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EFFECTS OF POLYACETYLENE COMPOUNDS FROM PANAX GINSENG C.A. MEYER ON CCl_4-INDUCED LIPID PEROXIDATION IN MOUSE LIVER

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ABSTRACT: *The inhibitory effect of three polyacetylene compounds, panaxydol, panaxynol and panaxytriol isolated from Panax ginseng C.A. Meyer on CCl_4-induced lipid peroxidation in vivo and in vitro hepatic microsomal lipid peroxidation induced by ADP-Fe^{3+}, NADPH and NADPH-cytochrome* ***P-450*** *reductase were investigated. Their effects on lowering the lipid peroxide levels both in serum and liver and lowering the serum enzyme (GOT, GPT, LDH) activities without the CCl_4-induction were also determined. Male ICR mice were pretreated i.p. with polyacetylene compounds or DL-α-tocopherol before administration of CCl_4 i.p. and 20 hr after the administration of CCl_4, serum and liver were analyzed. Hepatic microsome was isolated and used for the in vitro NADPH-dependent lipid peroxidation system. Except for panaxynol, treatment with polyacetylenes to control mice did not reduce the levels of lipid peroxides and serum enzyme activities. Panaxynol itself inhibited lipid peroxidation in the liver of normal mice. Polyacetylene compounds protected from the CCl_4-induced hepatic lipid peroxidation and lowered serum lipid peroxide levels. Polyacetylenes also inhibited the in vitro hepatic microsomal lipid peroxidation in a dose-dependent manner. The results suggest that panaxydol, panaxynol and panaxytriol seem to be the antioxidant components which contribute the anti-aging activities of Panax ginseng C.A. Meyer.*

Keywords: *Polyacetylene, Panax ginseng C.A. Meyer, carbon tetrachloride, microsomal lipid peroxidation, malondialdehyde.*

INTRODUCTION

For many years Panax ginseng C.A. Meyer has been considered as one of the most valuable medicines having mysterious effects for prevention of aging, tiredness, illness in biological systems such as cardiovascular system, central nervous system and liver (1-5). The study for ginseng has been limited to its fraction, extract levels or gin-

senoside levels. Recent investigations have identified some phenolic acids, maltol, vanillic and salicylic acid as the antioxidant components of Korean ginseng(6). Furthermore, some polyacetylene compounds were shown to have cytotoxic effects against some cancer cell lines(7-15).

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 oxidase (P-450) system of enzymes involved in the metabolism of lipid-soluble drugs and other compounds(16-19). The produced free radicals 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)(20).

NADPH-dependent lipid peroxidation is proposed to be initiated in the presence of ADP-perferryl ion by abstracting methylene hydrogen from the polyunsaturated fatty acids. The ADP-perferryl ion was proposed to be formed by the direct reduction of ADP-Fe^{+3}, catalyzed by NADPH-cytochrome P-450 reductase, and the subsequent reduction of ADP-Fe^{+2} with molecular oxygen(21).

In the present study, the inhibitory effects of three polyacetylene compounds, panaxydol, panaxynol and panaxytriol isolated from Panax ginseng C.A. Meyer on CCl_4-induced lipid peroxidation in mice were investigated and the correlations of the results with the inhibition of *in vitro* NADPH-dependent hepatic microsomal lipid peroxidation was determined. In addition, lowering of serum transaminases(GOT, GPT) and LDH activities and lipid peroxide levels both in serum and liver were determined after treatment of control mice with polyacetylene compounds.

