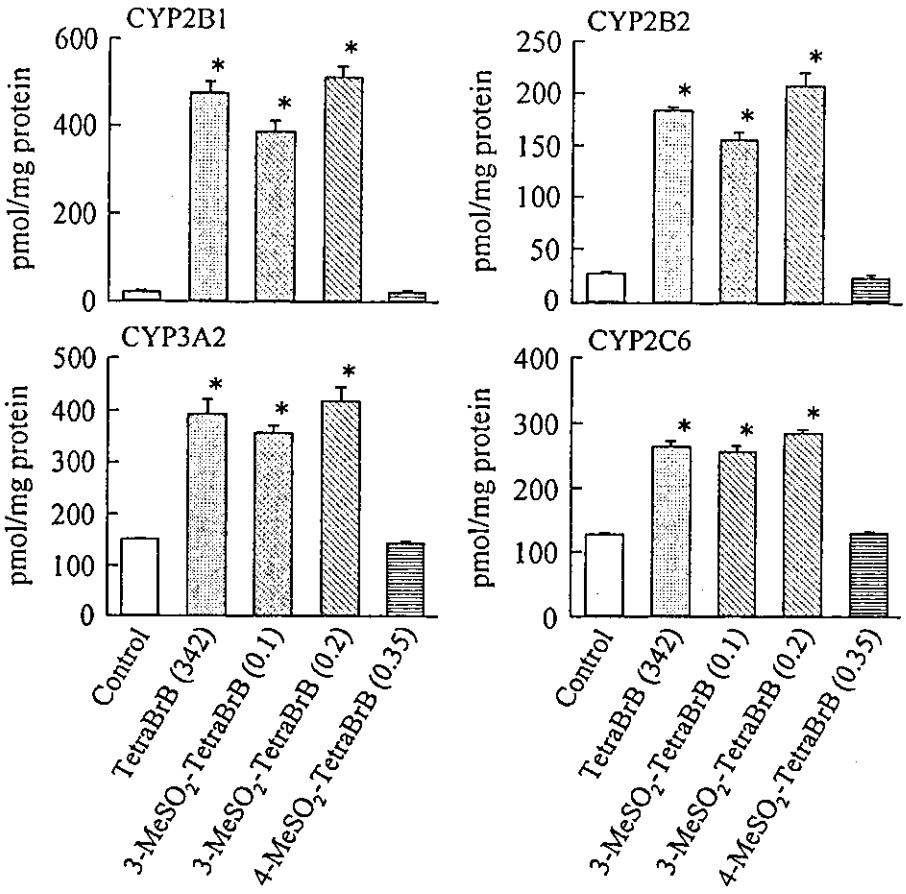

Figure 11. Relationship between the liver concentrations of 3- and 4-$MeSO_2$-TetraBrBs, and the contents of P450 forms after the administration of TetraBrB and its $MeSO_2$derivatives. The experimental conditions were the same as in figure 10. Assays were done under same conditions as described in figure 7. Results are the mean ± SEM (vertical bars) for four to six animals. The value in parentheses is a dose ($\mu mol\,kg^{-1}$) for each group. $^*P < 0.01$, significantly different from the control.

The prolonged induction of hepatic microsomal drug-metabolizing enzymes may be due to the prolonged presence of 3-$MeSO_2$-TetraBrB in the liver.

The dose-related increases in both the activities of drug-metabolizing enzymes and the contents of CYP2B1, CYP2B2, CYP3A2 and CYP2C6 after the administration of 3-$MeSO_2$-TetraBrB are considered to be due to the rise in the hepatic concentration of 3-$MeSO_2$-TetraBrB after dosing. The comparison of the inducing abilities of 3-$MeSO_2$-TetraBrB ($0.5\,\mu mol\,kg^{-1}$) with those of 3-$MeSO_2$-TetraCB ($1\,\mu mol\,kg^{-1}$), PB ($431\,\mu mol\,kg^{-1}$ twice), and the parent TetraBrB ($342\,\mu mol\,kg^{-1}$) indicated that 3-$MeSO_2$-TetraBrB has a comparatively pronounced inductive effect on the hepatic drug-metabolizing enzymes CYP2B1, CYP2B2, CYP3A2 and CYP2C6.

