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Effect of Methanethiol on the Xanthine Oxidase Activity of Liver and Serum in Rats

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Abstract

To evaluate the effect of methanethiol on the activity of xanthine oxidase in both liver and serum, the rats intraperitoneally received methanethiol. Injection of methanethiol in rats showed the more decreased xanthine oxidase activity of both liver and serum than that of the control group. Concomitantly, the xanthine oxidase activity in livers preincubated with methanethiol was decreased in vitro. The xanthine oxidase in livers preincubated with the methanethiol also showed more increased Km and similar Vmax value than those of the control. These results suggest that the methanethiol may induce a change in substrate binding affinity of xanthine oxidase.

Key words : methanethiol, xanthine oxidase

INTRODUCTION

Methanethiol is a product derived from methionine via the transamination pathway[1], and it also Can be generated by the action of mucosal thiol S-methyl transferase on hydrogen sulfite which is formed by anaerobic bacteria in the intestinal tract[2,3]. The toxicity of methanethiol has often been suggested as one of endogenous factors involved in the pathogenesis of hepatic encephalopathy[4]. Furthermore, methanethiol could cause the membrane damage and inhibition of some enzymes[5].

In our previous work[6] and a number of other reports[7-10], the higher levels of hepatic xanthine oxidase have been observed in an animal model fed a high protein diet. All the more, it was reported that the addition of an adequate quantity of DL-methionine to the diet increased liver xanthine oxidase to normal[11]. However, it is assumed that excess feeding of methionine may paradoxically inhibit the enzyme activity by the methanethiol induced from methionine.

Therefore, the purpose of this study is to investgate whether methanethiol treatment in rats will affect on the xanthine oxidase activity in both the liver and the serum. Concomitantly, the cause of change in enzyme activity will be clarified.

MATERIALS AND METHODS

Animal treatment

Male Sprague-Dawley rats weighing 220 to 240g fed a standard diet as described in Table 1 for a month were divided into two groups. One group was intraperitoneally injected with 0.1ml of 7.5% (v/v in saline) methanethiol. After 4hr, the methanethiol treatment was done once more with the same dose. The control group received only saline.

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Table 1. Composition of experimental diet (g/kg diet)

Ingredients	Quantities
Casein	200
Corn starch	674.36
Corn oil	64.85
Vitamin A and D mixture[a]	10.2
Vitamin E and K mixture[b]	2
Water soluble vitamin mixture[c]	3
Vitamin B_{12}[d]	1
Salt mixture[e]	40
α - Cellulose	20

[a] Vitamin A & D mixture : 51,000 unit of A and 5,100 unit of D dissolved in 100ml of corn oil.

[b] Vitamin E & K mixture : 5g of α -tocopherol and 0.2g of menadion dissolved in 200ml of corn oil.

[c] Water soluble vitamin mixture : contained (mg) ; choline chloride 2000, thiamine hydrochloride 10, riboflavin 20, nicotinic acid 120, pyridoxine 10, Ca-panthothenate 100, biotin 0.05, folic acid 4, inositol 500, *p*-aminobenzoic acid 100.

[d] Vitamin B_{12} : 5mg of vitamin B_{12} dissolved in 500ml of distilled water.

[e] Salt mixture : contained(g) ; $CaCO_3$, 300, potassium phosphate dibasic 322.5, $MgSO_4$, 102, Ca-phosphate monobasic 75, NaCl 167.5, ferric citrate 27.5, KI 0.8, $ZnCl_2$ 0.25, $CuSO_4 \cdot 5H_2O$ 0.3, $MnSO_4$ 5, molybdic acid 0.2.

Preparation of the liver

The animals were sacrified by exsanguination from abdominal aorta. Each liver was perfused with 0.9% saline solution through the portal vein, and then the liver was rapidly removed, cooled in ice, and a 20% homogenate was made in 0.25M sucrose. Each homogenates was centrifuged at 700 $\times g$ for 10min. The supernatant obtained was spun at 105,000$\times g$ for 60min at 4℃. The cytosolic supernatant was dialyzed against 100 volumes of 0. 25M sucrose solution for 12hr at 4℃. The dial-yzed supernatant was used for the determination of enzyme activity.