MATERIALS AND METHODS

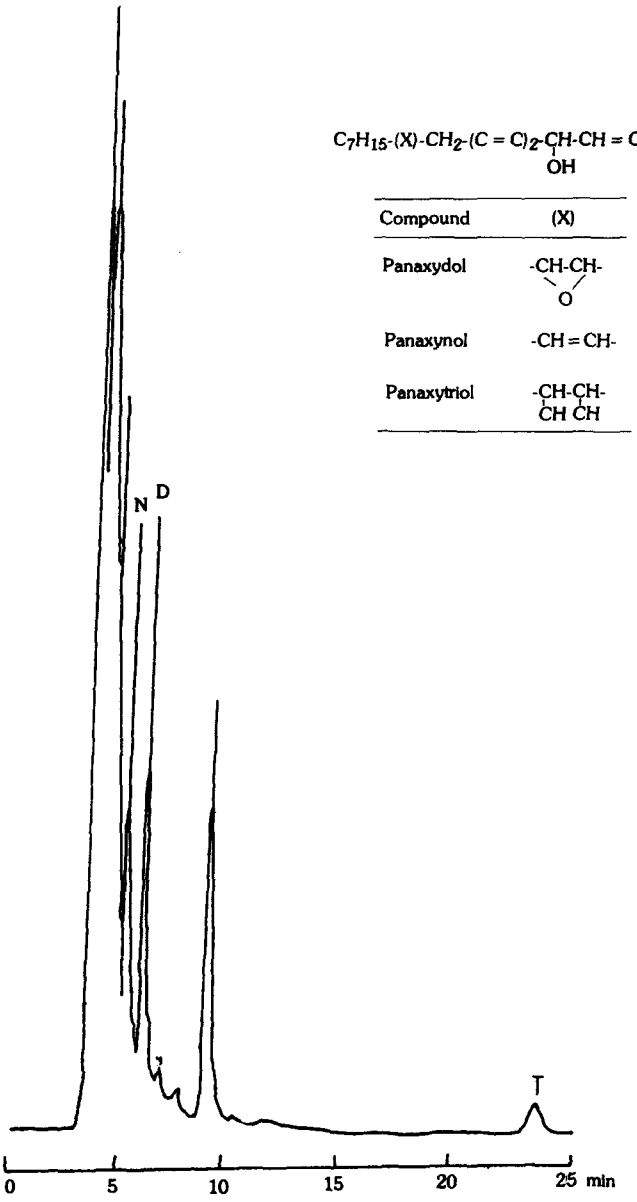
Materials

The chemicals used in the present study were obtained from the following sources: Nicotinamide adenine dinucleotide phosphate(NADPH), adenosine 5'-diphosphate (ADP) and bovine serum albumin(BSA) fromSigma chemical Co.; DL-α-tocopherol from Kanto chemical Co.; 2-thiobarbituric acid, 1,1,3,1-tetra methoxy propane and ferric chloride from Fluka chemical Co.; carbon tetrachloride and solvents for HPLC from J.T. Baker Chemical Co.; Silica gel 60 (70-230 mesh, ASTM) and silica gel 60F 254 plate (0.2 mm) from Merck Chemical Co. All other reagents used were of guaranteed reagent grade commercially available.

Isolation of polyacetylene compounds from Panax ginseng C.A. Meyer

Polyacetylene compounds from ginseng roots were isolated by the method of Ahn & Kim(13-14). Briefly, dried and pulverized red ginseng roots were extracted with distilled ethyl ether and were concentrated under nitrogen gas. The residue was repeatedly chromatographed over a silica gel column and eluted with petroleum/ethyl ether as the gradient solvent system. Panaxydol, panaxynol and panaxytriol were isolated by preparative HPLC under the following conditions;

column ; Allteck-NH_2 (250 × 10 mm)



C_7H_{15}-(X)-CH_2-(C = C)$_2$-CH-CH = CH_2
OH

Compound	(X)
Panaxydol	-CH-CH- (O)
Panaxynol	-CH = CH-
Panaxytriol	-CH-CH- (CH CH)

Fig. 1. HPLC chromatogram of petroleum ether extract of red ginseng. N,D,T denote panaxynol, panaxydol and panaxytriol. Chromatographic condition; column, Allteck-NH_2; eluent, n-hexane/iso-propanol (2:1); flow rate, 3ml/min; detection, UV 254nm.

eluent ; n-hexane/isopropanol (2:1)
flow rate ; 3.0 ml/min
detection ; UV 254 nm
system ; Waters Associates Model 244 equipped with Model 6000A solvent delivery system

HPLC chromatogram and the structure of polyacetylenes used in the study are shown in Fig. 1.

Animal treatment

Male ICR mice (26-28g) were obtained from the animal breeding room of the Korea Ginseng & Tobacco Research Institute (KGTRI) and were allowed free acess to food and water throughout the experiments. Mice were pretreated i.p. with polyacetylene compounds or DL-α-tocopherol (400ug/100g B.W.) 1 hr before the administration of carbon tetrachloride (12.5 ul/100g B.W.) i.p. dissolved in liquid paraffin. Control animals received equivalent volumes of the liquid paraffin carrier. After 20 hr administration of CCl_4, all animals were sacrificed and whole bloods were obtained by cardiac puncture to determine the serum enzyme levels and livers were removed for analysis of lipid peroxides. To investigate the antioxidant effects of polyacetylene compounds, polyacetylenes (400ug/100g B.W.) were administered to normal mice and liver and serum were analyzed 20 hr after the treatment.

