Korean J. Ginseng Sci.
Vol. 12, No. 2. 114–120 (1988)

Protective Effect of Ginseng on Bromobenzene-Induced Hepatotoxicity in Mice

Keun Huh, Byung-Su Jang and Jong-Min Park
Department of Pharmacology, College of Pharmacy, Yeungnam University Gyongsan 713-800, Korea
(Received December 7, 1988)

Abstract

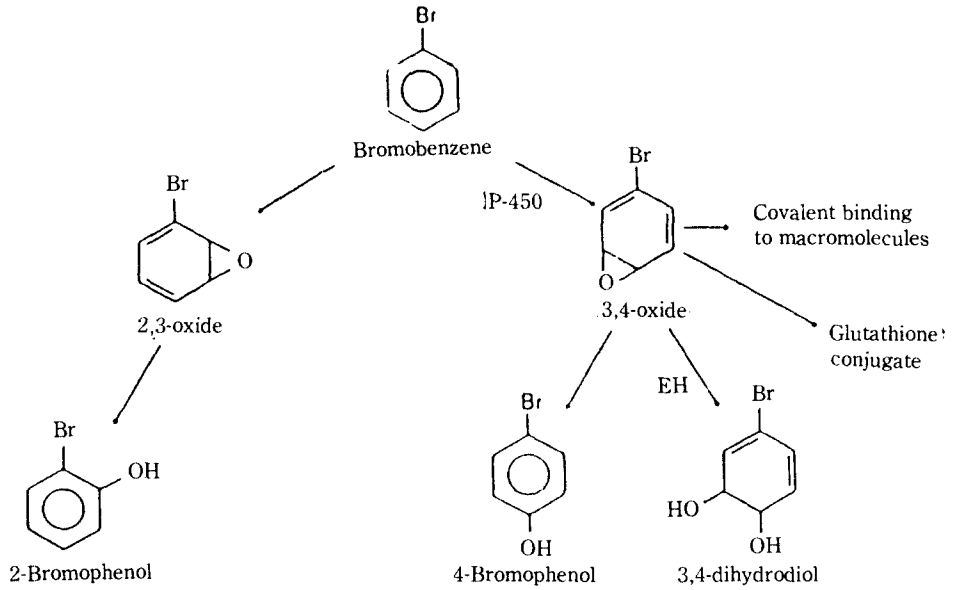
We have studied the mechanism by examining the effect of ginseng on the epoxide hydrolase which is catabolized the reactive intermetabolite of bromobenzene, and bromobenzene-induced hepatotoxicity. It was observed that ginseng saponin fraction protects against bromobenzene-induced hepatotoxicity in mice as evidenced 1. increased the epoxide hydrolase activity, 2. lower serum transaminase activity, 3. decreased the formation of lipid peroxide. These results suggested that the inducing effect of ginseng on the epoxide hydrolase is believed to be a possible detoxication mechanism for the bromobenzene toxicity in mice.

Introduction

Many researchs of *Panax ginseng* components on the defence mechanism in the body are being made intensively by many workers from various approaches.[1-4] Recently, a number of papers shown ginseng have some protective effect against various physico-chemical stress for xenobiotics involving mutagenic, carcinogenic and toxifying effect.[5-8]

A multitude of structurally different endogenous[9] and exogenous compounds containing an epoxide moiety have been isolated and characterized using sensitive and refined analytical methods.[10]

These epoxide can bind non-enzymatically and covalently to cellular macromolecules and, of course, also to endogenous compound of low molecular weight if a nucleophilic moiety such as an amino, a sulfhydryl or a hydroxy group is present.[11] The microsomal cytochrome P-450 system seems to be the major epoxide-producing progress in hepatocytes during metabolism of xenobiotics.[12] These toxic epoxides have been shown to be powerful active-site inhibitors for several enzymes, and to be mutagenic and carcinogenic in many studies.[13] The epoxide hydrolase is generally the most important enzyme involved in the metabolism of epoxide among the several pathways for epoxide degradation.[14] A number of different epoxide hydrolase catalyzes formation of a dihydrodiol and may be involved in cellular defense against oxidative stress during xenobiotic metabolism. This present study was undertaken to investigate the effect of ginseng on epoxide detoxication process and epoxide hydrolase activity using a model compound such as bromobenzene



Metabolism of bromobenzene. Oxidation may yield the nontoxic 2,3-oxide, which rapidly forms the phenol, or the toxic 3,4-oxide. As levels of glutathione (GSH) are depleted by the 3,4-oxide, covalent binding is increased. Other detoxication Pathways are formation of 4-bromophenol and hydration to 3,4-dihydrodiol.