The induction profiles of the drug-metabolizing enzymes and four PB-inducible forms of P450 of rat treated with 3-$MeSO_2$-TetraBrB were similar to those of rat treated with PB. This finding indicates that 3-$MeSO_2$-TetraBrB is a PB-like inducer of microsomal drug-metabolizing enzymes, analogous to the 3-$MeSO_2$ metabolites of PCBs.

It has been reported that the potent PB-like inducer, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, is 600 times more potent than PB as an inducer of *Cyp2b* in

mouse (Poland *et al.* 1980, 1981), and, by comparison, 3-MeSO$_2$-TetraBrB was 1700 times more potent than PB as an inducer of CYP2B1/2 in rat at an estimate in accordance with their doses and dose times. Therefore, 3-MeSO$_2$-TetraBrB is the most potent PB-like inducer of the CYP2B1 and CYP2B2 described to date, with an inducing potency several thousand times more than that of the parent TetraBrB.

When a 342 μmol kg^{-1} dose of TetraBrB was administered to rat, the hepatic concentration of 3-MeSO$_2$-TetraBrB 96 h after dosing was similar to that after the administration of 0.1–0.2 μmol kg^{-1} 3-MeSO$_2$-TetraBrB. The increases in the content of total P450 in the activities of 7-pentoxy- and 7-benzyloxyresorufin *O*-dealkylases and in the contents of CYP2B1, CYP2B2, CYP3A2 and CYP2C6 96 h after the administration of TetraBrB were almost equal to the increases 96 h after the administration of 3-MeSO$_2$-TetraBrB. Thus, 3-MeSO$_2$-TetraBrB is considered to play an important role in the induction mediated by the TetraBrB. On the other hand, 4-MeSO$_2$-TetraBrB did not appear to be involved in the induction process because it had no inducing effect, as was the case with the 4-MeSO$_2$ derivatives of PCB congeners (Kato *et al.* 1995a, b).

The above observations suggest that the inducing effect of TetraBrB on hepatic microsomal drug-metabolizing enzymes is attributable not to the action of TetraBrB *per se*, but to that of its MeSO$_2$ metabolite, 3-MeSO$_2$-TetraBrB.

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The contribution of 2,3,5-trichlorophenyl methyl sulfone, a metabolite of 1,2,4-trichlorobenzene, to the δ-aminolevulinic acid synthetase induction by 1,2,4-trichlorobenzene in rat liver

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Abstract

In the present study, we investigated the contribution of methylsulfonyl metabolite derived from 1,2,4-trichlorobenzene (1,2,4-TCB) on the δ-aminolevulinic acid (ALA) synthetase induction by the parent compound in rats. The time courses of increasing of hepatic microsomal total cytochrome P450 content after a single ip administration of 1,2,4-TCB (1.36 mmol/kg), and 2,3,5- and 2,4,5-trichlorophenyl methyl sulfones (2,3,5- and 2,4,5-$TCPSO_2Mes$) (50 μmol/kg each) were in parallel with those of increasing of the total heme content in liver microsomes. 1,2,4-TCB significantly increased the heme oxygenase activity, but 2,3,5- and 2,4,5-$TCPSO_2Mes$ did not. On the other hand, 1,2,4-TCB and 2,3,5-$TCPSO_2Me$ markedly enhanced the ALA synthetase activity. No change was observed in this enzyme activity after the administration of 2,4,5-$TCPSO_2Me$. After the administration of 1,2,4-TCB to the rats treated with DL-buthionine-(*S,R*)-sulfoximine (BSO) and to the non-BSO-treated rats, the concentrations of both 2,3,5- and 2,4,5-$TCPSO_2Mes$ were significantly lower in liver of the BSO-treated rats than in liver of the non-BSO-treated rats. Additionally, the 1,2,4-TCB did not elevate the ALA synthetase activity in the BSO-treated rats. On the other hand, the administration of 2,3,5-$TCPSO_2Me$ to BSO-treated rats resulted in induction of ALA synthetase. The results strongly suggest that the methyl sulfone derived from 1,2,4-TCB, i.e., 2,3,5-$TCPSO_2Me$, contributes highly to the induction of the ALA synthetase activity by the parent compound.