Chemical and enzymatic determinations

Lipid peroxidation of liver tissue and levels of serum lipid peroxide were determined by measuring the formation of malondialdehyde with thiobarbituric acid according to the method of Ohkawa *et al*(22) and Lee *et al*(23). Serum transaminase (GOT, GPT) activities were measured by the method of Reitman and Frankel(24) using a commercial kit. Activity of serum lactate dehydrogenase (LDH) was determined by a commercial kit which modified the method of Babson and Phillips(25).

Microsomal lipid peroxidation *in vitro*

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

NADPH-dependent microsomal lipid peroxidation reaction mixture contained 0.5mg of microsomal protein/ml, 1.7mM ADP, 0.1mM $FeCl_3$, 0.1mM NADPH and various concentrations of polyacetylene compounds or DL-α-tocopherol in 0.15M Tris-HCl buffer (pH 7.4). Incubations were carried out at 25°C for 30 min under an air atmosphere in a water bath. Lipid peroxidation was measured by the formation of the TBA-reactive material, malondialdohyde(MDA) using a method of Fairhurst *et al.*(27).

RESULTS

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

Treatment of polyacetylenes at 400ug per 100g B.W. i.p. did not influence lipid peroxide levels in serum when compared with those of liquid paraffin treated control. While panaxynol significantly inhibited the formation of lipid peroxide in the liver, panaxydol, panaxytriol and DL-α-tocopherol had no effect on the de novo lipid perx-

Table 1. 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 400ug / 100g. Values represent mass ± SD of 6 animals. An asterisk indicates value significantly 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 umoles / min / dl for LDH.

