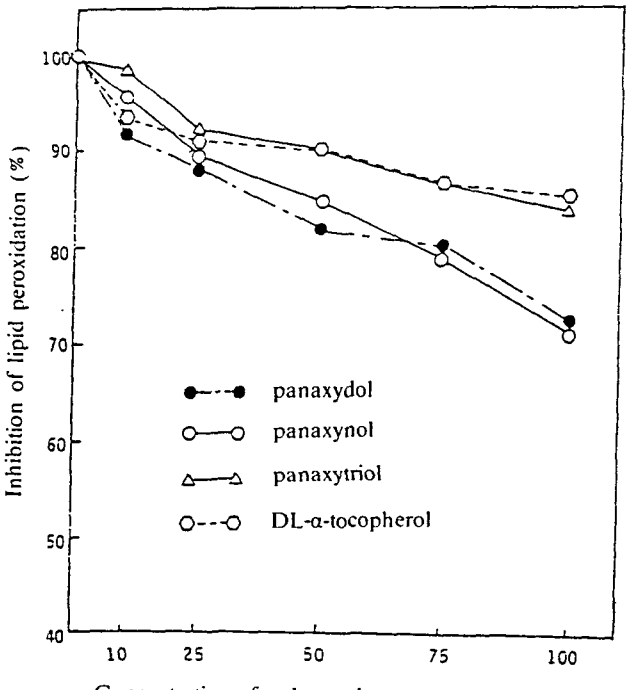

Fig. 4. Inhibition of various concentrations of polyacetylene compounds against microsomal lipid peroxidation *in vitro*. The microsomes obtained from CCl_4-treated mice were incubated with various concentrations of polyacetylenes or DL-α-tocopherol. Lipid peroxidation was induced by Ascorbate/Fe^{+2}.

initial events are accompanied by covalent binding of trichloromethyl radical largely to macromolecules (lipid and protein) of cell membrane after conversion of CCl_4 to CCl_3 radical and the destructive lipid peroxidation causes many pathologic events[20-22].

With this point of view, the effects of three polyacetylene compounds from *Panax ginseng* on lipid peroxidation were investigated and CCl_4 treatment was chosen as an animal model system of hepatic injury, primarily caused by lipid peroxidation. Polyacetylenes exerted protective effects upon hepatic lipid peroxidation and lowered serum lipid peroxide levels induced by CCl_4 in animals. Polyacetylene prevented leakage of lactic dehydrogenase to serum which was induced by CCl_4. They also inhibited *in vitro* hepatic microsomal lipid peroxidation in a dose-dependent manner. Even though pretreatment of polyacetylene compounds had no effect on loss of cytochrome P-450 and decrease in the activities of aniline hydroxylase and aminopyrine demethylase which were caused by CCl_4, they itself induced aniline hydroxylase and aminopyrine demethylase. At this point, we cannot relate the inhibition of lipid peroxidation and inhibition of cytochrome P-450-dependent mixed function oxidase system to elucidate the mechanism of action of polyacetylenes. However, our *in vivo* results together with results *in vitro* suggested that each polyacetylene itself or possibly its metabolites seem to act on liver microsome to inhibit lipid peroxidation whether it directly scavenge the radical or suppress the enzymes which are involved in prostaglandin metabolism like vitamin E[34]. The overall signficance of the study is that panaxydol, panaxynol and panaxytriol seem to be antioxidant components which contribute the antiaging activities of Korean ginseng.