Keywords: Chlorinated benzene; Methylsulfonyl metabolite; Heme oxygenase; Total cytochrome P450; Total heme; DL-buthionine-(*S,R*)-sulfoximine

1. Introduction

The chlorinated benzenes are important environmental contaminants that are widely used as chemical intermediates in the synthesis of organic compounds and as solvents, pesticides, dye carriers, space deodorants and moth repellents (US EPA, 1985; World Health Organization, 1991). A number of studies on the toxicity of chlorinated benzenes indicate that the liver and kidneys are their principal target sites (Chu et al., 1984; Côté et al., 1988). It has been reported that several chlorinated benzenes, e.g., *m*-dichlorobenzene (*m*-DCB) and 1,2,4-trichlorobenzene (1,2,4-TCB), have strong inducing effects on hepatic microsomal drug-metabolizing enzymes in rats (Ariyoshi et al., 1975a,b, 1981; Allis et al., 1992).

In our previous papers (Kimura et al., 1983; Kato et al., 1993), the administration of methylsulfonyl metabolites derived from *m*-DCB and 1,2,4-TCB resulted in the induction of hepatic microsomal mixed function oxidases in rats. The 3,5-dichlorophenyl methyl sulfone (3,5-$DCPSO_2Me$) from *m*-DCB and 2,3,5-trichlorophenyl methyl sulfone (2,3,5-$TCPSO_2Me$) from 1,2,4-TCB

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were found to be the potent inducers. We also showed that the inducing effects of *m*-DCB and 1,2,4-TCB on hepatic microsomal mixed function oxidases are not attributable to the action of *m*-DCB and 1,2,4-TCB per se but to those of their methylsulfonyl metabolites, respectively (Kato et al., 1986, 1993).

Several investigators have reported that *m*-DCB, 1,2,4-TCB and hexachlorobenzene (HCB) cause the inductions of δ-aminolevulinic acid (ALA) synthetase and heme oxygenase in liver, which are the rate-limiting enzymes in the biosynthesis and degradation of heme, respectively (Marver et al., 1966; Poland et al., 1971; Ariyoshi et al., 1975a,b, 1981; Kato et al., 1990). Furthermore, it has been reported that like HCB, *m*-DCB and 1,2,4-TCB cause hepatic porphyria in rats by the induction of ALA synthetase (Rimington and Ziegler, 1963; Poland et al., 1971). We have reported that 2,4- and 3,5-DCPSO$_2$Mes markedly increased the ALA synthetase activity (Kato et al., 1988). Therefore, the methylsulfonyl metabolites of 1,2,4-TCB, i.e., 2,3,5- and 2,4,5-TCPSO$_2$Mes, should stimulate the activity of ALA synthetase in the course of induction of the mixed function oxidase. Thus, it seems important to determine if the methylsulfonyl metabolites contribute to the inductive effects to ALA synthetase of chlorinated benzenes.

In the present study, we investigated the role of methyl sulfones derived from 1,2,4-TCB in the induction of ALA synthetase involved in heme regulation.

2. Materials and methods

2.1. Chemicals

2,3,5- and 2,4,5-TCPSO$_2$Mes (Fig. 1) were synthesized according to the method described in a previous paper (Kimura et al., 1984). 1,2,4-TCB (Fig. 1) was purchased from Kanto Chemicals Co., (Tokyo); and DL-buthionine-(*S*,*R*)-sulfoximine (BSO), from Sigma Chemical Co., (St. Louis, Mo). Nicotinamide adenine dinucleotide phosphate (oxidized form, $NADP^+$), glucose-6-phosphate disodium salt, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., (Osaka). Other chemicals were obtained as commercial reagent grades.

