Contribution of Methylsulfonyl Metabolites of 2,2',4,5,5'-Pentachlorobiphenyl to the Heme Metabolic Enzyme Induction by the Parent Compound in Rat Liver

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Introduction

A number of methylsulfonyl ($MeSO_2$) metabolites of polychlorinated biphenyls (PCBs) have been found in several mammalian species, such as seals, otters, polar bears, beluga whales and minks from the Canadian and Swedish environment [1-4] and in the Yusho patients from a Japanese environment [5, 6]. Recently, $MeSO_2$-PCBs have been determined in human milk, blood, liver and adipose tissue [7-9]. The main $MeSO_2$-PCBs in human milk and the tissues of human and mammals have been shown to be 3- and 4-$MeSO_2$ derivatives of PCBs with chlorine atoms in the 2,5- or 2,5,6-position, e.g. 3-$MeSO_2$- and 4-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyls (3-$MeSO_2$- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs) [1-4, 7, 9]. To our knowledge, the available information regarding the biological activities and toxicological effects of the $MeSO_2$ metabolites is limited.

In our preceding papers [10-12], we reported that nine 3-$MeSO_2$ metabolites of PCB congeners, such as 3-$MeSO_2$-2,2',4',5,5'-pentaCB and 3-$MeSO_2$-2,2',3',4',5,5'-hexachlorobiphenyl, were potent phenobarbital-type inducers of hepatic microsomal drug-metabolizing enzymes, while their isomeric 4-$MeSO_2$ metabolites were not. Additionally we suggested that the inducing effect of 2,2',4,5,5'-pentaCB on hepatic microsomal drug-metabolizing enzymes was not attributable to the action of 2,2',4,5,5'-pentaCB *per se*, but to that of its 3-$MeSO_2$ metabolite, 3-$MeSO_2$-2,2',4',5,5'-pentaCB [13].

We also previously showed that corresponding $MeSO_2$ metabolites derived from *m*-dichlorobenzene (*m*-DCB) was strong inducer of hepatic δ-aminolevulinic acid (ALA) synthetase in rats, and strongly suggested that the methyl sulfones derived from *m*-DCB, i.e., 2,4- and 3,5-dichlorophenyl methyl sulfones, contributed highly to the induction of ALA synthetase activity by the parent compound administration [14].

In the present study, we investigated the role of 3-methyl sulfone derived from 2,2',4,5,5'-pentaCB in the induction of ALA synthetase involved in heme regulation. Fig. 1 shows the chemical structures of 2,2',4,5,5'-pentaCB, and its 3- and 4-$MeSO_2$ derivatives used in this study.

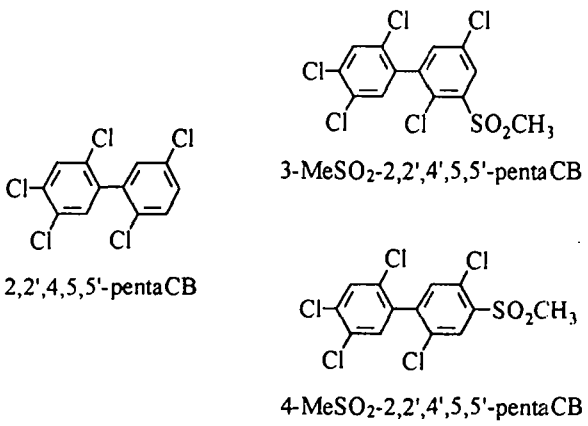

Fig. 1. Chemical structures of 2,2',4,5,5'-pentaCB and its methyl sulfone derivatives

Materials and Methods

Chemicals. 2,2',4,5,5'-pentaCB was synthesized according to the Cadogan coupling reactions [15]. 3- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs were prepared as described by previous studies [16]. The purity of these compounds was >99% when analyzed by gas chromatography. Other chemicals were obtained as commercial sources.

Animal treatments. Male Wistar rats, weighing about 200 g, were housed three or four per cage in the laboratory with free access to commercial chow and tap water, and maintained on a 12-hr dark/light cycle in a room with controlled temperature (24.5 ± 1°C) and humidity (55 ± 5%). Rats received an i.p. injection of 2,2',4,5,5'-pentaCB or its $MeSO_2$ derivatives dissolved in Panacete 810 (5 ml/kg). For the DL-buthionine-(*S,R*)-sulfoximine (BSO) treatment, rats were dosed s.c. with BSO (4 mmol/kg in 0.9% saline with the aid of 0.1 N NaOH to solubilize it) twice with a 6-hr interval between doses. Control animals received an equivalent volume of 0.9% saline (20 ml/kg). At 3 hr after the first BSO administration, rats received an i.p. injection of 2,2',4,5,5'-pentaCB or 3-$MeSO_2$-2,2',4',5,5'-pentaCB. Control animals were treated with an equivalent volume of the corresponding vehicle All rats were fasted for 18 hr prior to death. They were killed by decapitation at the designated time after the dosing.

