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Effect of Brief Treatment of Bromobenzene on the Liver Na^+/K^+-ATPase Activity in Rats

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랫트에 Bromobenzene의 급성 투여가 간조직 중 Na^+/K^+-ATPase 활성에 미치는 영향

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국문초록

Bromobenzene 투여에 의한 간조직중 ATPase 활성을 관찰할 목적으로 흰쥐에 bromobezene을 체중 kg당 400 mg을 복강으로 투여한 다음 4시간 후에 처치하여 다음과 같은 결과를 얻었다. bromobenzene 투여로 인한 체중당 간무게는 유의하게 증가되었으나 간조직중 단백질 함량은 감소되었다. 혈청중 alanine aminotransferase 활성은 대조군과 별다른 차이를 볼수 없었다. 따라서 본 실험조건에서 bromobenzene 처치 실험동물에서 간조직은 가역적상해로 생각되며 이러한 실험동물모델에 Na^+/K^+-ATPase 활성은 유의하게($p<0.05$) 증가되었으며 이때 V_{max} 치역시 대조군에 비하여 증가 되었다. 이때 간조직중 glutathione 함량은 감소되었으며 glutathione S-transferase 활성 및 cytochrome P-450 함량치는 증가되는 경향을 보였다.

Keywords: Bromobenzene, Na^+/K^+-ATPase

I. Introduction

Recently noxious xenobiotics in work place have brought out the injurious effect on the human health. Bromobenzene, a kind of xenobiotics, is a toxicant that is a byproduct in the industrial process, and it is widely used with a solvent in work place. Some studies indicate that hepatic microsomal enzymes metabolize bromobenzene to an active intermediate, bromobenzene 3, 4-oxide,[1] that produces hepatic necrosis *in vivo*[2,3] and the ability of bromobenzene to react with glutathione in the presence of hepatic microsome.[3,4] It is well generally known that the type of cell injury generated by a substance differs with acute and chronic exposes or large and small dose.[5,6] Therefore the style of bromobenzene exposure will effect on the type of cell injury, eg., reversable or irreversable. It has not been found out the paper on the mechanism of reversable injury by the bromobenzene.

In general, if the cell essentially suffers only minor or brief assault from a xenobiotics, it can survive the non-lethal cell injury.[5,6] Therefore, brief treatment of bromobenzene upon to the experimental animal may bring about reversible injury; hepatic cell swelling.

In the present study, the liver weight per body weight, serum levels of alanine aminotransferase (ALT) activity and the content of hepatic protein were determined to observe the extent of the liver damage. On the other hand, the Na^+/K^+-ATPase activity, glutathione(GSH) content and glutathione S-transferase(GST) activity were demonstrated to clarify the mechanism of the type of liver injury showing in the present experiment.

II. Materials and Methods

1. Animal treatment

Male sprague-dawley rats weighing about 200 g have bred on a diet purchased from Life Science Co. The animals were divided into a control group and a experimental groups, with each group containing 7 rats. Control group was treated with olive oil alone. Experimental group was done by injection of 400 mg/kg body wt. intraperitoneally and then both groups were sacrificed at 4 hours after the injection of bromobenzene

2. Preparation of liver enzyme

The animals were killed by exsanguination of abdominal aorta. The liver was exhaustively perfused with cold 0.9% saline through the protal vein. The liver of rats was rapidly removed and then homogenized in ice-cold 0.25M sucrose., Homogenate(20%) in 0.25M sucrose solution were centrifuged at 700×g for 10 min. The supernatants obtained were spun at 15,000×g for 30 min at 4℃. The postmitochondrial fractions were again centrifuged at 105,000×g for 60 min and the cytosolic and microsomal fractions were obtained.