2.2. Animal treatments

Male Wistar rats, weighing ≈200 g, were housed three or four per cage with free access to commercial chow (MF, Oriental Yeast Co.) and tap water, and maintained on a 12-h dark/light cycle (8:00 a.m.–8:00 p.m. light) in a room at a controlled temperature (24.5 ± 1 °C) and humidity (55 ± 5%).

Rats received an ip injection of 1,2,4-TCB or its methylsulfonyl derivatives dissolved in Panacete 810® (medium chain triglycerides, Nippon Oils and Fats Co. Ltd., Tokyo, Japan) (2.5 ml/kg). The doses of these compounds are noted in the legends to the figures and tables. Control animals received an appropriate amount of the corresponding vehicle. All rats were killed by decapitation at the designated time after the dosing.

For the BSO pretreatment, rats were dosed subcutaneously with BSO (4 mmol/kg in 0.9% saline with the aid of 0.1 N NaOH to solubilize it) twice with a 6-h interval between doses. Control animals received an equivalent volume of 0.9% saline (20 ml/kg). At 3 h after the first BSO administration, rats received an ip injection of 1,2,4-TCB or 2,3,5-TCPSO$_2$Me.

2.3. Preparation of hepatic homogenates

The livers were perfused in situ with ice-cold 1.15% KCl solution, quickly excised, minced and then homogenized with 3 vol. of an ice-cold solution of 0.9% NaCl–0.5 mM EDTA–10 mM tris-HCl buffer (pH 7.4) in a Potter–Elvehjem homogenizer with a Teflon pestle.

2.4. Preparation of hepatic microsomes and soluble fractions

The livers were removed after perfusion, rinsed with an ice-cold solution of 1.15% KCl, and homogenized in 2 vol. of an ice-cold solution of 1.15% KCl–50 mM potassium phosphate buffer (pH 7.4) by use of the above homogenizer. Microsomes were prepared by the method described previously (Kimura et al., 1983). The soluble fraction (105 000*g* supernatant), prepared from normal rat liver homogenate, was used as a source of biliverdin reductase for the determination of heme oxygenase activity. The protein contents of two fractions were de-

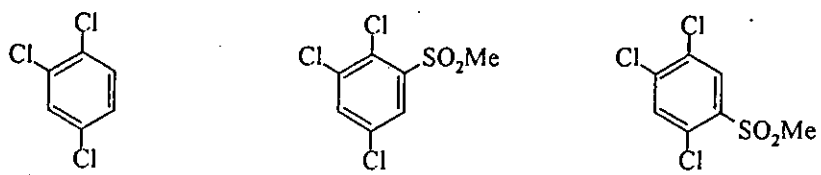

Fig. 1. Chemical structures of 1,2,4-TCB and its methyl sulfone derivatives.

termined according to the method of Lowry et al. (1951). Bovine serum albumin was used as the standard.

2.5. Enzyme assays

ALA synthetase activity of liver homogenates was determined by the method of Marver et al. (1966). Heme oxygenase activity of microsomes was measured by the method of Maines and Kappas (1974). Total cytochrome P450 and total heme contents in microsomes were estimated by the method of Omura and Sato (1964) and Matteis (1971), respectively.

2.6. Determination of methyl sulfones in liver

The concentration of 2,3,5- and 2,4,5-$TCPSO_2Me$s present in the liver was determined by HPLC as described previously (Kato et al., 1993).

2.7. Statistics

The data were analyzed by Student's *t*-test or Dunnet's test after the analysis of variance (ANOVA), depending on the nature of the experiment (Steel and Torrie, 1980).

3. Results

3.1. Changes in the heme metabolic enzyme activities of rat liver after the administration of 1,2,4-TCB, and 2,3,5- and 2,4,5-$TCPSO_2Me$s

Fig. 2 shows the time courses of activities of hepatic ALA synthetase and microsomal heme oxygenase, and contents of microsomal total heme and total cytochrome P450 after the ip administration of 1.36 mmol/kg of 1,2,4-TCB. The increase in ALA synthetase activity peaked at 24 h after the administration of 1,2,4-TCB. This increased enzyme activity returned to control levels by 72 h after the administration. A significant increase of heme oxygenase activity was observed 6 h after the injection of 1,2,4-TCB, and was maximum 24 h after the dosing. This enzyme activity was significantly increased even 72 h after the administration. The microsomal total heme content was elevated from 24 h after the dosing. The time course for the increase in total cytochrome P450 content paralleled that of the heme.

