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原 著

Effect of Dietary Protein on the Serum Xanthine Oxidase Activity in Methanethiol-treated Rats

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식이성단백질 함량이 Methanethiol 투여한 흰쥐의 혈청 Xanthine Oxidase 활성에 미치는 영향

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국 문 초 록

저 및 고단백식이로 성장한 흰쥐에 methanethiol 투여시 체중당 간무게 및 혈청 alanine aminotransferase 활성의 증가율은 고단백식이군(HP군) 보다 저단백식이군(LP군)이 높았으며, 이 때 혈청 xanthine oxidase 활성도는 HP군 보다 LP군이 높게 나타났다. 또한 혈청 중 요산함량 역시 HP군 보다 LP군에서 높게 나타났으며, 간조직의 uricase 활성은 LP군이 HP군 보다 낮게 나타났다. 따라서 LP군이 HP군 보다, methanethiol 투여로 인한 혈청 xanthine oxidase 활성이 높게 나타남은 식이중 단백함량을 낮출 때 methanethiol에 의한 간독성이 심하게 나타나기 때문이며, 또한 단백영양부족시 methanethiol의 폭로가 고요산혈증을 유도함을 시사해 주고 있다.

Keywords : High protein diet, low protein diet, xanthine oxidase, methanethiol, hypercuricemia.

Introduction

Methanethiol is a toxicant that is a byproduct in the industrial process (oil refinery),[1] and it is produced *in vivo* from methionine via transamination in case of its overintake.[2] And it also can be generated by the action of mucosal thiol S-methyltransferase on hydrogen sulfite which is formed by anaerobic bacteria in the intestinal tract.[3,4] The toxicity of methanethiol has often been suggested as one of endogenous factors involved in the pathogenesis of hepatic encephalopathy.[5] Furthermore, methanethiol could cause the membrane damage[6] and inhibition of some membrane protective enzymes.[7]

In the previous works,[6,8] the methanethiol has induced the liver damage and an injection of methanethiol to rats showed the more decreased xanthine oxidase activity both of liver and serum than that of its control group.[9] All the more, the depletion of dietary protein in methanethiol treated-animals induced the more severe damage of liver than those fed a high protein diet.[8] It is well known that the inflammation reaction of liver cell results in the increased permeability of cell membrane in case of liver damage.[10,11] For this reason, it is assumed that the more severe hepatic damage in methanethiol treated-rats fed a low protein diet (LP rats) comparing to those fed a high protein diet (HP rats) would rather increase the activity of serum xanthine oxidase, although HP rats show the decreased activity both of serum and liver xanthine oxidase. In the present study, the changes of xanthine oxidase activity both in liver and serum of LP or HP rats after injection of methanethiol were investigated by correlated liver weight per body weight and serum levels of alanine aminotransferase (ALT) activity, and

then the comparison of liver damage of LP rats with and without methanethiol will be demonstrated. On the other hand, the serum levels of uric acid and hepatic uricase activity were investigated between the two groups.

Materials and Methods

1. Animals and methanethiol treatment

Male Sprague-Dawley rats weighing 230 to 250 g had fed a high or low protein diet as described in Table 1 for a month were divided into two groups.

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

Ingredients	Low protein diet	High protein diet
Casein	70	200
Corn starch	804.36	674.36
Corn oil	54.8	54.8
Vitamin A & D mixture[a]	10.2	10.2
Vitamin E & K mixture[b]	2	2
Water soluble vitamin mixture[c]	3	3
Vitamin B_{12}[d]	1	1
Salt mixture[e]	40	40
α-Cellulose	20	20

[*4190.90 kcal]

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

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

[c] Water soluble vitamin mixture : contained (mg); choline chloride 2,000, 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} : 5 mg of vitamin B_{12} dissolved in 500 m*l* 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.

One group was intraperitoneally injected twice at interval of 4 hr with 0.1 m*l* of 7.5% (V/V in saline) methanethiol per 100 g body weight. Each control group received only saline. After 4 hr, the animals were sacrificed by exsanguination from abdominal aorta. Each liver was perfused with 0.9% NaCl through the portal vein, and then the liver was rapidly removed, weighing the weight of it and one portion of it cooled in ice, and a 20% homogenate was made in 0.25 M sucrose. Each homogenate was centrifuged at 700×g for 10 min. The supernatant obtained was span at 105,000×g for 1 hr at 4℃.

The cytosolic supernatant was dialyzed against 100 volumes of 0.25 M sucrose solution for 12 hr at 4℃. The dialyzed supernatant was used for the determination of enzyme activity.

2. Enzyme assay

The xanthine oxidase activity was measured by the method of Stirpe and Della Corte,[12] and Yoon.[13] The unit of enzyme activity defined as nmole uric acid formed per mg protein per min at 30℃. Hepatic uricase activity was determined by the method of Yonetani[14] and the unit was defined as the enzyme amount which oxidized 1×10^{-6} M uric acid per min per mg protein. Serum ALT activity was estimated according to the procedure described by Reitman and Frankel.[15] The unit of ALT is expressed as the Karmen[16] unit per m*l* of serum.