Enzyme assay

The xanthine oxidase activity was measured by the method of Stirpe and Della Corte[12], and Yoon[13]. Enzyme activity defined as *n* mole uric acid formed per mg protein per min at 30℃. Serum alanine aminotransferase (ALT) was estimated according to the procedure described by Reitman and Frankel[14]. The unit of ALT is expressed as the Karmen[15] unit per ml of serum.

Hepatic glutathione and protein assay

The glutathione contents were determined by the method of Ellman[16] and the content of protein in the liver extract was determined by the method of Lowry et al[17].

Student's t-test was used to establish significant differences in mean values between the control and treated groups.

RESULTS AND DISCUSSION

Changes of liver weight per body weight and serum ALT activities in methanethiol-treated rats

As shown in Table 2, injection of methanethiol in the rats showed about a 2.9 fold increase of serum ALT activities and a slightly decreasing tendency of hepatic glutathione compared to the control group. But the weight of the liver per body weight was similar to that of the control group. An insufficiency of liver function was identified in the present experiment.

Table 2. Effect of methanethiol treatment on the serum ALT activities, hepatic glutathione and liver weight per body weight in rats

Group experiments	Control	Methanethiol
ALT activity[#1]	25.33±2.17	73.20±5.53***
Hepatic glutathione[#2]	4.50±0.33	4.02±0.31
Liver wt/body(%)	3.79±0.11	3.73±0.12

Each value represents the mean ± SE of 6 rats.
[#1] ; karmen unit/ml of serum, [#2] ; μ moles/g of tissue
*** ; $p<0.001$

Xanthine oxidase activities of serum and liver in methanethiol treated rats

It has been observed that both the serum and liver xanthine oxidase activities were increased with liver damage such as viral hepatitis[18,19]. Even in liver damage induced by biological toxin (*Staphylococcus* toxin)[20] and xenobiotics (carbon tetrachloride)[21], xanthine oxidase activities in the liver or serum have been demonstrated to be elevated in an animal model. On the contrary, the present experiment herein showed that injection of methanethiol in the rats rather led to a 24% decrease in the activity of liver xanthine oxidase and slightly decreased activity of serum xanthine oxidase, in

Table 3. Effect of methanethiol treatment on the activities of xanthine oxidase in both liver and sera

Group		Control	Methanethiol
Xanthine oxidase	Liver[#1]	3.90±0.86	2.95±0.43
	Serum[#2]	20.00±0.31	17.86±2.94

Each value represents the mean ± SE of 6 rats.
[#1] ; *n* moles uric acid formed / min / mg protein
[#2] ; *n* moles uric acid formed / min / mg protein

spite of an insufficiency of liver function (Table 3).

It was assumed that a factor responsible for the decreased xanthine oxidase activity of both liver and serum in methanethiol-treated rats may be related to the inhibition action of methanethiol on the enzyme. Therefore, in the present experiment, an effect of methanethiol on the activity of xanthine oxidase in the liver was demonstrated *in vitro* to elucidate a cause of change in enzyme activity.

Effect of preincubation of liver extract with methanethiol *in vitro* on the xanthine oxidase activities

As shown in the Fig. 1, the activity of xanthine oxidase in preincubated liver extract (37.5mg of protein) with the methanethiol (15mg) at 37℃, was gradually decreased in proportion to preincubation time. Especially, the preincubation for 1 hr led to about 30% decreased activity of the xanthine oxidase to the control.

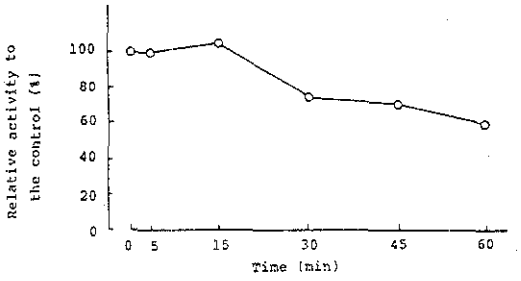

Fig. 1. Effect of preincubation of rats liver extract with methanethiol *in vitro* on the activities of xanthine oxidase. Each value is the mean of 3 experiments.