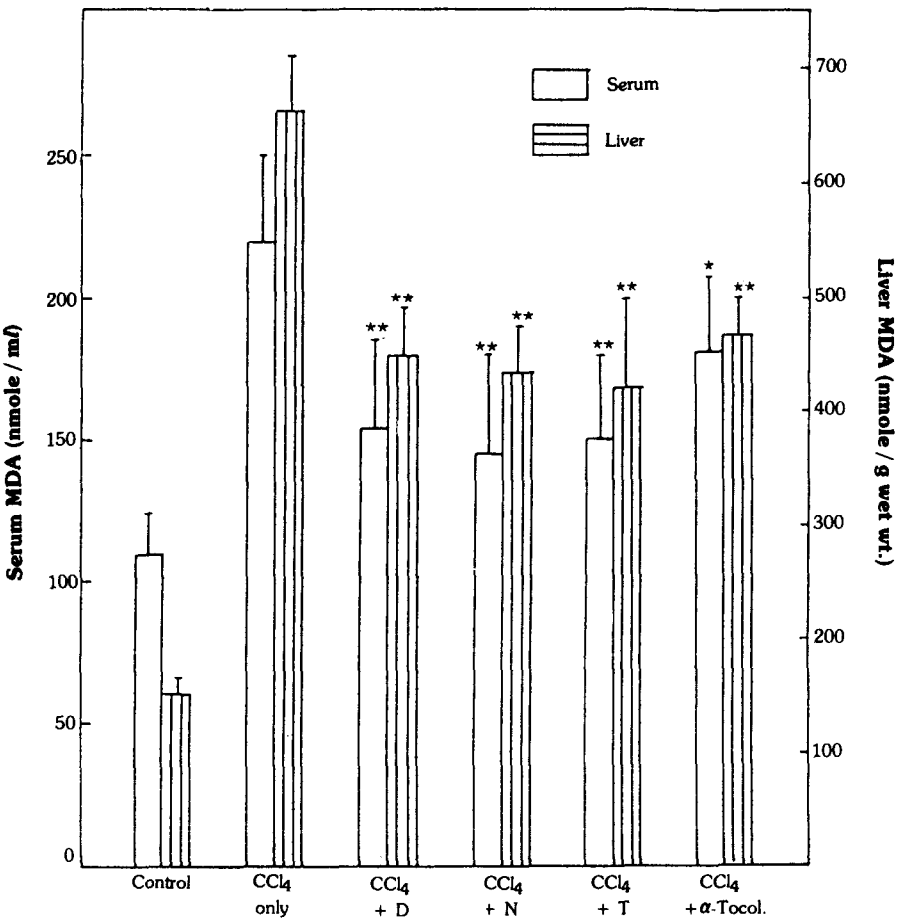


Fig. 2. Effect of polyacetylene compounds on lipid peroxide levels in CCl_4-treated mice. Mice received polyacetylenes (400ug / 100g BW. i.p.) 1 hr before administration of CCl_4. Values are means ± SD of 10 animals. D; panaxydol, N; panaxynol, T; panaxytriol, α-tocol; DL-α-tocopherol. An asterist indicates values significantly different from CCl_4-treated an animals.
*: $P<0.05$, **: $P<0.01$

Table 2. Effect of polyacetylene compounds on lipid peroxidation in mice liver microsomes.

Treatment	Rate of lipid peroxidation (MDA nmole / mg protein / min)	% control
None	0.72 ± 0.059	100
100uM panaxydol	0.47 ± 0.058	65
100uM panaxynol	0.36 ± 0.083	50
100uM panaxytriol	0.49 ± 0.094	68
100uM α-tochpherol	0.50 ± 0.053	70

Liver microsomes were obtained from mice treated with CCl_4 (12.5ul/100g B.W., i.p.) and incubated with 100uM polyacetylenes or DL-α-tocopherol. The incubation mixtures were subjected to TBA reaction for measuring lipid peroxide contents. Values represent the means ± SD of triplicate determinations.
MDA formed was determined using a MDA standard produced by the acid hydrolysis of 1,1,3,3-tetramethoxy propane.

idation in normal mice (Table 1). Blood samples for the determination of serum transaminase and LDH activities were collected 20 hr after the treatment. The result showed that treatment of polyacetylene compounds by itself had no effect on serum GOT, GPT and LDH levels.

Effect of polyacetylene compounds on lipid peroxide levels in CCl_4-treated mice.

Lipid peroxide contents of serum and liver homogenate from CCl_4-treated mice were increased to 2 times and 4.5 times that of the normal group, respectively. Pretreatment of panaxydol, panaxynol and panaxytriol prevented the increase of lipid peroxidation induced by CCl_4. As shown in Fig. 2, 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-tocotherol, polyacetylenes showed similar inhibitory effect against liver lipid peroxidation.

Inhibition of microsomal lipid peroxidation *in vitro*

Polyacetylene compounds, panaxydol, panaxynol and panaxytriol inhibited the CCl_4-inducible lipid peroxidation not only *in vivo* but also *in vitro* microsomal lipid peroxidation induced by ADP-Fe^{+3}, NADPH, and NADPH-cyt. P-450 reductase. When microsomal protein obtained from CCl_4-treated mouse was incubated with various concentrations of polyacetylenes or DL-α-tocopherol to reaction mixture *in vitro*, lipid peroxide formed during the incubation period was decreased in a concentration-dependent manner. There seems no differences in the inhibition rate among panaxydol, panaxynol, panaxytriol and DL-α-tocopherol up to 50uM concentration (Fig. 3). However, panaxynol showed the lowest rate of lipid peroxide formation among others at 100uM; the inhibition rates of lipid peroxidation with treatment of panaxydol, panaxynol, panaxytriol and DL-α-tocopherol were 65%, 50%, 68%, respectively.