Fig. 3 shows the time courses of the effect of 2,3,5-$TCPSO_2Me$ at a single ip dose of 50 μmol/kg on various heme related parameters. A significant increase of ALA synthetase activity was observed 6 h after the injection of 2,3,5-$TCPSO_2Me$, and this increase was still observed at 48 h. The heme oxygenase activity was barely decreased after 24–72 h. Significant increases in microsomal total heme and total cytochrome P450 contents were observed between 6 and 72 h after the administration of 2,3,5-$TCPSO_2Me$. On the other hand, no change was observed in both ALA synthetase and heme oxygenase activities after the administration of 2,4,5-$TCPSO_2Me$ (50 μmol/kg) (data not shown). Slight increases (7–26%) in total heme and total cytochrome P450 contents were only observed 12–72 h after the administration of 2,4,5-$TCPSO_2Me$.

3.2. Methyl sulfone concentrations in liver after the administration of 1,2,4-TCB and 2,3,5-$TCPSO_2Me$ to BSO-treated rats

BSO has been reported to be a specific inhibitor of γ-glutamylcysteine synthetase in the liver, leading to a marked lowering of the hepatic glutathione level (Griffith and Meister, 1979; Meister, 1983; Drew and Miners, 1984; White et al., 1984). Thus, 1,2,4-TCB or 2,3,5-$TCPSO_2Me$ was administered to the BSO-treated rats, and the determination of 2,3,5- and 2,4,5-$TCPSO_2Me$s concentrations in liver was performed 21 h later. The time of determination was chosen from a preliminary experiment, which showed this to be the time when the concentrations of both methyl sulfones were lowest. As shown in Table 1, the administration of 1,2,4-TCB to the BSO-treated rats caused the concentrations of the two methyl sulfones in the liver to decrease by 77–94%, as compared with the non-BSO-rats. On the other hand, no significant difference was found in the methyl sulfone concentration in liver between non-BSO-treated and BSO-treated rats after the administration of 2,3,5-$TCPSO_2Me$. 2,4,5-$TCPSO_2Me$ was not detected in the liver (data not shown).

3.3. Effects of 1,2,4-TCB and 2,3,5-$TCPSO_2Me$ on hepatic ALA synthetase in BSO-treated rats

Table 2 shows the effects of treatment with 1,2,4-TCB or 2,3,5-$TCPSO_2Me$ on ALA synthetase in BSO-treated rat liver. BSO alone did not affect ALA synthetase activity. The administration of 1,2,4-TCB to non-BSO-treated rats significantly increased the ALA synthetase activity. In BSO-treated rats, however, 1,2,4-TCB had no significant increase in the ALA synthetase activity. When 2,3,5-$TCPSO_2Me$ was administered to BSO-treated rats, the activity of ALA synthetase was significantly increased, and the degree of increase in the enzyme was almost the same as that observed in non-BSO-treated rats.

4. Discussion

In the present study, 1,2,4-TCB markedly enhanced the ALA synthetase activity. This finding coincided with the result reported by Ariyoshi et al. (1975a,b, 1981).