These results, therefore, indicate that the methanethiol could directly inhibit the activity of the xanthine oxidase. The cause of decreasing activity of the enzyme *in vitro* was clarified on the determination of enzyme activity over a wide range of substrate concentration in the absence as well as in the presence of methanethiol.

Effect of methanethiol on the kinetics of dialyzed liver cytosolic xanthine oxidase

Km and Vmax values of hepatic xanthine oxidase were determined with the liver cytosol (37.5mg of protein) preincubated with 15mg of methanethiol for 30min at 37℃.

As shown in Fig. 2, the xanthine oxidase in liver

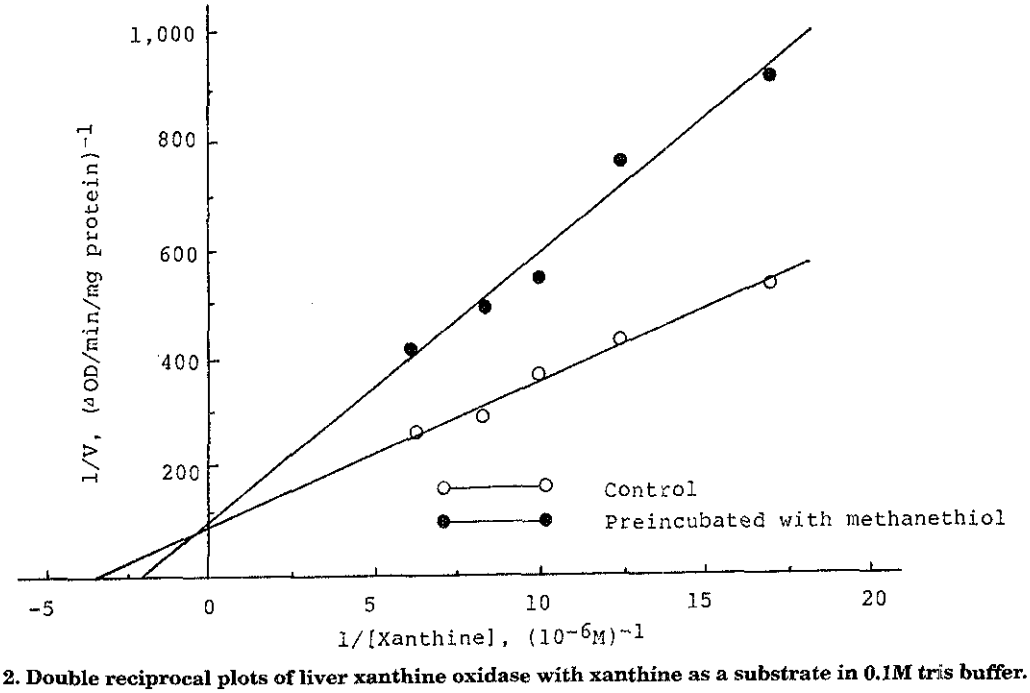

Fig. 2. Double reciprocal plots of liver xanthine oxidase with xanthine as a substrate in 0.1M tris buffer.

preincubated with the methanethiol showed 0.52 μM of Km value and that in the control preincubated in the absence of methanthiol showed 0.29μM. This means that the xanthine oxidase in liver preincubated with the methanethiol was revealed to be the more increased Km value than that of the control. On the other hand, there was no difference in the Vmax value of liver xanthine oxidase between the control and the preincubated with methanethiol.

Therefore, it is likely that the decreasing activity in the liver xanthine oxidase of methanethiol-treated rats may be induced by an effect on a change in substrate binding affinity of the enzyme protein, but further research in this field is needed.

