Korean J. of Pharmacology
Vol. 25, No. 1, pp.87-91, 1989

Effect of Panax Ginseng in Mouse Liver with Ethnol-Induced Monoamine Oxidase Activity

Keun Huh, Sang-Il Lee*, Min-Ik Song, Uk-Seob Shin and Jong-Min Park

Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 713-800
**Department of Food and Nutrition, Keimyung Junior College, Daegu 705-030, Korea*

ABSTRACT

Effect of ginseng butanol fractions on the hepatic mitochondrial monoamine oxidase activity with ethanol treatment was investigated in this experiment. Ethanol treatment, either acutely or chronically, increased the hepatic mitochondrial monoamine oxidase activity compared to control group. Whereas, treatment with ginseng butanol fractions lowered the ethaol-induced monoamine oxidase activity. Acetaldehyde, the major metabolite of ethanol, significantly increased the hepatic mitochondrial monoamine oxidase activity more than ethanol did. It was also observed that ginseng butanol fractions reduced the increase of the hepatic mitochondial monoamine oxidase activity by acetaldehyde. From these results, it is suggested that ginseng butanol fractions may be associated with the modulation of the hepatic mitochondrial monoamine oxidase activity in ethanol-treated animals.

Key Words: Monoamine Oxidase, MAO, Ethanol, Pyrazole, Acetadehyde, Tyramine

INTRODUCTION

Ginseng has been utilized as a restorative and tonic agent for thousand years. Recently, Ginseng has been reported to have many pharmacological actions: analgesic, anticonvulsant and central nervous system (CNS)-depressant actions (Takagi *et al.*, 1972; Saito *et al.*, 1973; Nabato *et al.*, 1973). The CNS is obviously affected by ethanol, and ethanol is a depressant of the CNS (Klemm, 1979). More recently, it is reported that ingestion of ethanol, either acutely or chronically, causes in the structure and function of hepatocellular organelles (Schilling and Reitz, 1980; Teschke *et al.*, 1981; Chin and Goldstein. 1981; Prasad *et al.*, 1985). Moreover, acetaldehyde, the product of ethanol oxidation, is considered to be toxic because of its reactivity (Sakurai *et al.*, 1980; Hagihara *et al.*, 1981). Acetaldehyde could exacerbate the neurologic, hepatic and cardiac complications of alcoholism (Cohen *et al.*, 1975; Pettersson *et al.*, 1977; Pikkarainen *et al.*, 1981). These studies provide evidence that ingestion of ethanol may alter the metabolism of biogenic amines. Monoamine oxidase (MAO, EC 1.4.3.4) is tightly bound to the outer membrane of mitochondria (Smith, 1980; Fowler and Tipton, 1981), and plays a very important role in the deamination of monoamine analogues (Murphy, 1978; Yu *et al.*, 1981; Benedette *et al.*, 1983). Although ginseng prevent the ethanol-induced biological dysfunction (Huh and Choi, 1979; Joo *et al.*, 1979), effect of ginseng on the ethanol-induced MAO activity has not been completely elucidated yet.

Therefore, in this experiment concerned with ethanol-monoamine interactions, it was undertaken to study the effect of ginseng butanol fraction on the hepatic mitochondrial MAO activity by treatment with ethanol and acetaldehyde in mice.

To whom all correspondences should be addressed; Keun Huh. Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 713-800,

MATERIALS AND METHODS

Chemicals

Pyrozole and bovine serum albumin were purchased from Sigma Chemical Co., ethanol from Fluka Chemical Co., acetaldehyde from Hayashi Pure Chemical Co., tyramine hydrochloride from

Aldrich Chemical Co.. All other chemicals were of analytical reagent grade if available.

Animals and treatment

Male mice (ICR, 20-25 g) were used for all studies, and were acclimatized in an 12hr light-12hr dark cycle animal room for at least 2 weeks prior to use. Animals were allowed free access to food and water but were deprived of food for the 16hr prior to sacrifice. In these acute studies, mice received ethanol (1 g/kg) i.p. 90 min before decapitation, and acetaldehyde (100 mg/kg) i.p. 30 min before decapitation. Pyrazole (200 mg/kg) was injected i.p. to mice 2hr before ethanol treatment. In the chronic studies, mice were fed 5% (v/v) ethanol or water for 2 months. Ginseng butanol fractions (4 mg/kg) was injected i.p. 3hr before decapitation.

Preparation of mitochondrial fraction

Mice were killed and liver was perfused in situ with cold 0.15M sodium chloride solution through the portal vein and quickly removed. After homogenizing, homogenate was centrifuged at 600 $\times$g for 10min and the resulting supernatant was centrifuged at 10,000$\times$g for 30 min. The mitochondrial pellets obtained were suspended in the 0.1 M phosphate buffer (pH 7.5) and centrifuged again for 30 min at 10,000$\times$g. The pellets were resuspended in the same buffer and used immediately.