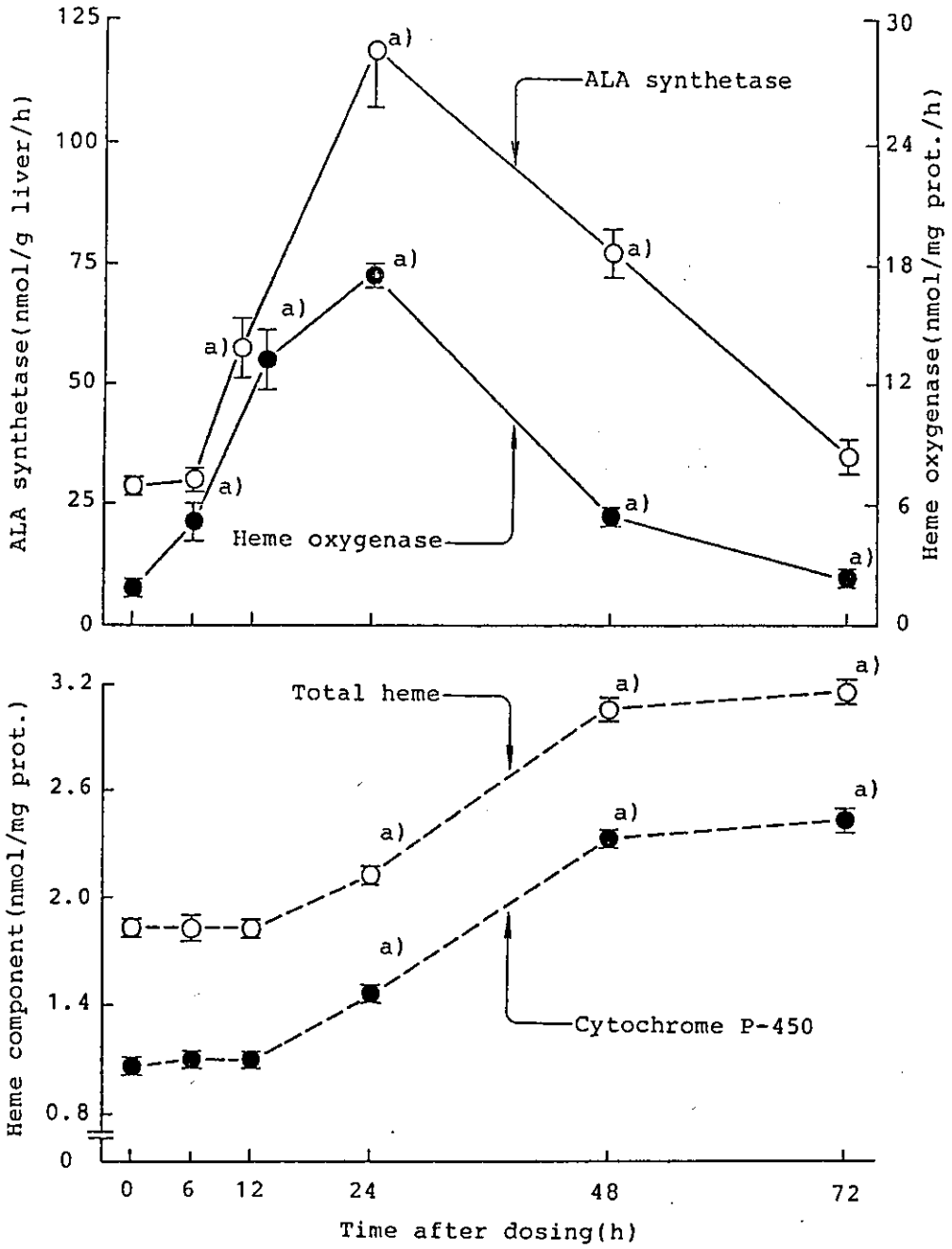

Fig. 2. Effects of 1,2,4-TCB administration on various heme-related parameters in rat liver. Rats were given ip a 1.36 mmol/kg dose of 1,2,4-TCB and killed at the appropriate times after the administration. Each point represents the mean ± SE (vertical bars) for five to six animals. (a) Significantly different from control (0 h), $p < 0.02$.

2,3,5-TCPSO$_2$Me also significantly increased the ALA synthetase activity, but 2,4,5-TCPSO$_2$Me did not. This observation suggests that 2,3,5-TCPSO$_2$Me is the inducer of the hepatic ALA synthetase, that the methyl sulfone plays a principal role in the inducing effect of 1,2,4-TCB on the hepatic ALA synthetase.

