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Trihalomethane Induced Alterations in the Content of Metallothionein and in the Activities of Heme Metabolizing Enzymes in Rats

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Trihalomethanes(THMs) have been reported as widespread contaminants in municipal drinking water(Bellar et al. 1974; Rook 1974; Dowty et al. 1975). Since carcinogenic, mutagenic and physiological effects of THMs were shown in animals(Tardiff 1977; Cantor et al. 1978; Davidson et al. 1982), the presence of THMs in drinking water might adversely affect human health.

Previous studies in our laboratories demonstrated that acute treatments by some halogenated aliphatic hydrocarbons to rats increased the activities of δ-aminolevulinic acid(δ-ALA) synthetase and heme oxygenase , which are the initial and rate-limiting enzymes in the biosynthesis and degradation of heme, respectively, and caused a loss of hemoprotein, cytochrome P-450 (Yamaguchi et al. 1983). In addition, we observed an increase of hepatic metallothionein(MT) content, which is considered to play a central role in homeostasis of essential metals and in protection from metal toxicity(unpublished data). However, little information is available about the relationship between the chlorine- or bromine-content of THMs and their biological or toxicological effects in living animals.

Therefore, in this study, we investigated the detailed effects of THMs on the above parameters as biological indicators, because these parameters can respond sensitively to foreign compounds even in the absence of appreciable injury to liver cells.

MATERIALS AND METHODS

D-Glucose-6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH were purchased from Boehringer Mannheim-Yamanouchi Co., Ltd.,(Tokyo, Japan), and NADP was obtained from Oriental Yeast Co.,LTd.,(Tokyo, Japan). Bovine serum albumin and δ-aminolevulinic acid were purchased from Sigma Chemical Co., (St. Louis, USA) and Daiichi Pure Chemicals Co., Ltd.,(Tokyo. Japan), respectively. Bromodichloromethane($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), tribromomethane($CHBr_3$) and hemin were obtained from Tokyo Kasei Kogyo Co.,Ltd.,(Tokyo, Japan). Trichloromethane($CHCl_3$) was obtained from Wako Pure Chemical Industries Ltd.,(Osaka, Japan) and used following purification by redistillation. Other chemicals of reagent grade were obtained from commercial sources and used without further purification.

Male Wistar rats weighing about 130g were used in all experiments. Animals

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received normal rats chow and water ad libitum. Rats were given THMs orally, dissolved in olive oil, at 4 ml per dose per kg of body weight. Control animals were given oral doses of olive oil alone at 4 ml/kg. A single dose of THMs was 20.0 % of each LD_{50}.

After treatment with THMs rats were sacrificed by decapitation, and the livers were perfused in situ with a cold 0.9% NaCl solution, removed, washed and weighed. The livers were homogenized with 4 volumes of 0.25M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. The microsomes and cytosol were prepared as described previously(Ariyoshi et al.1990).

Metallothionein concentration was determined by the cadmium-heme method according to Onosaka et al.(1978). The activity of δ-ALA synthetase was measured in liver homogenates by the method of Marver et al.(1966). Heme oxygenase activity was calculated from the amount of bilirubin formed using an extinction coefficient of 40 $mM^{-1}cm^{-1}$ between 464nm and 530nm as described by Maines and Kappas(1976). Cytochrome P-450 (P-450) content was determined according to the method of Omura and Sato(1964) using an extinction coefficient of 91 mM^{-1} cm^{-1} between 450nm and 490nm following carbon monoxide bubbling. Protein concentration was estimated according to the method of Lowry et al.(1951) using bovine serum albumin as a standard. Statistical variations among the experiments were evaluated by the Student's t-test. All results are expressed as mean ± S.E..

RESULTS AND DISCUSSION

The results for time dependent effects on the hepatic MT contents following a single administration of THMs to rats are summarized in Table 1. Twenty percent doses of the LD_{50} of THMs were used in this study, since that dose did not indicate a hepatotoxic effect as judged by serum GOT and GPT activities in rats treated with $CHBr_3$ (unpublished data).

The concentration of hepatic MT was increased significantly 6 hr after the administration of $CHBr_3$ and $CHCl_3$, though a marked enhancement of MT level was seen at 12 hr after each THM treatment. This increase approached the control level at 24 hr after treatment.

Although the induction of hepatic MT was observed in this experiment 12 hr after treatment with doses of THMs, it is interesting that hepatic MT content was increased markedly by THMs having chlorine atoms compared to those containing bromine. It might be due to the number of chlorine atoms in the THMs.

However, the physiological significance for increased MT levels by THMs treatment is unclear, but the induction of MT seems to reflect the adaptation phenomenon in the living body to acute phase stimuli, such as protecting against cell damage, imparting stress resistance or maintaining physiological homeostasis.

Figure 1 shows the activities of δ-ALA synthetase, heme oxygenase and the content of P-450 in the liver of rats treated with THMs.