3. Biochemical analysis

Hepatic GSH content was determined by the method of Ellman[7] the color development of glutathione with 5,5'-dithiobis(2-nitrobenzoic acid). Hepatic GST activity was determined by the method of Habig et al.[8] Serum levels of ALT activity was estimated using kit prepared with the method of Reitman and Frankel.[9] Microsomal cytochrome P-450 content was determined by the Omura and Sato[10] method. And hepatic Na^+/K^+-ATPase activity was determined by the method of Dunham.[11] And for an enzyme kinetics, hepatic Na^+/K^+-ATPase activities determined using the liver dialyzing homogenate with various concentrations of ATP as substrate(pH 8.0) and then Km and Vmax value were calculated from double reciprocal plots of enzyme activity. The protein contents in enzymes solution were estimated by the method of Lowry et al.[12]

III. Results and Discussion

Table 1. Effect of bromobenzene administration on the % ratio of liver wt./ body wt., serum levels of ALT and hepatic protein content in rats

Experiments \ Groups	Control	Bromobenzene
Liver wt./body wt.(%)	3.52±0.17	5.39±0.72*
Serum ALT[1]	30.13±4.03	39.40±6.50
Protein[2]	111.71±3.84	89.01±4.68**

The assay procedure was described in the experimental methods.
Each value represents the mean±S.E. of 7 rats
Unit ; [1] Karmen unit/ml, [2] mg/g. wet. liver
Significantly different from the control(*; $p<0.05$, **; $p<0.01$)

1. Effect of bromobenzene treatment on the liver weight per body weight(%), serum levels of ALT activity and hepatic protein contents in rats.

As shown in Table 1, liver weight per body weight(%) was increased about 53% and hepatic protein content was decreased about 20%., at 4hrs after injection of one dose of bromobenzene. At the same time serum ALT activity was tend to increase but it did not mean statistical significance. These findings indicate that one dose injection of bromobenzene to the animal may lead to the effect of slight injury i.e., reversible injury.

Especially increased liver weight and concomitant decreased content of protein just indicate the hepatic cellular swelling. It may be also suggested that the rat's livers at 4hrs after injection of bromobenzene led to a slight injury, which can be pathologically define the reversible injury.

The most general forms of the reversible cell injury were hypoxia, physical agent, chemicals, infectious agent.[13] Among these causing factors on cell injury, a study on effect of chemical agent; especially bromobenzene on the reversible injury could not be well known.

2. Effect of bromobenzene treatment on the hepatic cytochrome P-450, GSH content and GST activity

It is now well established that hepatotoxicity produced by xenobiotics including bromobenzene is mediated by cytochrome P-450-dependent formation of electrophilic metabolite and proceded by GSH depletion.[1-4] It is well revealed that bromoben-

Table 2. Effect of bromobenzene administration on the hepatic cytochrome P-450, glutathione contents and Glutathione S-transferase activity in rats

Experiments \ Groups	Control	Bromobenzene
Cytochrome P-450[1]	0.310±0.040	0.422±0.016
Glutathione[2]	5.00±0.43	3.41±0.64
GST[3]	560.00±40.40	672.00±75.60

The assay procedure was described in the experimental methods.
Each value represents the mean±S.E. of 7 rats.
Unit ; 1) n moles/mg protein
2) μ moles/g of tissue
3) 2, 4-dinitrobenzene-glutathione conjugate n moles/mg protein/min

zene biotransformed into bromobenzene 3,4-oxide which lead to cell injury in body. This bromobenzene metabolic intermediate, bromobenzene 3, 4-oxide is detoxicated by the conjugated with GSH chiefly in liver organ.[1]

As shown in Table 2, the rats showed a tendency of increased content of hepatic cytochrome P-450 and decreased content of GSH by the bromobenzene treatment, but GST activity was appeared to be increased tendency compared with control.