1,2,4-TCB markedly increased the heme oxygenase activity, however 2,3,5- and 2,4,5-TCPSO$_2$Mes did not. These results indicate that the inducing effect of 1,2,4-TCB on the hepatic microsomal heme oxygenase is not attributable to its methylsulfonyl metabolites.

The ip administration of 1,2,4-TCB caused an increase in total cytochrome P450 content. This result coincided with the result reported by Ariyoshi et al. (1975a,b, 1981). The time courses of increasing of the total cytochrome P450 content after the administration of 1,2,4-TCB and 2,3,5-TCPSO$_2$Me were in parallel with those of increasing of the total heme content. These

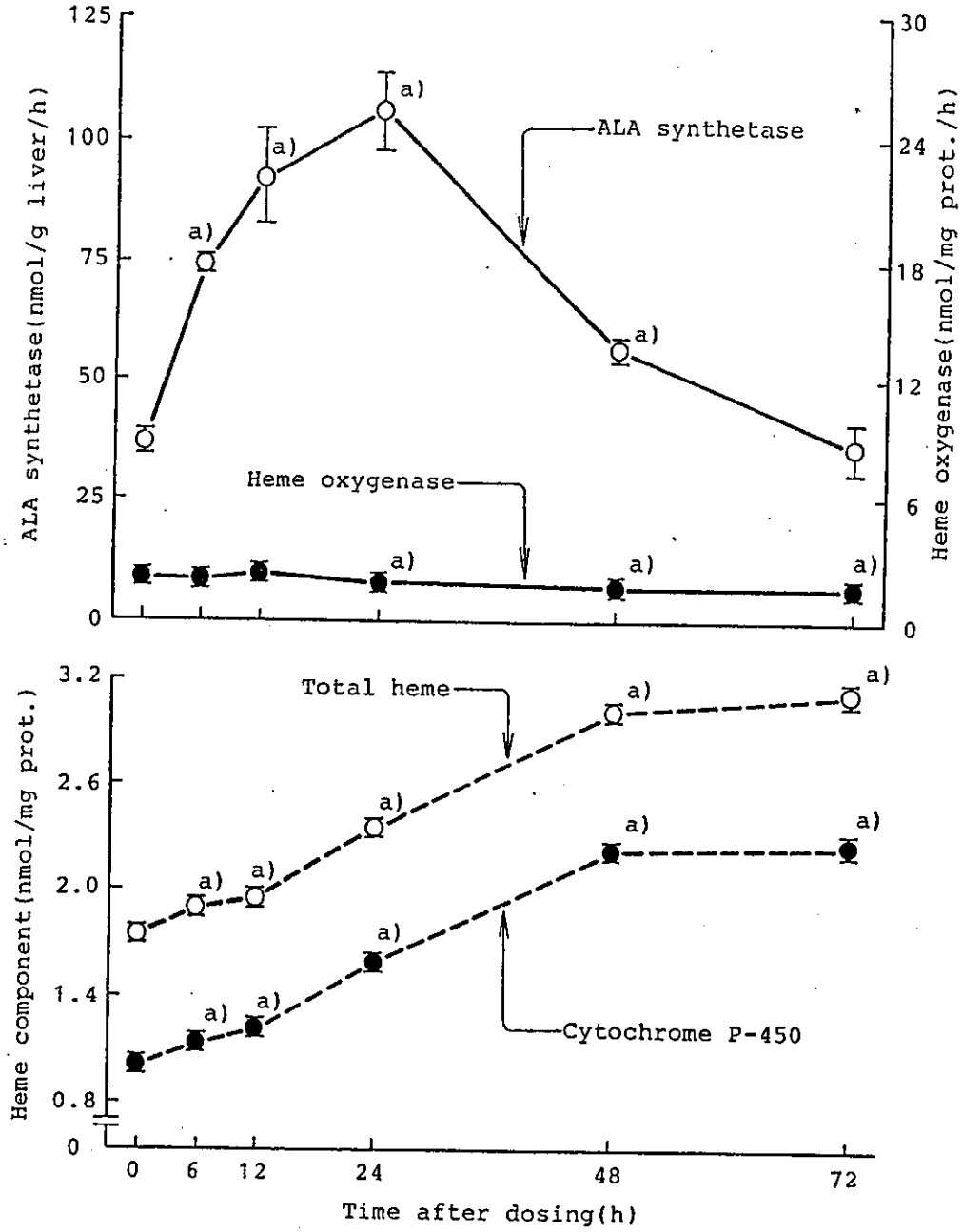

Fig. 3. Effects of 2,3,5-TCPSO$_2$Me administration on various heme-related parameters in rat liver. Rats were given ip a 50 μmol/kg dose of 2,3,5-TCPSO$_2$Me and killed at the appropriate times after the administration. Each point represents the mean ± SE (vertical bars) for four to seven animals. (a) Significantly different from control (0 h), $p < 0.05$.

results suggest that the induction of total cytochrome P450 after the administration of 1,2,4-TCB and 2,3,5-TCPSO$_2$Me are closely related to the heme metabolism.

2,3,5- and 2,4,5-TCPSO$_2$Mes in the liver were present at much lower concentrations in the BSO-treated rats dosed with 1,2,4-TCB than in non-BSO-treated rats. Additionally, in the BSO-treated rats, the induction of ALA synthetase did not take place after 1,2,4-TCB dosing. On the other hand, the administration of 2,3,5-TCPSO$_2$Me to BSO-treated rats resulted in a similar hepatic concentration of the methyl sulfone to that in non-BSO-treated rats, and the extent of induction of the ALA synthetase was almost the same as that in the non-BSO-treated rats. These findings suggest that the inducting effect of 1,2,4-TCB on hepatic ALA synthetase is not attributable to the action of 1,2,4-TCB per se but to that of its 2,3,5-methylsulfonyl metabolite.