Materials and Methods

Materials

Bovine serum albumin (BSA) and trans-stilbene oxide were purchased from Sigma Chemical Company, Na salt of thiobarbituric acid from Nakarai Chemical Co. and bromobenzene from Hayashi Chemical Co. All other reagents were of reagent grade commercially available.

Animals

Male ICR-mice weight of 20 to 25g were used for these experiments. The control group received saline intraperitoneally and the other group received ginseng saponin fraction daily for 5 days. Bromobenzene was injected intraperitoneally to mice daily for 2 days. All the animals had free access to food and water but deprived of the 26 hr prior to sacrifice.

Enzymatic determinations

Hepatic cytosolic epoxide hydrolase activity was measured by the method of Hammock *et al.*[15] with trans-stilbene oxide as substrate. In brief, its enzyme activity was determined by measuring the amounts of decreased trans-stilbene oxide. Under the assay conditions used, the initial rates of trans-stilbene oxide disappearance demonstrated linear function with time and protein concentration. Serum aminotransferase (ALT,AST) activities were measured by the method of Reitman and Frankel[16] using a commercial kit.

Chemical determinations

Lipid peroxidation of liver tissue was followed by measuring the formation of malondialdehyde with thiobarbituric acid according to the method of Ohkawa *et al.*[17] Protein was determined by the method of Lowry *et al.*[18] using bovine serum albumin as the standard.

Student's t-test was used to estabilished significant different of mean values between the control and treated group.

Results and Discussion

Hepatic cytosolic epoxide hydrolase activities with ginseng saponin treatment are shown in Table I. The enzyme activity of control mice was 8.24 n moles/mg protein/min. In the hepatic cytosolic fraction of ginseng saponin-treated mice, the enzyme activities were 8.86, 9.43 and 11.39 n moles/mg protein/min with an increment of dose, respectively. The enzyme activity was significantly elavated as compared to control group when ginseng saponin (10 mg/kg) was injected to mice.

To study the effect of ginseng saponin on the kinetic parameters, initial rates of cytosolic epoxide hydrolase activity measured as a function of variable concentrations of trans-stilbene oxide (Fig. 1). As shown in Fig. 1, there was no change in the K_m value in the ginseng saponin-treated mice, compared to control mice. When plotted on double reciprocal form, the V_{max} value was increased about 1.3 fold by the treatment of ginseng saponin. Thus, the characteristics of the increase in the enzyme activity may result from a change in the quantity of enzyme proteins, rather than activation of enzyme activities due to other factors.

In order to obtain further information concerning to the effect of ginseng saponin on the cytosolic epoxide hydrolase activity, after bromobenzene treatment as model for hepatic injuries, we demonstrated the influence of each fraction on the cytosolic epoxide hydrolase activity. The cytosolic epoxide hydrolase activity was increased by saponin fraction-treated group as compared to control group. Moreover, the increment of its enzyme activity was more significant in triol saponin-treated group. However, when bromobenzene was injected, significant decrease of its enzyme activity was observed. In addition, pretreatment of ginseng saponin fraction before the bromobenzene maintained the enzyme activity at control levels. (Fig. 2).

Table 1. Dose response of ginseng saponin fraction on the hepatic cytosolic epoxide hydrolase activity in mice.

Treatment (mg/kg)	Epoxide hydrolase activity (n moles/mg protein/min)
0	8.24 ± 0.64
1	8.86 ± 0.71
5	9.43 ± 0.84
10	11.39 ± 0.87*
20	11.52 ± 0.93*

Ginseng saponin fraction (1,5,10 and 20 mg/kg) was injected to mice intraperitoneally daily for 5 days. Mice were decapitated 24 hr after the last administration. Values are mean ± S.E. of 5 animals in each group. *; p 0.05.