δ-ALA synthetase activity was enhanced significantly at 12 and 24 hr after each THM administration except at 12 hr by $CHCl_3$. These enhanced activities gradually returned to normal level 48 hr after $CHCl_3$ and $CHBrCl_2$ treatment, whereas the

Table 1. Time course of changes in the hepatic metallothionein content of rats after treatment with trihalomethanes

Experiment	Treatment	Dose (mg/kg)	Metallothionein content (μg/mg protein) Time after administration (hr) 6	12	24
	Control	--	0.15 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
A	$CHBr_3$	408	0.23 ± 0.02*	0.30 ± 0.05*	0.16 ± 0.01
	$CHCl_3$	192	0.33 ± 0.03**	0.52 ± 0.07*	0.16 ± 0.01
	Control	--	0.15 ± 0.02	0.15 ± 0.01	0.13 ± 0.02
B	$CHBrCl_2$	86	0.24 ± 0.04	0.41 ± 0.06*	0.15 ± 0.01
	$CHBr_2Cl$	74	0.24 ± 0.04	0.30 ± 0.04*	0.18 ± 0.21

Animals were orally administered trihalomethanes at a single dose of 1/5 of each LD_{50}, and were sacrificed at 6, 12 and 24 hrs after administration.

Values are the mean ± S.E. of 3 to 4 rats.

Significantly different from corresponding mean of control (*$P<0.05$, **$P<0.02$).

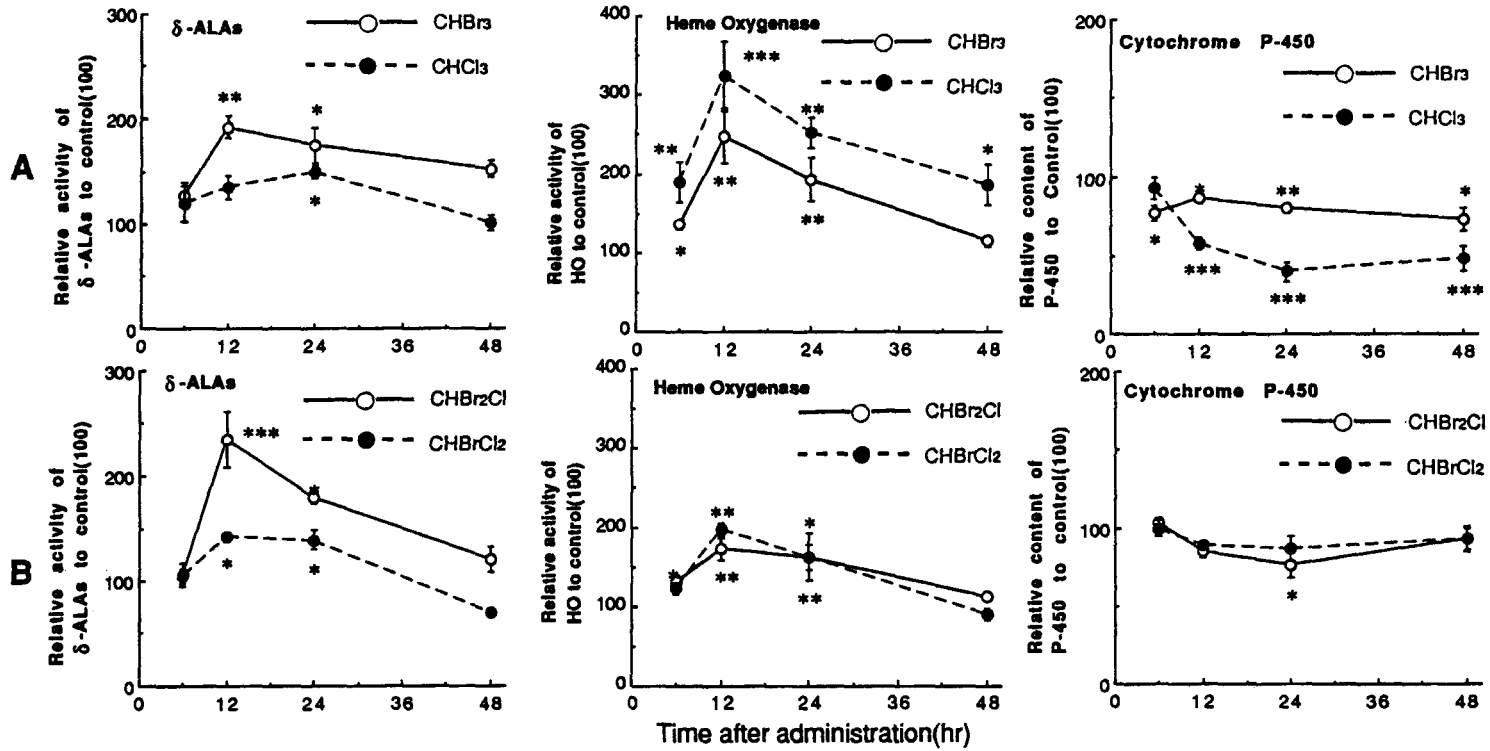

Figure 1. Time course of changes of activities of δ-ALAs and heme oxygenase and of loss of cytochrome P-450 content after administration of trihalomethanes