References

1. Kim, N. D., Hahn, B. Y., Lee, E. B., Hong J. Y., Kim, M. H. and Jin, C. B. (1979): Studies of ginseng on the antistress effect. Korean J. Pharmacol. 10:61-66.
2. Otsuka, H., Komiya, H., Fujioka, S., Goto, M., Hiramatsu, K. and Fujimura, H (1981): Studies on antiinflamatory agents. Yakugaku Zasshi 101: 1113-1117.
3. Kaneko, H., Nakanishi, K., Murakami, A. and Kuwashima, K. (1981): Studies on the clinical effect of Korean red ginseng powder (4). Linshio & Kenkyu, 58: 145-149.
4. Takagi, K., Saito, H. and Nabata, H. (1972): Pharmacological studies of *Panax ginseng* root. Japanese J. Pharmacol. 22: 245-249.
5. Brekhman, I. I and Dardymov, I. V. (1969): New substances of plant origin which increase nonspecific resistance. Ann. Rev. Pharmacol. 9: 419-430.
6. Park, C. W., Lim, J. K., Chung, M. H. and Chang, K. C. (1983): Effect of ginseng components on the action of oxygen radicals to the generation of skin collagen. Yongoik Bokoseo, Korea Ginseng & Tobacco Research Institute.
7. Paik, T. H., Hong J. T. and Hong S. U. (1982): Studies on the antioxygenic substances in *Panax ginseng* roots. 1. The antioxidative action of various solvent extracts of *Panax ginseng* roots. Korean J. of Food Sci. & Tech. 14(2): 130-135.
8. Kim, M. W., Choi, K. J., Cho, M. H. and Hong S. K. (1980): Study on the components of the antioxidant activity of *Panax ginseng*. J. Korean Agricultural Chem. Soc. 23(3): 173-177.
9. Lee, J. H., Woun, B. R. and Lee, C. S. (1978): Ultrastructural changes in the mouse liver cell treated with ginseng extract for damaged liver by carbon tetrachloride. Korean J. Vet. Res. 18(2): 87-95.
10. Lee, H. B. (1978): A study on the antioxidant activity of Korean ginseng. Chungbuk National University Nonmunjip (Natural Science) 17: 232-238.
11. Han, B. H., Park, M. H., Han, Y. N. and Shin, S. C. (1984):Studies on the antioxidant components of Korean ginseng. 4. Antifatigue active components. Ann. Rep. Nat. Prod. Res. Ins. 73-77.
12. Lee, T. Y.(1984): Biological activities of non-saponin fractions occurred in *Panax ginseng* and its relatives. Yongoik Bokoseo, Korean Ginseng and Tobacco Research Institute.
13. Poplawski, J., Wrobel, J. T. and Glinka, T. (1980): Panaxydol, a new polyacetylenic epoxide from *Panax ginseng* root. Phytochem. 19: 1539-1541.
14. Dabrowski, Z., Wrobel, J. T. and Wojtasiewicz, K. (1980):Structure of an acetylenic compound from *Panax ginseng*. Phytochem. 19: 2464-2465.
15. Shim, S. C., Koh, H. Y. and Han, B. H (1983): Polyacetylenes from *Panax ginseng* roots. Bull. Korean Chem. Soc. 4: 183-188.
16. Shim, S.C. and Chang, S.K. (1987): New polyacetylene compounds from *Panax ginseng* C.A. Meyer. Bull. Korean Chem. Soc. 8: 272-275.
17. Ahn, B. Z. and Kim, S. I. (1985): Antineoplastic natural products and the analogue VI. Arch. Pharm. Res. 8: 283-284.
18. Kim, S. I. (1988) Studies on the cytotoxic components of the Korean ginseng roots. Ph. D. dissertation, College of Pharmacy, Chungnam National University.
19. Hwang, W. I. and Oh, S. K. (1986): Effects of petroleum ether extract of ginesng root on some enzyme activity in human colon cancer cells. Korean J. Ginseng Sci. 10: 27-35.
20. Recknagel, R. O. and Lombardi, B. (1961): Studies of biochemical changes in subcellular particles of rat liver and their relationship to a new hypothesis regarding the pathogenesis of carbon tetrachloride fat accumulation. J. Biol. Chem. 236: 564-569.
21. Alpers, D. H., Solin, M. and Isselbacher, K. J. (1968): The role of lipid peroxidation in the pathogenesis of carbon tetrachloride-induced liver injury. Mol. Pharmacol. 4: 566-573.
22. Noguchi, T., Fong, K. L., Lai, E. K., Olson, L. and McCay, P. B (1982): Selective early loss of peptides in liver microsomes of CCl_4-treated rat. Biochem. Pharm. 31: 609-614.
23. Noguchi, T., Fong, K. L., Lai, E. K., Alexander, S. S., King, M. W., Olson, L., Poyer, J. L.and McCay, P. B. (1982): Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. Biochem. Pharm. 31: 615-624.
24. Cosgrove, J. P., Church, D. F. and Pryor, W. A. (1987): The kinetics of the autoxidaion of polyunsaturated fatty acids. Lipids 22: 299-304.
25. Ohkawa, H., Ohishi, N. and Yagi, K. (1979): Assay

