3-METHYLSULFONYL-2,2',4',5-TETRABROMOBIPHENYL, A METABOLITE OF 2,2',4',5-TETRABROMOBIPHENYL INDUCES CYP2B1/2 IN RATS

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Introduction

A number of the methylsulfonyl ($MeSO_2$) metabolites of polychlorinated biphenyls (PCBs) have been found in several species of animals in Canada and Sweden[1-4] and in healthy humans, as well as in Yusho patients in Japan[5, 6].

In our previous papers[7-9], we reported that the nine 3-$MeSO_2$ metabolites of PCBs were the potent inducers of hepatic microsomal drug-metabolizing enzymes in rats. Additionally we suggested that some 3- and 4-$MeSO_2$ metabolites may act as liver tumor promoters, based upon the results from *in vitro* intercellular communication assay[10]. Recently we showed that the seven 3-$MeSO_2$ and two 4-$MeSO_2$ metabolites of tetra-, penta- and hexachlorobiphenyls reduced serum thyroxine level and increased serum thyroid stimulating hormone level in rats[11-13], suggesting that the metabolites may act as endocrine-disrupters.

Polybrominated biphenyls (PBrBs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a great variety of electrical appliances. The biological activities and toxicological significance of $MeSO_2$ metabolites of PBrB have not been clarified.

In this study, therefore, we attempted to identify the sulfur-containing metabolites in the liver and feces of rats administered with a PBrB congener, 2,2',4',5-tetrabromobiphenyl (2,2',4',5-tetraBrB), and investigated the effects of 3- and 4-$MeSO_2$ metabolites of 2,2',4',5-tetraBrB, and compared their activities with their parent compound, on the drug-metabolizing enzyme system. The purpose of the present study was to clarify the role of 3- and 4-$MeSO_2$ metabolites in altering the hepatic microsomal drug-metabolizing enzyme system by 2,2',4',5-tetraBrB.

Materials and Methods

Chemicals. 2,2',4',5-tetraBrB was synthesized by using the Cadogan coupling reactions[14]. The $MeSO_2$-PBrBs were prepared as described elsewhere[15]. The purity of these compounds was >99% when analyzed by gas chromatography (GC). All other chemicals, with appropriate purity, were commercially obtained.

Animal treatments. Male Wistar rats, weighing 180-200 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 (8:00 A.M.-8:00 P.M. light) in a room with controlled temperature (24.5 ± 1%) and humidity (55 ± 5%). Rats received an intraperitoneal injection of 2,2',4',5-tetraBrB (342 μmol/kg) or its $MeSO_2$ derivatives (0.05-2.0 μmol/kg) dissolved in Panacete 810 (5 ml/kg).

Control animals received an equivalent volume of vehicle. All rats were starved for 18 hr prior to killing by decapitation at designated time after dosing.

Microsomal preparation and enzyme assays. Microsomes were prepared according to the procedure described previously[7]. The protein content was determined by the method of Lowry *et al.*[16] with bovine serum albumin as a standard. Total cytochrome P450 content was estimated according to the method of Omura and Sato[17]. The activity of alkoxyresorufin *O*-dealkylase in microsomes was determined by the method of Burke *et al.*[18]. The immunoblotting and immunochemical quantitation were performed as described by Imaoka *et al.*[19].

Separation and identification of metabolites. The analysis of sulfur-containing metabolites of 2,2',4',5-tetraBrB in liver and feces was based on the method described Bergman *et al.*[2].

Determination of 2,2',4',5-tetraBrB and its $MeSO_2$-PBrBs in liver. The concentrations of 2,2',4',5-tetraBrB and its $MeSO_2$-PBrBs present in liver were determined by analyzing the n-hexane extracts from the samples by GC[2].

Results and Discussion

3- and 4-methylthio-2,2',4',5-tetraBrBs were detected in the feces, and 3- and 4-$MeSO_2$-2,2',4',5-tetraBrBs were detected in the liver of rats dosed with 2,2',4',5-tetraBrB.

The administration of 3-$MeSO_2$-2,2',4',5-tetraBrB (0.35 μmol/kg) to rats significantly increased both the content of total cytochrome P450 and the activities of 7-benzyloxy-, 7-ethoxy- and 7-pentoxyresorufin *O*-dealkylase after 48-168 hr. 3-$MeSO_2$-2,2',4',5-tetraBrB produced a dose-related increase in the hepatic concentration. The 3-$MeSO_2$ derivative produced nearly dose-related increases both in the content of cytochrome P450 and in the extent of metabolization of 7-benzyloxy-, 7-ethoxy- and 7-pentoxyresorufin of liver microsomes in the range of 0.05-2.0 μmol/kg. The inducing effect of the 3-$MeSO_2$ derivative (0.2 μmol/kg) on both the content of cytochrome P450 and the activities of three enzymes was higher than that of parent compound (342 μmol/kg) (Table 1). Major phenobarbital (PB)-inducible forms, CYP2B1, CYP2B2, CYP3A2 and CYP2C6 were dramatically induced by the 3-$MeSO_2$ derivative. In contrast, 4-$MeSO_2$ derivative of 2,2',4',5-tetraBrB had no effect on the drug-metabolizing enzyme activities and these P450 forms.