Determination of monoamine oxidase activity

MAO activity was measured by the amount of ammonia formed according to Nagatsu and Yagi (1966) with tyramine as substrate. Briefly, the reaction was terminated by the addition of 0.767 N H_2SO_4 and 2 % (w/v) Na_2WO_4. MAO activity was expressed by the amount of ammonia formed, which was measured by the reaction with phenol reagent and hypochlorite solution spectrophotometrically at 625 nm. Protein was determined with bovine serum albumin as standard (Lowry *et al.*, 1951).

RESULTS

Effect of ginseng on the hepatic MAO activity in acute ethanol-treated mice

Treatment with ginseng butanol fraction (4 mg/kg) alone did not influence the hepatic mitochondrial MAO activity as compared to the saline-treated controls (Table 1).

However, when ethanol was injected to mice, a significant increase of hepatic mitochondrial MAO activity was observed. The increase of hepatic mitochondrial MAO activity caused by ethanol treatment was markedly reduced in the ginseng butanol fraction-pretreated group.

Effect of ginseng on the hepatic MAO activity in chronic ethanol-treated mice

Table 2 shows the effect of ginseng butanol fraction on the hepatic mitochondrial MAO activ-

Table 1. Effect of ginseng butanol fraction on the hepatic MAO activity in acute ethanol-treated mice

Treatment	Specific Activity (n moles/mg protein/min)
Control	2.64$\pm$0.18
Ethanol	3.43$\pm$0.21*
Ginseng butanol fraction	2.58$\pm$0.20
Ethanol+Ginseng butanol fraction	2.87$\pm$0.23

Mice received ethanol(1 g/kg) intraperitoneally 90 min before sacrifice. Ginseng butanol fraction (4 mg/kg) was injected 3 hr before sacrifice. The assay procedure was described in the materials and methods. Values are means$\pm$S.E. for 5 animals. Significantly different from control (*;$p<0.05$).

Table 2. Effect of ginseng butanol fraction on the hepatic MAO activity in chronic ethanol-treated mice

Treatment	Specific Activity (n moles/mg protein/min)
Control	2.64$\pm$0.18
Ethanol	3.57$\pm$0.23*
Ginseng butanol fraction	2.58$\pm$0.20
Ethanol+Ginseng butanol fraction	3.08$\pm$0.23

Mice received ethanol (5%, v/v) orally for 2 months. The other conditions are the same as described in Table 1. (*:$p<0.05$).

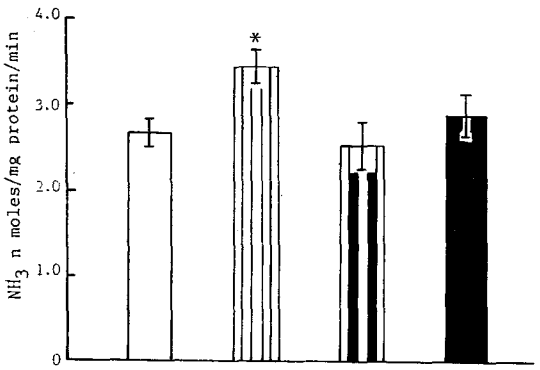

Fig 1. Effect of pyrazole on the hepatic MAO activity in acute ethanol-treated mice.
Pyrazole (200 mg/kg) was injected i.p. 2 hr before sacrifice. The other conditions are the same as described in Table I. Control, □ ; ethanol, ▥ ; pyrazole, ▯ ; ethanol+ pyrazole, ■. (*; $p<0.05$).

ity in chronic ethanol-treated mice. The hepatic mitochondrial MAO activity was significantly elevated as compared to control group when ethanol fed chronicaly to mice for 2 months, and the result obtained was slightly increased than that of acute-ethanol treated group. Whereas, in the ginseng butanol fraction-treated group, it was observed that the hepatic MAO activity was similar to that of control group.

Effect of pyrazole on the hepatic MAO activity in acute ethanol-treated mice

Effect of pyrazole on the hepatic mitochondrial MAO activity with ethanol treatment is shown in Fig 1. It is well known that pyrazole inhibited the alcohol dehydrogenase which catalyzes the oxidation of ethanol to acetaldehyde (Krikum and Cederbaum, 1984). When pyrazole was injected to the control mice, the hepatic mitochondrial MAO activity obtained was similar to that of control group. Pretreatment with pyrazole has markedly blocked the increase of the hepatic mitochondrial MAO activity with ethanol treatment.

Effect of ginseng on the hepatic MAO activity in acetaldehyde-treated mice

Effect of ginseng butanol fraction on the hepatic mitochondrial MAO activity in acetaldehyde-treated mice is shown in Table 3. The hepatic mitochondrial MAO activity of control mice was 2.64 n moles/mg protein/min. The enzyme activity was significantly elevated as compared to the control group when acetaldehyde (100mg/kg) was injected to mice. However, when ginseng butanol fraction was pretreated, a significant decrease in the hepatic MAO activity was observed.