The alteration in heme contents by chemical agents and physiological and pathological factors, with few exceptions, is mainly regulated by the activities of ALA

Table 1
Effects of BSO treatment on liver concentrations of methyl sulfones after the administration of 1,2,4-TCB and 2,3,5-TCPSO$_2$Me to rats

Animal	Methyl sulfone concentration (nmol/g)		
	1,2,4-TCB-administered		2,3,5-TCPSO$_2$Me-administered
	2,3,5-TCPSO$_2$Me	2,4,5-TCPSO$_2$Me	2,3,5-TCPSO$_2$Me
Non-BSO-treated	14.5 ± 1.6	3.2 ± 0.8	8.6 ± 0.6
BSO-treated	3.4 ± 0.1[a]	0.2 ± 0.1[a]	10.3 ± 0.6

The rats, which were treated with BSO as described in the text, were given ip 1,2,4-TCB (1.36 mmol/kg) or 2,3,5-TCPSO$_2$Me (50 μmol/kg), and killed 21 h after the administration. Results are expressed as the mean ± SE for five to six animals.
[a] Significantly different from intact group, $p < 0.02$.

Table 2
Effects of 1,2,4-TCB and 2,3,5-TCPSO$_2$Me on ALA synthetase activity in liver of BSO-treated rats

Animal	ALA synthetase activity (nmol/g liver/h)		
	Control	1,2,4-TCB	2,3,5-TCPSO$_2$Me
Non-BSO-treated	41.48 ± 2.34	157.42 ± 10.49[a]	131.75 ± 10.85[a]
BSO-treated	44.67 ± 4.32	43.12 ± 4.71	113.69 ± 6.81[a]

Treatment of rats was carried out the same as described in the legend to Table 1. Results are expressed as the mean ± SE for six animals.
[a] Significantly different from control, $p < 0.01$.

synthetase and heme oxygenase in a reciprocal way (Maines, 1982). In the present study, the increase in total cytochrome P450 content caused by 1,2,4-TCB administration is thought to be the consequence of excess of heme biosynthesis through the prolonged induction of ALA synthetase by its metabolite, 2,3,5-TCPSO$_2$Me. It is suggested that the enhanced heme synthesis overrides the destruction of heme, particularly the cytochrome P450 heme, which results from the induction of heme oxygenase by 1,2,4-TCB or some metabolite(s) other than the methyl sulfone.

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