The inducing ability of 3-$MeSO_2$-2,2',4',5-tetraBrB (0.5 μmol/kg) on both the activities of three enzymes and the contents of CYP2B1/2 was the same degree as that of 3-$MeSO_2$-2,2',4',5-tetrachlorobiphenyl (3-$MeSO_2$-2,2',4',5-tetraCB) (1 μmol/kg) or PB (431 μmol/kg twice at a 24 hr interval) (Table 1). The induction profiles of these enzymes and CYP isozymes of rats treated with 3-$MeSO_2$-2,2',4',5-tetraBrB were similar to those of PB (Table 1). It is noticeable that 3-$MeSO_2$ metabolite of 2,2',4',5-tetraBrB is a potent PB-type inducers of microsomal drug-metabolizing enzymes, CYP2B1 and CYP2B2 at levels several thousand fold lower than required for equivalent induction by parent PBrB, while its isomeric 4-$MeSO_2$ metabolite is not.

The extent of both the hepatic accumulation of the 3-$MeSO_2$ metabolite and the induction of the enzymes and CYP2B1/2 after the administration of 2,2',4',5-tetraBrB (342 μmol/kg) was almost the same as those after the administration of 3-$MeSO_2$-2,2',4',5-tetraBrB (0.1 μmol/kg) (Table 1). The relationship between hepatic concentration of 3-$MeSO_2$ metabolite and the extent and profile of induction of enzymes after the administration of 2,2',4',5-tetraBrB and its 3-$MeSO_2$ metabolite suggests that the 3-$MeSO_2$ metabolite contributes prominently to the induction of microsomal drug-metabolizing enzyme by 2,2',4',5-tetraBrB.

Table 1. Effects of 2,2',4',5-tetraBrB, its methyl sulfone derivatives, 3-$MeSO_2$-2,2',4',5-tetraCB and phenobarbital on the content of total cytochrome P450 and the activities of drug-metabolizing enzymes of liver microsomes in rats

Treatment	Dose (μmol/kg)	Total cytochrome P450 [a]	7-Benzyloxy-resorufin *O*-dealkylase [b]	7-Pentoxy-resorufin *O*-dealkylase [b]	7-Ethoxy-resorufin *O*-dealkylase [b]
Control		0.99 ± 0.01	0.15 ± 0.005	0.04 ± 0.002	0.18 ± 0.006
2,2',4',5-tetraBrB	342	1.43 ± 0.14*	2.45 ± 0.26*	0.50 ± 0.03*	0.24 ± 0.02
3-$MeSO_2$-2,2',4',5-tetraBrB	0.1	1.38 ± 0.05*	2.03 ± 0.21*	0.41 ± 0.04*	0.38 ± 0.04*
3-$MeSO_2$-2,2',4',5-tetraBrB	0.2	1.61 ± 0.05*	4.45 ± 0.58*	0.78 ± 0.07*	0.48 ± 0.03*
3-$MeSO_2$-2,2',4',5-tetraBrB	0.35	1.96 ± 0.08*	8.61 ± 0.75*	1.41 ± 0.12*	0.63 ± 0.03*
3-$MeSO_2$-2,2',4',5-tetraBrB	0.5	2.24 ± 0.11*	9.12 ± 0.61*	1.51 ± 0.08*	0.63 ± 0.04*
4-$MeSO_2$-2,2',4',5-tetraBrB	0.35	1.03 ± 0.02	0.15 ± 0.004	0.04 ± 0.003	0.18 ± 0.01
3-$MeSO_2$-2,2',4',5-tetraCB	1	2.23 ± 0.04*	7.44 ± 0.18*	1.45 ± 0.03*	0.55 ± 0.01*
Phenobarbital	431×2	2.25 ± 0.06*	8.40 ± 0.31*	1.47 ± 0.06*	0.55 ± 0.01*

Rats were given i.p. 2,2',4',5-tetraBrB, its methyl sulfone derivatives or 3-$MeSO_2$-2,2',4',5-tetraCB and killed 96 hr after the administration. Phenobarbital was injected i.p. into rats twice with a 24 hr interval and the rats were killed 24 hr after the second injection. Results are expressed as the mean ± S.E. for three to eight animals.

[a] nmol/mg protein.

[b] nmol resorufin/mg protein/min.

*$P<0.05$, significantly different from the control.

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