Table 3. Effect of ginseng butanol fraction on the hepatic MAO activity in acetaldehyde-treated mice

Treatment	Specivic Activity (n moles/mg protein/min)
Control	2.64±0.18
Acetaldehyde	3.82±0.36*
Ginseng butanol fraction	2.58±0.20
Acetaldehyde+Ginseng butanol fraction	3.27±0.35

Mice received acetaldehyde (100 mg/kg) i.p. 30 min before sacrifice. The other conditions are the same as described in Table 1. (*:$p<0.05$)

DISCUSSION

The oxidative deamination of a number of primary and secondary amines has been shown to be catalyzed by monoamine oxidase (Murphy, 1978; Tabakoff *et al.*, 1985). Monoamine oxidase is an intrinsic protein of the outer mitochondrial membrane (Smith, 1980).

It is evident from the data presented here that the ginseng butanol fraction may regulate the ethanol-induced monoamine oxidase activity. In the previous report, it is reported that acute treatment with ethanol caused to increase the hepatic mitochondrial MAO activities (Huh *et al.*, 1988). It was also observed that chronic ethanol treatment increased the hepatic mitochondrial MAO activity in mice. When ginseng butanol fracton was injected to the ethanol-treated mice, the increasing effect caused by ethanol was markedly reduced.

But the increase of the hepatic mitochondrial MAO activities can be either due to an action of ethanol or due to an action of its metabolite, acetaldehyde. It is well known that pyrazole is an

effective inhibitor of alcohol dehydrogenase and is often used to assess the metabolic consequences produced by oxidation of ethanol by alcohol dehydrogenase (Krikum and Cederbaum, 1984). Therefore, to differentiate between these possibilities, we measured the hepatic mitochondrial MAO activity after pyrazole pretreatment. It was observed that effect of ethanol on the hepatic mitochondrial MAO activity in mice was diminished with pyrazole pretreatment. Thus, the characteristics of the increase in the enzyme activity may result from an action of acetaldehyde rather than an action of ethanol. Since the pyrazole pretreatment in the ethanol-treated mice reduced the hepatic mitochondrial MAO activity. Acetaldehyde, the intermediate of ethanol, was injected to ensure the this possibility. Acetaldehyde treatment was more significantly increased the hepatic MAO activity than ethanol did.

This result indicated that the increase of the hepatic MAO activity may be to an actaldehyde rather than ethanol. Increasing effect of the hepatic MAO activity caused by acetaldehyde treatment was blocked by ginseng butanol fraction pretreatment.

As we consider that pretreatment with ginseng butanol fraction increased the activity of aldehyde dehydrogenase which metabolized the acetaldehyde, and decreased the acetaldehyde level (Huh *et al.*, 1985) a possibility of prevention in ginseng butanol fraction-pretreated mice may be also considerable.

From the above results, it is supposed that ginseng butanol fraction shows a preventive effect against the hepatic MAO activity which were induced by ethanol and acetaldehyde, but further research in this field is needed.

REFERENCES

Benedetti MS, Boucher T, Carlsson A and Fowler CJ: *Interstinal metabolism of tyramine by both forms of monoamine oxidase in the rat. Biochem Pharmacol 32:47-52, 1983*

Chin JH and Goldstein DB: *Membrane-disordering action of ethanol, Variation with membrane cholesterol content and depth of the spin label probe. Mol Pharmacol 19:425-431, 1981*

Cohen G, MacNamee D and Dembiec D: *Elevation in blood acetaldehyde by pargyline during ethanol administration. Biochem Pharmacol 24:313-316, 1975*

Fowler CJ and Tipton K: *Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. Biochem Pharmacol 30:3329-3332, 1981*

Hagihara S, Sameshima Y, Kobayashi M and Obo F: *Behavior of acetaldehyde transporte in blood. Biochem Pharmacol 30:657-661, 1981*

Huh K, Park JM, Lee SI and Choi JW: *The effect of ginseng butanol fraction on the acetaldehyde metabolism in mice. Yakhak Hoeji 29:18-26, 1985*

Huh K and Choi JW: *Effect of ginseng saponin on xanthine oxidase activity after ethanol treatment in mouse liver. Yakhak Hoeji 23:173-179, 1979*

Joo CN, Koo JH and Kang BH: *Biochemical studies of ginseng saponins (XIV), Effect of ginseng saponins on alcohol oxidation. Korean J Biochem 12:81-90, 1979*

Klemm WR: *Effects of ethanol on nerve impulse activity. In, Biochemistry and Pharmacology of ethanol, Vol. 2. (Majchrowicz E and Noble EP eds.) Plenum Press, New York, 1979, pp 243-267*