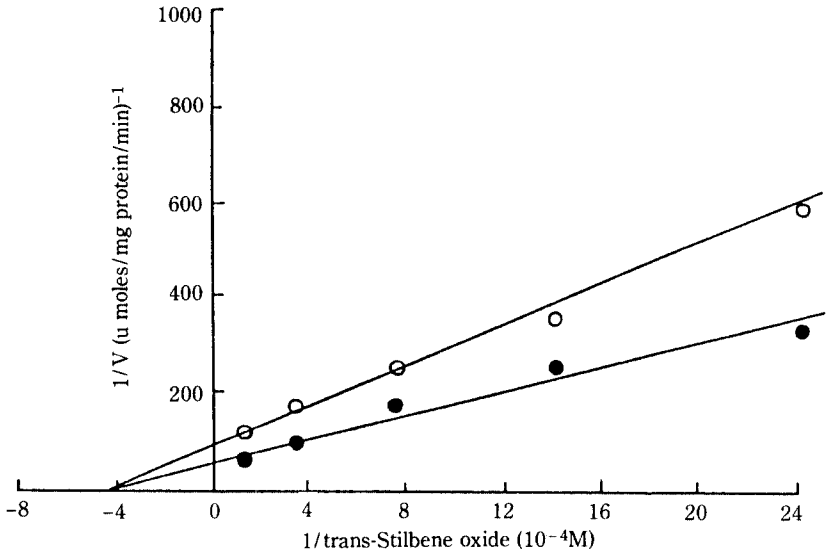


Fig. 1. Double reciprocal plots of the hepatic cytosolic epoxide hydrolase activity in mice for trans-sitilbene oxide.

Mice received ginseng saponin fraction (10 mg/kg) i.p. daily for 5 days. Bromobenzene (310 mg/kg) was injected i.p. to mice for 2 days. Data points represent the mean 3 experiments. Control, ○ ; ginseng saponin fraction, ●

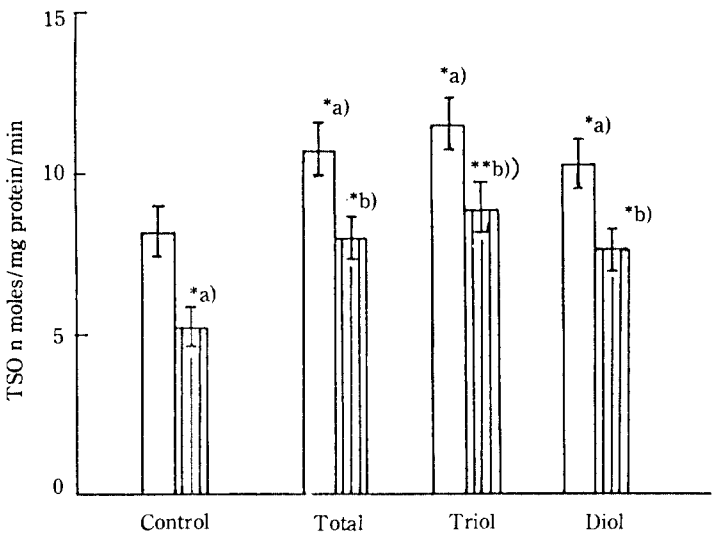


Fig. 2. Effect of total, triol and diol saponin fraction on the hepatic cytosolic epoxide hydrolase activity in bromobenzene-treated mice.

Mice received each fraction (10 mg/kg) i.p. daily for 5 days. Mice were decapitated 24 hr after the last dose of bromobenzene (310 mg/kg). Values are means ± S.E. of 5 animals. Saponin fraction, □ ; bromobenzene, ▨ . a) Significantly different from control group, b) Significantly different from bromobenzene-treated group. *$p<0.05$, **$p<0.01$.