The average activity and content in the control (mean ± S.E.) were : A) δ-ALAs: 51.7 ± 2.6 (nmole δ-ALA / g liver / hr) ; H O: 1.25 ± 0.05 (nmole bilirubin / mg protein / hr) ; P-450: 0.90 ± 0.03 (nmole / mg protein) ; B) δ-ALAs: 48.9 ± 3.6 (nmole δ-ALA / g liver / hr) ; H O: 1.15 ± 0.07 (nmole bilirubin / mg protein / hr) ; P-450: 0.93 ± 0.05 (nmole / mg protein) * P<0.05 , **P<0.02, ***P<0.01

elevated levels by $CHBr_3$ and $CHBr_2Cl$ continued at 48 hr after dosing compared with that of the respective control. However, these levels were statistically insignificant.

In addition, each THM increased the heme oxygenase activity markedly 6, 12 and 24 hr after treatment except at 6 hr following $CHBrCl_2$ administration. A significant enhancement by $CHCl_3$ continued to be seen, 48 hr after dosing.

In contrast, P-450 content was depressed markedly by treatment with $CHCl_3$ and $CHBr_3$, and the depressed level by $CHCl_3$ was larger than that of $CHBr_3$. No significant difference appeared to the P-450 content after administration of $CHBrCl_2$ and $CHBr_2Cl$ except at 24 hr($CHBr_2Cl$). In which the content showed a tendency to be slightly decreased 12 and 24 hr after dosing.

In this study, we found that THMs, especially $CHBr_3$ and $CHBr_2Cl$, significantly enhanced the δ-ALA synthetase activity at 12 and 24 hr, whereas $CHBr_3$ and $CHCl_3$ significantly depressed P-450 content. Since it generally has been considered that the induction of δ-ALA synthetase precedes the enhancement of P-450 content(Baron and Tephly 1970), the influences of THMs on P-450 content might be associated with the stimulation of heme degradation or with the inhibition of heme utilization.

As expected we observed a marked increase of heme oxygenase activity by THMs administration to 200 to 300% of control level(Figure 1). Heme is known to exercise a negative feed back control on the activity of porphyrin biosynthetic pathway at the level of δ-ALA synthetase. Therefore, the regulation of δ-ALA synthetase and of heme oxygenase in the liver are interrelated.

As shown in Figure 1, $CHCl_3$ stimulated a high level of heme oxygenase activity or a low level of δ-ALA synthetase activity when compared to the respective enzyme activities by $CHBr_3$. $CHCl_3$ could induced an intense, rapid and prolonged degradation of heme or P-450 heme compared to $CHBr_3$. On the other hand, $CHBr_3$ could increase hepatic heme biosynthesis continually through a prolonged induction of δ-ALA synthetase compared to $CHCl_3$. The magnitude of different actions on the enzymes in heme metabolic pathways lead to an alteration of P-450 content.

Since liver is the principal organ for biosynthesis, metabolism and biotransformation of various endogenous or exogenous compounds, liver appears to play a major role in the acute phase responses.

Therefore, the induction of MT and the alteration of key enzymes in heme biosynthesis and degradation and the depression of P-450 seem to reflect the adaptation phenomena by the living body to acute phase stimuli.

Chu et al.(1982) reported that THMs, fed to rats for 90 days in drinking water, can produce biochemical, hematological and histological changes even in small concentrations(1.2-1.5 mg/rat/day). However, those changes were reversible in rats after exposure was terminated. We also observed no significant effects on the above parameters from THM treatment, such as $CHBr_3$ or $CHBr_2Cl$, at relatively

Table 2. Subacute effects of trihalomethanes administration on the hepatic contents of hemoprotein and metallothionein and on the activities of heme metabolizing enzymes

		Control	$CHBr_3$	$CHBr_2Cl$
Body weight	initial(g)	123 ± 2	126 ± 1	126 ± 1
	final (g)	159 ± 1	160 ± 2	160 ± 3
Liver weight (g/100g body weight)		4.76 ± 0.10	5.19 ± 0.15	5.13 ± 0.24
Cytochrome P-450 (nmole/mg protein)		0.95 ± 0.03	0.97 ± 0.05	0.96 ± 0.05
Metallothionein (μg/ mg protein)		0.13 ± 0.05	0.16 ± 0.03	0.15 ± 0.02
δ-Aminolevulinic Acid synthetase (*nmole δ-ALA/g liver/hr*)		50.4 ± 3.5	56.6 ± 1.0	53.5 ± 3.7
Heme oxygenase (nmole bilirubin/mg protein/hr)		1.29 ± 0.35	2.09 ± 0.16	1.69 ± 0.21

Animals were given orally $CHBr_3$(204 mg/kg) or $CHBr_2Cl$(37 mg/kg) once a day for 7 days and were sacrificed 24hrs after the last administration. Values are the mean ± S.E. of 4 to 6 rats

low doses(10 .0% of LD_{50}) of once daily for 7 days(Table 2).

Further investigations will be required to determine the relationship between chemical structures and biological activities of aliphatic halogenated hydrocarbons.

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