3. Serum uric acid and liver protein determination

The serum uric acid was determined by the method of Yonetani.[14] The protein in the liver extract was determined by the method of Lowry *et al.*[17]

Results and Discussion

Effect of protein level in diet and methanethiol on the liver weight per body weight and serum levels of ALT activity have been shown in Table 2.

The liver weight per body weight was somewhat larger in LP rats with methanethiol than in those without methanethiol. But the differences were not be found between HP rats with methanethiol

Table 2. Effect of methanethiol on the changes of organ weight per body weight and the serum ALT activities in rats fed a low or high protein diet

Groups / Parameters	High protein diet		Low protein diet	
	Control (6)	Methanethiol (6)	Control (6)	Methanethiol (7)
Liver weight/100 g body wt.	2.80± 0.08	2.75± 0.09	2.33± 0.10	2.57± 0.09
Serum ALT[1]	25.50± 2.18	73.49± 5.57***	29.52± 2.80	145.39± 23.00***

The assay procedure was described in the experimental methods.
Each value represents the mean± S.E. of number described as above.
*** Significantly different from the control group ($p<0.001$)
Unit : [1]Karmen unit/m*l* of serum

Table 3. Effect of methanethiol treatment on the activities of xanthine oxidase in liver or serum in rats fed a low or high protein diet

Groups / Specimens	High protein diet		Low protein diet	
	Control (6)	Methanethiol (6)	Control (6)	Methanethiol (7)
Liver[1]	3.10± 0.68	2.34± 0.34	0.62± 0.07	0.66± 0.08
Serum[2]	22.00± 0.34	20.74± 2.34	27.50± 2.49	35.53± 3.20

The asssay procedure was described in the experimental methods.
Each value represents the mean± S.E. of numbers described as above.
Unit : [1]nmole uric acid/mg protein/min, [2]μmole uric acid/*l* of serum/min.

and those without methanethiol. The increasing rate of serum ALT activity in LP rats with methanethiol was about 4.9 fold and that in HP rats with methanethiol about 3 fold to the control group. The present results would suggested that the liver damage was greater in LP rats with methanethiol than in HP with methanethiol. The present results were similiar with our previous reports.[6,8] In the previous study,[9] the xanthine oxidase activity both in serum and liver were decreased by the injection of methanethiol to the rats fed a normal protein diet.

As shown in the Table 3, on the contrary the liver xanthine oxidase activities were not significantly found differences between LP rats with and without methanethiol, but the serum levels of xanthine oxidase activity were 29% increased in methanethiol treated LP rats comparing to the control group, although the liver and serum xanthine oxidase activity rather somewhat decreased in HP rats with methanethiol comparing with the control group, the HP rats without methanethiol.

This indicates that the depletion of dietary protein induced the more severe liver damage and it could cause the increased activity of serum xanthine oxidase. Furthermore, it is well established that inflammation reaction in case of liver damage induces the increased permeability of cell membrane and it can be attribute to leak of the enzyme into blood stream, rising in serum levels.[10,11] For this reason, and in the present results, it is assumed that increased activity of serum xanthine oxidase in methanethiol treated LP rats would be due to more severe damage of liver comparing with HP rats treated with methanethiol.

In the present result described as in Table 4, increased level of serum uric acid in LP rats with and without methanethiol comparing with HP rats can be also a cause of increased xanthine oxidase activity in LP rats with and without methanethiol, since hyperuricemia could be induced in a case of liver damage in the previous report.[18]

But, it can not be ruled out that the increasing cause of serum uric acid in LP rats with and without methanethiol compared to the HP rats could be due to the more significantly de-

Table 4. Effect of methanethiol treatment on the serum levels of uric acid in rats fed a low or high protein diet

Groups		Uric acid(mg/dl)
High protein diet	Control (6)	2.16± 0.26
	Methanethiol (6)	2.48± 0.35
Low protein diet	Control (6)	2.55± 0.48
	Methanethiol (7)	3.18± 0.34

The assay procedure was described in the experimental methods.
Each value represents the mean± S.E. of numbers described as above.

Table 5. Effect of methanethiol treatment on the activities of liver uricase in rats fed a low or high protein diet

Groups		Hepatic uricase activities[1)]
High protein diet	Control (6)	2.00± 0.14
	Methanethiol (6)	1.51± 0.08*[a]
Low protein diet	Control (6)	1.02± 0.06*[b]
	Methanethiol (7)	0.86± 0.08

The assay procedure was described in the experimental methods.
Each value represents the mean± S.E. of numbers described as above.
*[a] Significantly different from the control group ($p<0.05$)
*[b] Significantly different from the high protein diet group ($p<0.001$)
Unit : [1)] 1 unit; mg protein that oxidized 1 μmole uric acid/min

creased activity of uricase[19)] which act on the uric acid as catalyst in LP rats with and without methanethiol than in HP rats as shown in Table 5.

In conclusion, it can be shown that depletion in dietary protein can exert on hepatic methanethiol toxicity, and induce the increased activity of hepatic and serum xanthine oxidase with the increased levels of serum uric acid.

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