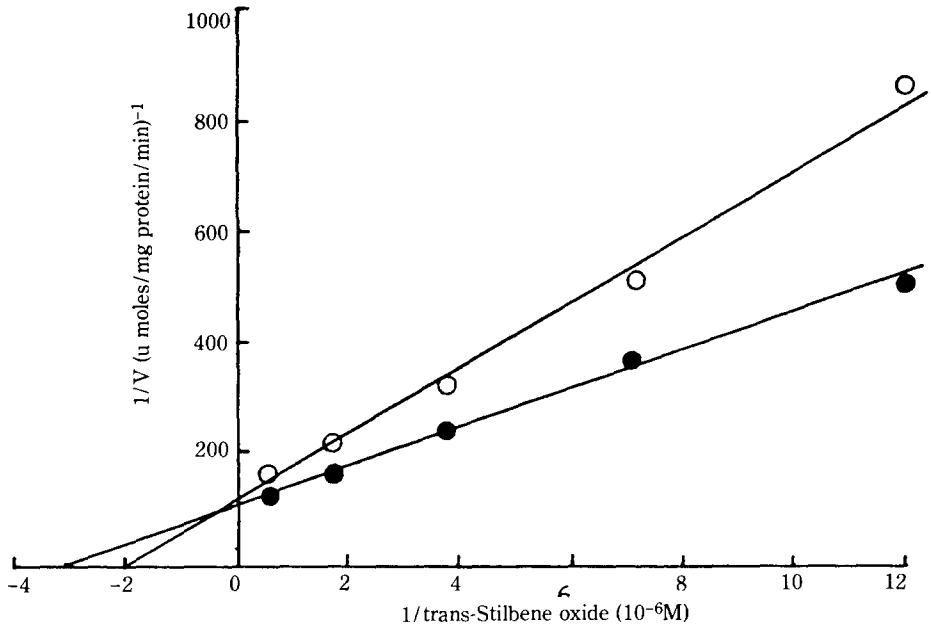


Fig. 3. Double reciprocal plots of the hepatic cytosolic epoxide hydrolase activity for trans-stilbene oxide. The reaction mixture contained 0.1M phosphate buffer (pH 7.0), various concentration of trans-stilbene oxide, enzyme solution and with or without ginseng total saponin fraction. Data points represent the mean of 3 experiments. Control, ○ ; total saponin fraction, ●

Data was not shown in this paper, epoxide hydrolase activity was increased at low dose level. On the other hand, epoxide hydrolase activity was decreased at high dose, by the addition of ginseng saponon fraction *in vitro*. Thus, we studied the effect of ginseng saponin on the kinetic parameter *in vitro*. (Fig. 3). In the presence of total saponin fraction (5×10^{-5} g/m*l*), the K_m value of cytosolic epoxide hydrolase for trans-stilbene oxide was decreased. On the other hand, V_{max} value was not changed. This result suggested that ginseng saponin fraction may increase substrate affinity.

The average increase of serum ALT activity after 24 hr was 5.5 fold, and the increase of serum AST activity was 4.0 fold after bromobenzene administration compared to control. This increment of enzyme activity indicated that bromobenzene treatment induced the hepatic lesion. On the other hand, each ginseng saponin fraction pretreatment reduced the serum ALT and AST activity by bromobenzene. This results indicate that ginseng saponin fraction may improve the bromobenzene-induced hepatic disfunction (Table 2).

Each ginseng saponin treatment had not changed the lipid peroxide content. However, the lipid peroxide level in the bromobenzene-treated group was increase to about 2.5 fold of control. But the increase in lipid peroxide level in the each ginseng saponin pretreated group was less than that of the group given bromobenzene alone (Table 3).

From the above results, it was observed that ginseng saponin fraction protects against bromobenzene hepatotoxicity in mice. These results demonstrated the most likely mechanism for protective effect of ginseng saponin is the induction of epoxide hydrolase activity. Further

Table 2. Effect of ginseng saponin on the serum alanine and aspartate amino-transferase (ALT, AST) activities in bromobenzene-treated mice.

Treatment	Aminotransferase activity(unit/ml of serum)	
	ALT	AST
Control	28.3 ± 5.6	42.1 ± 6.8
Total saponin	30.7 ± 6.4	45.5 ± 7.2
Triol saponin	29.8 ± 4.9	44.3 ± 8.1
Diol saponin	31.6 ± 7.2	47.2 ± 9.0
Bromobenzene	154.0 ± 24.0***(a)	172.4 ± 28.5**(a)
Total saponin + Bromobenzene	107.5 ± 21.3**(a)	120.7 ± 20.2**(a)
Triol saponin + Bromobenzene	57.0 ± 15.4**(b)	61.3 ± 16.2**(b)
Diol saponin + Bromobenzene	131.7 ± 21.3**(a)	149.6 ± 30.8**(a)

Mice received each fraction (10 mg/kg) i.p. daily for 5 days. Mice were decapitated 24 hr after the last dose of bromobenzene(310 mg/kg). Values are mean ± S.E. of 5 animals. (a): significantly different from control group(**, $p<0.01$, ***, $p<0.001$). (b): significantly different from bromobenzene-treated group(**, p 0.01).

Table 3. Effect of ginseng saponin fraction on the formation of lipid peroxide in bromobenzene-treated mouse liver.