Biochemical analyses. Hepatic homogenates and microsomes were prepared according to the procedure described previously [14]. The protein content was determined by the method of Lowry *et al.* [17] with bovine serum albumin as a standard. ALA synthetase activity of homogenates was determined by the method of Marver *et al.* [18]. Heme oxygenase activity of microsomes was measured by the method of Maines and Kappas [19]. Total cytochrome P450 content was estimated according to the method of Omura and Sato [20]. Microsomal total heme content was determined by the method of Matteis [21].

Determination of 2,2',4,5,5'-pentaCB, and 3- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs in liver. The clean-up and determination of 2,2',4,5,5'-pentaCB and its $MeSO_2$ derivatives from liver were done as described previously [22].

Results and Discussion

The time courses of the effects of a single i.p. administration of 2,2',4,5,5'-pentaCB (342 μmol/kg) and 3-$MeSO_2$-2,2',4',5,5'-pentaCB (2 μmol/kg) on hepatic microsomal total cytochrome P450 content were almost in parallel with those on the total heme content in liver microsomes. No change was observed in heme oxygenase activity after administration of 2,2',4,5,5'-pentaCB, whereas this activity after injection of 3- or 4-$MeSO_2$-2,2',4',5,5'-pentaCB was significantly decreased. On the other hand, 2,2',4,5,5'-pentaCB and 3-$MeSO_2$-2,2',4',5,5'-pentaCB markedly enhanced ALA synthetase activity, but 4-$MeSO_2$-2,2',4',5,5'-pentaCB did not.

No significant difference was found in 2,2',4,5,5'-pentaCB concentration in liver between non-BSO-treated and BSO-treated rats after administration of 2,2',4,5,5'-pentaCB. In the liver of BSO-treated rats dosed with 2,2',4,5,5'-pentaCB (514 μmol/kg), 3- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs were present at significantly lower concentration than in non-BSO-treated rats (Table 1). Additionally, 2,2',4,5,5'-pentaCB did not elevate ALA synthetase activity in BSO-treated rats (Table 2). On the other hand, both the concentration of the methyl sulfone and the induction of ALA synthetase in the liver of BSO-treated rats dosed with 3-$MeSO_2$-2,2',4',5,5'-pentaCB (2 μmol/kg) were almost the same as those of non-BSO-treated rats after the administration of 3-$MeSO_2$-2,2',4',5,5'-pentaCB (Tables 1 and 2).

Table 1. Effects of BSO treatment on liver concentrations of 2,2',4,5,5'-pentaCB and its methyl sulfones after administration of 2,2',4,5,5'-pentaCB and 3-$MeSO_2$-2,2',4',5,5'-pentaCB to rats

	Concentration (nmol/g)			
	2,2',4,5,5'-pentaCB administered			3-$MeSO_2$-2,2',4',5,5'-pentaCB administered
Animal	2,2',4,5,5'-pentaCB	3-$MeSO_2$-2,2',4',5,5'-pentaCB	4-$MeSO_2$-2,2',4',5,5'-pentaCB	3-$MeSO_2$-2,2',4',5,5'-pentaCB
Non-BSO-treated	1.53 ± 0.13	0.16 ± 0.03	0.28 ± 0.05	1.24 ± 0.08
BSO-treated	1.53 ± 0.07	0.09 ± 0.01*	0.16 ± 0.02*	1.18 ± 0.09

Rats were given i.p. 2,2',4,5,5'-pentaCB (514 μmol/kg) or 3-$MeSO_2$-2,2',4',5,5'-pentaCB (2 μmol/kg) and killed 24 hr after the administration. Results are expressed as the mean ± S.E. for 4-10 animals.
*$P<0.05$, significantly different from intact group.

Table 2. Effects of 2,2',4,5,5'-pentaCB and 3-$MeSO_2$-2,2',4',5,5'-pentaCB on ALA synthetase activity in liver of BSO-treated rats

	ALA synthetase activity (nmol/g liver/hr)				
Animal	Control	2,2',4,5,5'-pentaCB	Effect (%)	3-$MeSO_2$-2,2',4',5,5'-pentaCB	Effect (%)
Non-BSO-treated	41.7 ± 6.3	80.5 ± 11.7*	+93	68.9 ± 8.3*	+65
BSO-treated	50.7 ± 4.7	56.8 ± 6.8	+12	84.4 ± 2.2*	+66

Treatment of rats was carried out the same as described in the legend to Table 1. Results are expressed as the mean ± S.E. for 4-8 animals.
*$P<0.05$, significantly different from control.

The results strongly suggest that the methyl sulfone derived from 2,2',4,5,5'-pentaCB , i.e., 3-$MeSO_2$-2,2',4',5,5'-pentaCB, contribute highly to the induction of the ALA synthetase activity by the parent compound administration.

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