According to the other report[1] and the present experimental findings, the bromobenzene-treated animals might produce bromobenzene 3, 4-oxide. Just such free radical was thought to be responsible for cell membrane damage and this led to defects in membrane permeability. Concomittantly such defects may be the result of a series of event involving ATP depletion. ATP depletion is primarily responsible for acute cellular swelling.[13]

It is well known that failure of active transport, owing to diminished ATP concentration and enhanced ATPase activity, causes sodium to accumulate intracellulary with diffusion of potassium out of cell.[13]

3. Effect of bromobenzene treatment on the Na^+/K^+ dependent ATPase activity, and its Km and Vmax value

As shown in Table 3, injection of bromobenzene to the rats showed about 1.8 fold significantly increased activity of liver Na^+/K^+-ATPase activity

Table 3. Effect of bromobenzene administration on the hepatic Na^+/K^+-ATPase activities in rats

Treatment	Na^+/K^+-ATPase (n moles pi/mg protein/hr)
Control	146.00±34.00
Bromobenzene	258.00±25.00*

The assay procedure was described in the experimental methods.
Each value represents the mean±S. E. of 7 rats.
*; Significantly different from the control($p<0.05$)

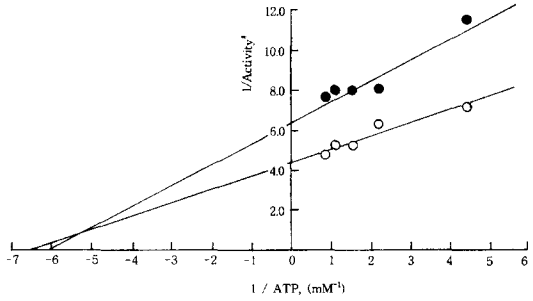

Fig. 1. Double reciprocal plots of hepatic Na^+/K^+-ATPase activity with ATP as substrate in control and bromobenzene-treated rats. Each value represents the mean of 3 experiments with homogenate from pooled liver of each group. #; (pi nmoles/mg protein/hr)×10^{-3}, ●——●; Control, ○——○; Bromobenzene-treated group .

compared to control ($p<0.05$).

This result indicates that an enhancement of Na^+/K^+-ATPase activity by brief treatment of bromobenzene to the animals may inply the failure of active transport, and it can be responsible for swelling of hepatocyte.

Furthermore, to clarify the cause of the increased activity of Na^+/K^+-ATPase, Km and Vmax value in liver Na^+/K^+-ATPase were demonstrated.

Fig, 1 shows the K_m and V_{max} value in hepatic Na^+/K^+-ATPase activity with ATP as substrate. The Km value for Na^+/K^+-ATPase activity with ATP as substrate was calculated to be 6.08 in control and 6.48 mmole in bromobenzene-treated animals. On the other hand, V_{max} value was calculated to be 160.2 in control and 236.3 nmoles/mg protein/hr in bromobenzene-treated animals.

Therefore, K_m value in bromobenzen-treated rats was similiar with the control. But V_{max} was 1.5

fold increased in bromobenzene-treated rats liver compared to the control. According to the present result, in the cause with the facts of increased of Na^+/K^+-ATPase, injection of bromobenzene to the rats effects the Na^+/K^+-ATPase enzyme protein synthesis. And it may be due to influence upon the active transport of liver cell membrane.

In conclusion, the present study supported the facts that a slight injury induced by injection of single dose of bromobenzene to the animal may be responsible for the depletion of active transport in liver cell membrane.

IV. Summary

To evaluate an effect of brief treatment of bromobenzene on the liver Na^+/K^+-ATPase activity, the single dose of bromobenzene (400 mg/kg body weight, i.p.) was injected to the rats and then sacrificed at 4 hrs after treatment.

The bromobenzene-treated animals showed more increased liver weight per body weight(%) and decreased hepatic protein content than control group. At the same time serume alanine aminotransferase (ALT) activity was tend to increase but it did not mean statistical significance.

The animals showed a tendency of increased content of hepatic cytochrome P-450 and decreased GSH content by the bromobenzene treatment.

On the other hand, injection of bromobenzene to the rats showed significantly increased activity($p< 0.05$) of liver Na^+/K^+-ATPase activity compared with control.

Concomitantly in the enzyme preparation of pooled liver homogenate, Km value in bromobenzene-treated rats was similar with the control, but V_{max} was 1.5 fold increased in bromobenzene-treated rats compared to the control.

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