for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 : 351-358.
26. Lee, J. Mo, S. and Lee, T. Y. (1983) : Effect of ferricion on the enhancement of the reaction between 2-thiobarbituric acid and serum under anaerobic atmosphere. Korean Biochem. J. 16 : 348-359.
27. Omura, T. and Sato, R. (1964) : The carbon monoxide-binding pigment of liver microsome. J. Biol. Chem. 239 : 2370-2385.
28. Kiso, Y., Tohkin, M., Jikino, H., Hattori, M., Sakamoto, T. and Namba, T. (1984) : Mechanism of antihepatotoxic activity of glycyrrhizin. I : Effect on free radical generation and lipid peroxidation. Planta Medica 50 : 298-302.
29. Wills, E. D. (1969) : Lipid peroxide formation in microsomes : Relationship of hydroxylation to lipid peroxide formation. Biochem. J. 113 : 333-341.
30. Lowry, O. H., Roserbrough, N. J., Farr, A. L. and Randall, R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 : 265-275.
31. Fairhurst, S., Barbr, D. J., Clark, B. and Horton, A. A. (1982) : Studies on paracetamol-induced lipid peroxidation. Toxicol. 23 : 249-259.
32. Wong, S. F., Halliwell, B., Richmond, R. and Skowroneck, W. R. (1981) : The role of superoxide and hydroxyl radicals in the degradation of hyaluronic acid induced by metal ions and by ascorbic acid. J. Inorganic Biochem. 14 : 127-134.
33. Takahai, M. and Yoshikura, M. (1964) : Studies on the components of *Panax ginseng* C. A. Meyer. III. On the etheral extract of ginseng radix alba (3). J. Pharmac. Soc. Japan 84 : 757-759.
34. Gwebu, E. T., Trewyne, R. W., Cornwell, D. G. and Panganamala, R. V. (1980) : Vitamin E and inhibition of platelet lipoxygenase. Res. Commun. Chem. Pathol. Pharmacol. 28 : 361-376.

F. Sandberg : For Europeans, it is important whether polyacetylenes really are extracted in ethanol which is the most widely used solvent for ginseng extract in European market. And have you tried polyacetylenes for clinical use?

H. Kim (Jun) : At this time, we can not use polyacetylenes as a single medicine for clinical study. The significance of this study is the antioxidant action of polyacetylenes which may contribute the antiaging activity of Korean ginseng. I think polyacetylenes are included in Korean ginseng products which are also available in European market.

S. C. Shim : I would like to comment on Professor Sandberg's question. Most polyacetylenes are lipid soluble but some are water-soluble. Therefore, polyacetylenes may exist in ethanol and water extract of ginseng.

고려인삼의 폴리아세틸렌 성분이 과산화 지질 형성에 미치는 영향

김혜영, 이유희, 김신일, 진승하

한국인삼연초연구소

고려인삼에서 분리한 폴리아세틸렌 성분인 파낙시돌, 파낙시놀과 파낙시트리올의 사염화탄소로 유도된 마우스와 흰쥐의 지질과산화와 효소적 또는 비효소적으로 유도된 시험관내 과산화 지질 형성에 미치는 영향을 관찰하였다. 정상 또는 사염화탄소 처리된 마우스와 흰쥐에 대한 폴리아세틸렌의 효과를 혈청과 간 과산화 지질수준과 혈청효소 (GOT, GPT, LDH) 활성을 측정함으로써 관찰하였다. 간 마이크로좀 내 cytochrome P-450 함량과 aniline hydroxylase와 aminopyrine demethylase 활성도 측정되었다. 정상 마우스에 폴리아세틸렌을 처리한 경우 파낙시놀의 경우를 제외하곤 간과 혈청의 과산화 지질 형성과 혈청효소들의 활성에 변화가 없었으며, 파낙시놀은 간의 지질 과산화를 억제하였다. 폴리아세틸렌 성분들은 사염화탄소로 유도된 간의 과산화지질형성에 대한 보호작용을 나타내었고, 혈청지질과산화 수준을 낮추었다. 또한 사염화탄소로 유도된 LDH의 혈액내 유출에 대한 보호작용이 있으나, 혈청 GOT와 GPT 수준엔 영향을 주지 않았다. 사염화탄소는 cytochrome P-450 함량과 aniline hydroxylase, aminopyrine demethylase 활성을 낮추었으며, 이 경우 폴리아세틸렌은 효과를 나타내지 못하였다.반면, 사염화탄소 없이 폴리아세틸렌만 처리한경우, 파낙시돌과 파낙시놀은 aniline hydroxylase를, 세폴리아세틸렌 성분은 aminopyrine demethylase를 유도하였으며, cytochrome P-450엔 영향을 주지 않았다. 시험관내 간 마이크로좀의 지질 과산화는 폴리아세틸렌 첨가시 농도에 비례하여 억제되었다.