REFERENCES

1. Blom, H. J., Jacintha, P. A. M., Yap, S. H. and Tangerman, A. : Methanethiol and dimethylsulfide formation from 3-methylthiopropionate in human and rat hepatocytes. *Biochem. Biophys. Acta.*, **972**, 131 (1988)
2. Kirk, E. : The quantity and composition of human colonic flatus. *Gastroenterol.*, **12**, 782 (1949)
3. Sogaard, H. : Hydrogen sulfide producing variants of *Escherichia coli*. *Acta Vet. Scand.*, **16**, 31 (1975)
4. Zieve, L., Doizaki, W. M. and Zieve, F. J. : Synergism between the mercaptants and ammonia or fatty acids in the production of coma. : A possible role for mercaptans in the pathogenesis of hepatic coma. *J. Lab. Clin. Med.*, **83**, 28 (1974)
5. Finkelstein, A. and Benevenga, N. J. : The effect of methanethiol and methionine toxicity on the activities of cytochrome oxidase and enzyme involved in protein from peroxidative damage. *J. Nutr.*, **116**, 204 (1986)
6. Yoon, C. G. and Kang, H. Y. : Effect of the peritoneal injection of alcohol on hyperuricemia in rats previously fed low protein. *J. Korean Publ. Hlth. Asso.*, **12**, 81 (1986)
7. Rowe, P. B. and Wyngaarden, J. B. : The mechanism of dietary alterations in rat hepatic xanthine oxidase levels. *J. Biol. Chem.*, **241**, 5571 (1966)
8. Carrozza, G., Livrea, G., Manasseri, L. and Murraca, U. : Differential effect of gluten and casein diets on rat liver HMP shunt dehydrogenase. *J. Nutr.*, **104**, 819 (1974)
9. Muramatsu, K. and Ashida, K. : Effect of dietary protein level on growth and liver enzyme activities of rats. *J. Nutr.*, **76**, 143 (1962)
10. Muramatsu, K. and Ashida, K. : Relationship between the nutritive value of dietary protein and liver xanthine oxidase activity in young rats. *Agric. Biol. Chem.*, **26**, 25 (1962)
11. Williams, J. N., Denton, A. E. and Elivehjem, C. A. : Effect of methionine deficiency upon enzyme activity in the rat. *Proc. Soc. Exp. Biol. Med.*, **72**, 386 (1949)
12. Stirpe, F. and Della Corte, E. : The regulation of rat liver xanthine oxidase. *J. Biol. Chem.*, **244**, 3855 (1969)
13. Yoon, C. G. : A modified colorimetric assay for the xanthine oxidase in rat liver extracts. *Keimyung Research Journal* (Keimyung Junior College), **2**, 295(1984)
14. Reitman, S. and Frankel, S. : A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, **28**, 56 (1957)
15. Karmen, A., Wroblewski, F. and LaDue, J. S. : Transaminase activity in human blood. *J. Clin. Invest.*, **34**, 126 (1955)
16. Ellman, G. L. : Tissue sulfhydryl group. *Arch. Biochem. Biophys.*, **82**, 70 (1959)
17. 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)
18. Shammaa, M. H., Nasrallah, S. M. and Al-Khalidi, U. A. S. : Serum xanthine oxidase an experience with 2,000 patients. *Digestive Disease*, **18**, 15 (1973)
19. Ziegler, D. W., Hutchinson, H. D. and Kisscing, R. E. : Induction of xanthine oxidase by virus infections in newborn mice. *Infect. Immune*, **3**, 237 (1971)
20. Tubaro, E., Banci, F., Lotti, B. and Croce, C. : Xanthine oxidase activation in animal liver during infectious process. *Arzeim-Forch*(Drug Res.), **26**, 2185 (1976)
21. Fowler, J. S. L. : Carbon tetrachloride metabolism in the rabbit. *Br. J. Pharmacol.*, **37**, 733 (1969)

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흰쥐에 Methanethiol 투여가 간 및 혈청 Xanthine Oxidase 활성에 미치는 영향

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요 약

Methanethiol을 흰쥐에 투여시 혈청중 alanine aminotransferase 활성이 대조군 보다 약 3배의 유의한 증가를 보였으며, 이때 간 및 혈청중 xanthine oxidase 활성은 감소되는 경향을 보였다. 또한 간효소액과 methanethiol의 혼액을 37℃에서 preincuabation 시킨후 in vitro에서 xanthine oxidase활성을 측정 하였을때 본 효소의 활성이 억제됨과 동시에 기질인 xanthine의 량을 변화 시켜가면서 관찰한 kinetic 실험에서도 대조 시험에 비하여 Km치가 높게 나타났다.