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Preventive Effect of Ginseng Butanol Fraction against Acetaldehyde -Induced Acute Toxicity

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Abstract□The objective of this study was to investigate the preventive effect of ginseng on acetaldehyde-induced acute toxicity in mice. Compared to the control group, treatment with acetaldehyde inhibited the hepatic cytosolic xanthine oxidase activity with increase in dose. The inhibition of enzyme activity was not changed after dialysis. Pretreatment with ginseng butanol fraction prevented the inhibition of enzyme activity by acetaldehyde. In conjunction with the our previous results (*Yakhak Hoeji*, **29**, 18 (1985)), these results suggest that the most likely mechanism for the observed preventive effects of ginseng against the acetaldehyde-induced acute toxicity may be the decrease hepatic acetaldehyde level.

Keywords□*Panax ginseng*, C.A. Meyer, acetaldehyde-induced toxicity, xanthine oxidase

Introduction

Acetaldehyde, a product of ethanol oxidation, is considered to be toxic because of its reactivity.[1-4] Acetaldehyde could exacerbate the neurologic, hepatic and cardiac complications of alcoholism. The liver is generally considered as the main organ capable of oxidizing ethanol to acetaldehyde.[4,7] It is well known that acetaldehyde causes membrane damages and inhibition of several enzyme activities.[8-10] However, the effect of acetaldehyde on the hepatic cytosolic xanthine oxidase (EC 1.2.3.2) activity has not been completely elucidated. The hepatic cytosolic xanthine oxidase is a terminal oxidase of purine catabolism in nucleotide metabolic pathway.[11,12] Therefore, the present work was undertaken to study the effect of ginseng butanol fraction on the hepatic cytosolic xanthine oxidase activity in acetaldehyde-intoxicated mice.

Materials and Methods

Chemicals

Acetaldehyde was purchased from Hayashi Chemical Co., xanthine and uric acid sodium salt from Nakarai Chemical Co., bovine serum albumin from Sigma Chemical Co. All other reagents were of reagent grade commercially available.

Animals and treatment

Male ICR-mice weighing 20 to 25g were used for these experiments. The control group received saline intraperitoneally and the other group received acetaldehyde 30 min before decapitation. Ginseng butanol fraction was injected intraperitoneally 120 min before decapitation. All the animals had free access to food and water but deprived of the 16 hours prior to sacrifice.

Prepration of hepatic cytosolic fraction

After decapitation, the liver was extensively perfused with cold saline through the portal vein until uniformly pale and quickly excised. The piece of liver was homogenized in 4 vol. of 0.25M sucrose. The homogenate was sequentially centrifuged at $600\times g$, $10,000\times g$ and $105,000\times g$. Then, the $105,000\times g$ supernatant was used as the cytosolic fractions.

Assay for xanthine oxidase

Hepatic cytosolic xanthine oxidase activity was

measured by the method of Stripe *et al.*[13] with xanthine as substrate. Briefly, incubation mixtures consisted of 0.1M potassium phosphate buffer (pH 7.5) containing 60 μM xanthine and cytosolic fraction. Incubations were terminated by the addition of 20% trichloroacetic acid. Enzyme activity was defined as formed uric acid in n moles/mg protein/min. Under the assav conditions used, the initial rates of uric acid formation was linear function of time and protein concentration. Protein was determined by the method of Lowry *et al.*[14] using bovine serum albumin as standard.

Results and Discussion

The hepatic cytosolic xanthine oxidase catalyzes the oxidation of many purine and pyrimidine derivatives.[15] Xanthine oxidase activities of hepatic cytosolic fractions of mice treated with acetaldehyde are shown in Table 1.

The enzyme activity of saline-treated mice was 1.84 n moles/mg protein/min. In the hepatic cytosolic fraction of acetaldehyde-treated mice, the enzyme activities were decreased 1.62, 1.22, and 0.93 n moles/mg protein/min with an increase of dose. The enzyme activity was significantly decreased, as compared to the control group, when acetaldehyde was injected to mice with doses of bigher than 60 mg/kg. The xanthine oxidase activity can be inhibited either due to an acute toxicity by acetaldehyde treatment[16] or due to an action of acetaldehyde as substrate.[17]

Table 1. Dose response of acetaldehyde on the hepatic cytosolic xanthine oxidase activity in mice

Dose(mg/kg) (acetaldehyde)	Specific activity (n moles/mg protein/min)	Percentage inhibition (%)
0	1.84 ± 0.12	—
30	1.62 ± 0.14	13
60	1.22 ± 0.13*	34
100	0.93 ± 0.21*	49

Mice were decapitated 30 min after administration of acetaldehyde. The assav procedure was described in the experimental methods. Values are mean ± S.E. for 5 animals.

* Significantly different from control ($p<0.01$).

To study the action of acetaldehyde on the xanthine oxidase inhibition, each enzyme solution was dialysed for 5 hours against 3 liter of the 0.1M potassium phosphate buffer (pH 7.5). The results are shown in Table 2. There was no significant change in the enzyme activities after dialysis in the cytosolic fractions obtained from acetaldehyde-treated mice. Thus, the characteristics of the inhibition in the enzyme activity may result from an acute toxicity by acetaldehyde treatment rather than an action of acetaldehyde as substrate.