Treatment	Malondialdehyde (n moles/g of tissue)
Control	15.91 ± 2.93
Total saponin	15.14 ± 2.62
Triol saponin	14.23 ± 3.65
Diol saponin	14.79 ± 3.54
Bromobenzene	38.65 ± 4.97**
Total saponin + Bromobenzene	31.35 ± 5.26*
Triol saponin + Bromobenzene	24.39 ± 5.71
Diol saponin + Bromobenzene	35.72 ± 6.42*

Mice received each fraction (10 mg/kg) i.p. daily for 5 days. Mice were decapitated 24 hr after the last dose of bromobenzene (310 mg/kg). Values are mean ± S.E. of 5 animals. *, $p<0.05$; **, $p<0.01$.

experiments to confirm this postulate are under development.

References

1. Abe, K. and I. Saitow, The active principle of Korean ginseng. *Japan Med. World*, **2**(6), 166-168 (1922).
2. Yamakami, I., The effect of sulfurous acid gas on the components and pharmacological action of ginseng. Chosen Yakugaka Zasshi, **15**(1), 254-268 (1935).
3. Kim, Y.S., Influence of *Panax ginseng* on the hypotherma in rats elicited by various drugs. *Korean J. Pharmacol.*, **2**(1), 87-97 (1966).
4. Koo, K.H. and Joo, C.N., Clinical study on the efficacy of *Panax ginseng* C.A. Meyer on acute viral(B)

hepatitis. *Korean J. Ginseng Sci.*, **2**(1), 115-132 (1983).

5. Abbott, B.J., Leiter, J., Hartwell, J.L., Perdue, Jr. R.E. and S.A. Schepartz, Screening data from the cancer chemotheraphy national service center screening laboratories. XXXII. Plant extracts. *Cancer Res.*, **26**, 391-508 (1963).
6. Lazarev, N.N., The non-specific resistance of the body and the neoplastic process. VII. Mezhdunan Protivorakovyi Kongress, (1962).
7. Lee, K.D. and Huemer, R.P., Antitumoral activity of *Panax ginseng* extracts. *Jap. J. Pharmacol.*, **21**, 299-301 (1971).
8. Chung, H.K., Cho, C.S. and Kim, C.H., Studies on the antimutagenicity of ginseng extracts. *Korean J. Ginseng Sci.*, **8**, 1-7 (1984).
9. Watabe, T., Ichihara, S. and Sawahata, T., Hepatic microsomal biotransformation of 1,3,5(10), 16-Estratetraen-3-ol to 16,17-Epiestriol and Estriol via 16,17 -and 16,17-Epoxy-1,3,5(10)-estratrien-3-ols. *J. Biol. Chem.*, **254**, 10720-10727 (1979).
10. Johan, M. and Joseph, W.D., Cytosolic epoxide hydrolase. *Chem. Biol. Interactions.* **64**, 207-249 (1988).
11. Milewski, C.H., Dzieduszycka, M. Smulkowski, M. and Borowski, E., Epoxypeptide : A novel group of metabolic inhibitors in prokaryotic and eukaryotic micro-organisms. *Drugs Exp. Clin. Res.*, VIII, 11-20 (1982).
12. Boyland, E. and Wolf, G., Metabolism of polycyclic compounds 6. Conversion of phenantherene into dihydroxydihydrophenanthrenes, *Biochem. J.*, **47**, 64-69 (1950).
13. Bolt, H.M., Laib, R.J., Filser, J.G., Reactive metabolites and carcinogenicity of halogenated ethylenes. *Biochem. Pharmacol.*, **31**, 1-4 (1982).
14. Wang, P., Meijer, J. and Guengerich, F.P., Purification of human liver cytosolic epoxide hydrolase and comparison to the microsomal enzyme. *Biochemistry*, **21**, 5769-5776 (1982).
15. Hammock, B.D., Hasegawa, L.S., Spectrophotometeric assay for mammalian cytosolic epoxide hydrolase using trans-stilbene oxide as substrate. *Biochem. Pharmacol.*, **31**(11), 1979-1984 (1982).
16. Reitman, S. and Frankel, S., A colorimetric method for the determination of serum glutamic oxaloacetic acid glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, **28**, 56 (1957).
17. Ohkawa, H., Ohishi, N. and Yaki, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351-358 (1979).
18. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951).