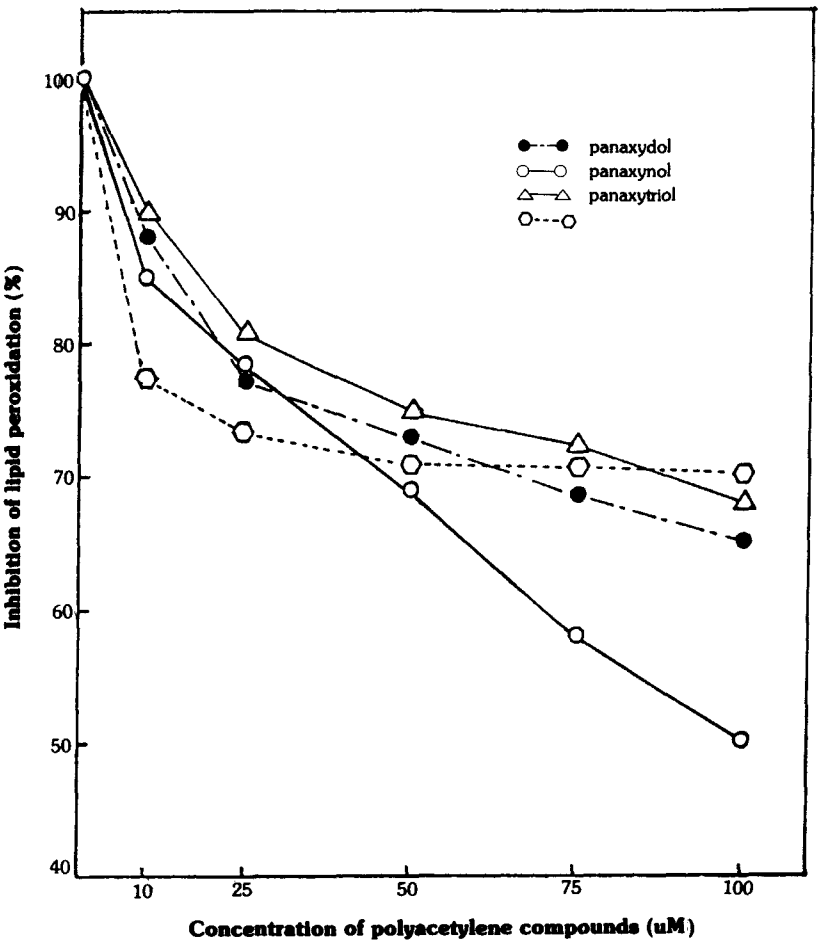


Fig. 3. Inhibition of various concentrations of polyacetylene compounds against microsomal lipid peroxidation *in vitro*. The microsomes obtained from CCl_4-treated mice were incubated with various concentrations of polyacetylenes or DL-α-tocopherol and incubation mixtures were subjected to TBA reaction for measuring lipid peroxide contents.

DISCUSSION

Panaxynol was the first polyacetylene compound isolated from ginseng root by Takahash *et al*(7,8), other C_{17} polyacetylene compounds from ginseng were isolated by Poplawsk *et al*(9), Dabrowski *et al*(10), Shim *et al*(11,12) and Ahn and Kim(13,14). 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*(12-15) and crude polyacetylenes named as panaxyne had antioxidant activity(28), the biological significance of polyacetylene compounds from ginseng has been rarely known.

Liver injury caused by carbon tetrachloride is the generally accepted model for the study of lipid peroxidation. The initial events are accompanied by covalent binding of trichloromethyl radical largely to macromolecules (lipids and proteins) of cell membrane after conversion of CCl_4 to CCl_3 radical and the destructive lipid peroxidation causes many pathologic events(16-19).

With this point of view, the inhibitory effects of three polyacetylene compounds isolated from Panax ginseng on lipid peroxidation were investigated and CCl_4 treated mice were chosen as the animal model of hepatic injury, primarily caused by lipid peroxidation. All three polyacetylenes exerted protective effects upon CCl_4-induced hepatic lipid peroxidation and lowered serum lipid peroxide levels in mice. They also inhibited *in vitro* hepatic microsomal lipid peroxidation in a dose-dependent manner. Although the polyacetylenes by itself did not have the lowering influence on the levels of serum enzyme (GOT, GPT, LDH) and lipid peroxide both in serum and liver of normal mice, the panaxynol did.

It is thought that the metabolism of unsaturated fatty acids, such as arachidonic acid to prostaglandins may have a role in increasing lipoperoxide levels, Gwebu *et al*(29) reported that Vitamin E inhibited lipoperoxygenase an important enzyme involved in the metabolism from arachidonic acid to prostaglandins (leucotrienes). Even though the degree of inhibitory effect of polyacetylenes against liver lipid peroxidation were similar to that produced by DL-α-tocopherol at the same concentration *in vivo*, the mode of action is not clear yet. Our *in vivo* results together with the results *in vitro* have 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 inhibit the microsomal mixed function oxidases which convert CCl_4 to CCl_3 radical or supress the enzymes which are involved in prostaglandin metabolims is not known. The overall significance of this study is that panaxydol, panaxynol and panaxytriol seem to be the antioxidant components of Panax ginseng C.A. Meyer.

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