Fig. 1. shows the effect of ginseng butanol fraction on the inhibition of xanthine oxidase in acetaldehyde-treated group. Treatment with ginseng butanol fraction by itself was not changed the hepatic cytosolic xanthine oxidase activity when compared to control group. On the other hand, the enzyme activity in the acetaldehyde-treated group was inhibited to about 34% of the control group. But the inhibition of xanthine oxidase in the ginseng butanol fraction-pretreated group was less than that of the group given acetaldehyde alone.

Considering that the ginseng butanol fraction increases the activity of aldehyde dehydrogenase which metabolizes the acetaldehyde,[18] giseng butanol fraction may exert a preventive effect on the acetaldehyde-induced inhibition of xanthine oxidase by promoting the degradation of acetaldehyde.

From the above results, acetaldehyde, although a metabolite of ethanol, produces severe hepatic disfunction when overdosed. It suggested that the significant inhibition of hepatic cytosolic xanthine oxidase activity may attribute the changes of purine catabolism, and that the ginseng butanol

Table 2. Changes in the hepatic cytosolic xanthine oxidase activities after dialysis

Control	before dialysis	1.84 ± 0.12	—
	after dialysis	1.81 ± 0.13	2
Acetal-	before dialysis	1.22 ± 0.13*	34
dehyde	after dialysis	1.28 ± 0.11*	31

The experimental conditions are the same as in Table 1.

* Significantly different from control ($p<0.01$).

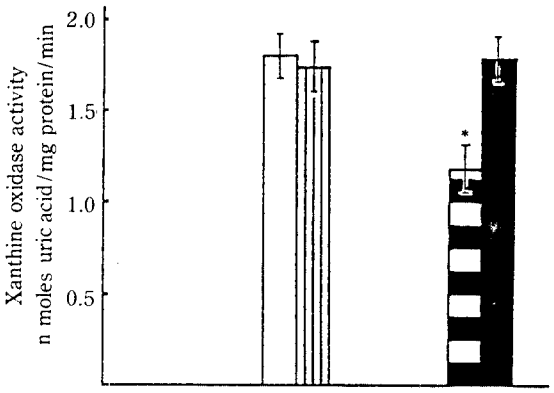

Fig. 1. Effect of ginseng butanol fraction on the hepatic cytosolic xanthine oxidase activity in acetaldehyde-treated mice.
Acetaldehyde (60 mg/kg) was injected intraperitoneally after ginseng butanol fraction treatment (4 mg/kg i.p.). The assay procedure was described in the experimental methods. Values are mean ± S.E. for 5 animals. Control, □ : ginseng butanol fraction, ▥ : acetaldehye, ▤: ginseng butanol fraction + acetaldehyde, ■. *Significantly different from control ($p<0.01$).

fraction may produce a preventive effect against the acute organ toxicities induced by acetaldehyde.

Literature Lited

1. Sakurai, T., Bing, O.H.L., Huber, G. and Abelmann, W.H.: *J. Pharmacol. Exp. Ther.* **214,** 219 (1980).
2. Hagihara, S., Sameshima, Y., Kobayashi, M. and Obo, F.: *Biochem. Pharmacol.* **30,** 657 (1981).
3. Pikkarainen, P.H., Gordon, E.R., Lebsack, M.E. and Lieber, C.S.: *Biochem. Pharmacol.* **30,** 799 (1981).
4. Dawson, A.G.: *Biochem. Pharmacol.* **32,** 2157 (1983).
5. Pettersson, H. and Kiessling, K.H.: *Biochem. Pharmacol.* **26,** 237 (1977).
6. Cohen, G., MacNamee, D. and Dembiec, D.: *Biochem. Pharmacol.* **24,** 313 (1975).
7. Teschke, R., Moreno, F. and Petrides, : *Biochem. Pharmacol.* **30,** 1745 (1981).
8. Dicker, E. and Cederbaum, A.: *Arch. Biochem. Biophys.* **217,** 441 (1981).
9. Cederbaum, A.I. and Dicker, E.: *Biochem. Pharmacol.* **30,** 3079 (1981).
10. Johnson, D.E., Chiao, Y.B., Gavaler, J.S. and Van-Thiel, D.H.: *Biochem. Pharmacol.* **30,** 1827 (1981).
11. Woolfolk, C.A. and Downard, J.S.: *J. Bacteriol.* **130,** 1175 (1977).
12. Boland, M.J., Blevins, D.G. and Randall, D.D.: *Arch. Biochem. Biophys.* **222,** 435 (1983).
13. Stripe, F. and Della Corte, E.: *J. Biol. Chem.* **244,** 3855 (1969).
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: *J. Biol. Chem.* **193,** 265 (1951).
15. Duke, E.J., Jovce, P. and Ryan, J.P.: *Biochem. J.* **131,** 187 (1973).
16. Rahwan, R.G.: *Life Sci.* **15,** 617 (1975).
17. Kellogg III, E.W. and Fridovich, I.: *J. Biol. Chem.* **250,** 8812 (1975).
18. Huh, K., Park, J.M., Lee, S.I. and Choi, J.W.: *Yakhak Hoeji.* **29,** 18 (1985).