Krikun G and Cederbaum A: *Increased microsomal oxidation of alcohols after pyrazole treatment and its similarities to the induction by ethanol consumption. Biochim Biophys Acta 801:131-137, 1984*

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: *Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275, 1951*

Murphy DL: *Substrate-selective monoamine oxidases-inhibitor, tissue, species and functional differences. Biochem Pharmacol 27:1889-1893, 1978*

Nabata H, Saito H and Takagi K: *Pharmacological studies of neutral saponins (CNS) of panax ginseng root. Japan J Pharmacol 23:29-41, 1973*

Nagatsu T and Yagi K: *A simple of monoamine oxidase and D-amino acid oxidase by measuring ammonia. J Biochem 60:219-221, 1966*

Pettersson H and Kiessling KH: *Acetaldehyde occurrence in cerebrospinal fluid during ethanol oxidation in rats and its dependence on the blood level and on dietary factors. Biochem Pharmacol 26:237-240, 1977*

Pikkarainen PH, Gordon ER, Lebsack ME and Lieter CS: *Determinants of plasma free acetaldehyde levels during the oxidation of ethanol, Effects of chronic ethanol feeding. Biochem Pharmacol 30:799-802, 1981*

Prasas JS, Crankshaw DL, Erickson RR, Elliott CE, Husby AD and Holtzman JL: *Studies on the effect of chronic consumption of moderate*

amounts of ethanol on male rat hepatic microsomal drug-metabolizing activity. Biochem. Pharmacol 34:3427-3431, 1985

Saito H, Morita M and Takagi K: *Pharmacological studies of panax gineseng leaves. Japan J Pharmacol 23:43-56, 1973*

Sakurai T, Bing OHL, Huber G and Abekmann WH: *Effects of acetaldehyde upon mechanical properties of isolated rat papillary muscle. J Pharmacol Exp Ther 214:219, 1980*

Schilling RJ and Reitz RC: *A mechanism for ethanol-induced damage to liver mitochondrial structure and function. Biochim Biophys Acta 603:266-277, 1980*

Smith GS: *Changes in monoamine oxidase activity associated with the uncoupling of rat liver mitochondria. FEBS Letters 121:303-305, 1980*

Tabakoff B, Lee JM, Leon-Jones FD and Hoffman PL: *Ethanol inhibits the activity of the B form of monoamine oxidase in human platelet and brain tissue. Psychopharmacol 87:152-156, 1985*

Takagi K, Saito H and Natata H: *Pharmacological studies of panax ginseng root: Estimation of pharmacological actions of panax ginseng root. Japn J Pharmacol 22:245-259, 1972*

Teschke R, Moreno F and Petrides AS: *Hepatic microsomal ethanol oxidizing system (MEOS): Respective roles of ethanol and carbohydrates for the enhanced activity after chronic alcohol consumption. Biochem Pharmacol 30:1745-1715, 1981*

Yu PH, Barclay S, Davis B and Boulton AA: *Deuterium isotope effects on the enzymatic oxidative deamination of trace amines. Biochem Pharmacol 30:3089-3094, 1981*

= 국문초록 =

Ethanol이 유도한 간장중 MAO 활성변동에 미치는 인삼의 영향

영남대학교 약학대학 약리학교실, 계명실업전문대학 식품영양학과*

허 근 · 이상일* · 송민익 · 신억섭 · 박종민

본 실험에서는 ethanol 및 acetaldehyde가 유도하는 MAO 활성변동에 인삼 butanol 분획이 미치는 영향을 검토하였다.

Ethanol(1 g/kg)을 mouse 복강내로 투여하였을 때 간장 중 MAO 활성이 현저히 증가하였으며 이러한 작용은 인삼butanol 분획 전처리로 개선되었다. 또한 pyrazole(200 mg/kg)을 전처리하였을 때 ethanol에 의해 증가된 간장중 MAO활성이 정상수준으로 감소되었다. 만성적으로 ethanol을 투여한 경우에 있어서도 MAO활성이 증가되었으며 인삼 butaonl분획 전처리로 MAO 활성이 감소됨이 관찰되었다.

Ethanol의 대사산물인 acetaldehyde(100 mg/kg)을 복강내로 투여하고 관찰한 실험에서도 간장중 MAO활성이 현저하게 증가되었으며 인삼 butanol분획을 전처리함으로써 정상수준 가깝게 MAO활성이 감소되었다.

이 연구결과로 ethanol에 의한 간장중 MAO활성증가는 ethanol의 대사산물인 acetaldehyde에 기인된 것으로 생각되어지며 인삼 butanol분획이 acetaldehyde에 의해 유도되는 간장중 MAO활성변동을 조절하고 있을 것으로 사료된다.