Induction of Hepatic Microsomal Drug-Metabolizing Enzymes by Methylsulfonyl Metabolites of PCBs in Rats

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INTRODUCTION

Methylsulfonyl($MeSO_2$) derivatives of polychlorinated biphenyls(PCBs) were first identified in blubber from seals in the Baltic[1]. Since then, a number of these substances have been demonstrated in animals[2-4]. Recently, main substances in the seal blubber were identified as 3- and 4-$MeSO_2$-PCB isomers chlorinated at 2- and 5-positions of at least one of the phenyl rings, 3-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl(pentaCB), 4-$MeSO_2$-2,2',4',5,5'-pentaCB and 4-$MeSO_2$-2,2',3',4',5-pentaCB[5]. Even in healthy people, $MeSO_2$-PCB isomers were found in adipose tissue at concentration as high as those of PCBs[6]. However, the biological activities and toxicologic effects of the $MeSO_2$-PCB metabolites of animals has been studied little.

In preceding papers[7], we reported that corresponding dichlorophenyl methyl sulfones ($DCPSO_2Mes$) were detected in several tissues and urine of rats dosed with *m*-dichlorobenzene(DCB). We also showed that the administration of these $DCPSO_2Mes$ resulted in strong induction of hepatic microsomal drug-metabolizing enzymes in rats[8].

In this study, we investigated the effects of some PCB congeners and their $MeSO_2$-PCBs on the drug-metabolizing enzyme system.

MATERIALS AND METHODS

Chemicals. 2,3',4',5-tetrachlorobiphenyl(tetraCB)(IU-70), 2,2',3',4',5-pentaCB (IU-87), 2,2',4',5,5'-pentaCB(IU-101) and 2,2',3',4',5,5'-hexachlorobiphenyl (hexaCB)(IU-141) were synthesized according to the Cadogan coupling reactions[9]. The $MeSO_2$-PCBs were prepared as described elsewhere[10]. These compounds were confirmed not to show any peaks other than that of individual compounds in the gas chromatogram. 3-Hydroxybenzo[*a*]pyrene was the kind gift of Dr. Hiroo Kinoshita of Kyushu University, Japan. Other chemicals were obtained as commercial reagent grades.

Animal Treatments. Male Wistar rats weighing about 200 g were used in the present study. They were housed in an air-conditioned room with free access to a

commercial chow and tap water.

Rats received an i.p. injection of PCBs or their $MeSO_2$ derivatives (3- and 4-$MeSO_2$-PCBs). For control, the animals were treated with an equvalent volume of the vehicle. All rats were starved for about 18 hr prior to death and then killed by decapitation at 96 hr after the dosing.

Biochemical analyses. Microsomes were prepared according to the procedure described previously[8]. The protein content was determined by the method of Lowry *et al*[11]. Aminopyrine *N*-demethylase and aniline hydroxylase activities were assayed as reported previously[8]. Cytochromes P-450 and b_5 contents were estimated according to the method of Omura and Sato[12,13]. 7-Ethoxycoumarine *O*-deethylase and benzo[*a*]pyrene hydroxylase activities were determined by the method of Guengerich[14], and Nebert and Gelboin[15], respectivelly.

Separation and Identification of Metabolites. The analysis of sulfur-containing metabolites of four PCB congeners in blood and feces was based on the method described by Bergman *et al*[4].

Determination of PCBs and their $MeSO_2$-PCBs in blood and tissues. Preparation of gas chromatography(GC) samples from the blood and tissues was carried out by the method of Bergman *et al*[4]. with some modification. The sample was submitted to GC which was performed on a Shimadzu GC-8A equipped with an electron capture detector. A glass column of 2.0 m length and 3.2 mm i.d. was used. It contained OV-17 (2%) on Chromosorb W (AW-DMCS)(60-80 mesh). The oven temperature was 240°C. The carrier gas used was nitrogen, and the flow rate was 50 ml/min.

RESULTS AND DISCUSSION

The corresponding 3- and 4-$MeSO_2$-PCB metabolites were detected in the blood, liver, kidney, adipose tissue and feces of rats dosed with 2,3',4',5-tetraCB, 2,2',3',4',5- and 2,2',4',5,5'-pentaCBs and 2,2',3',4',5,5'-hexaCB(342 μmol/kg each). Among the tissues studied, the adipose tissue had the highest concentration of the 3- and 4-$MeSO_2$-PCBs after dosing of every PCBs. 3- and 4-$MeSO_2$-2,3',4',5-tetraCBs remained above 2,3',4',5-tetraCB level in the blood, liver and kidney until 16 days after the administration of 2,3',4',5-tetraCB.

2,3',4',5-tetraCB, 2,2',3',4',5- and 2,2',4',5,5'-pentaCBs and 2,2',3',4',5,5'-hexaCB(342 μmol/kg each) increased the contents of cytochromes P-450 and b_5, and the activities of aminopyrine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase and benzo[*a*]pyrene hydroxylase of rat liver microsomes. A single injection of 3-$MeSO_2$-2,3',4',5-tetraCB(10 μmol/kg), 3-$MeSO_2$-2,2',3',4',5- and 3-$MeSO_2$-2,2',4',5,5'-pentaCBs(0.5 μmol/kg each) and 3-$MeSO_2$-2,2',3',4',5,5'-hexaCB(2 μmol/kg) caused the significant increase in the contents of cytochromes P-450 and b_5, and the activities of aminopyrine *N*-demetylase, 7-ethoxycoumarin *O*-deethylase and benzo[*a*]pyrene hydroxylase. No increase was observed in aniline hydroxylase activity after the administration of four PCBs and their 3-methyl sulfone derivatives. The induction profiles of the hepatic microsomal drug-metabolizing enzymes of rats treated with four PCBs and their 3-$MeSO_2$-PCBs were similar to that of rats treated with phenobarbital(PB) but was different from that of rats treated with 3-methylcholanthrene.

4-$MeSO_2$ isomers, 4-$MeSO_2$-2,3',4',5-tetraCB, 4-$MeSO_2$-2,2',3',4',5- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs and 4-$MeSO_2$-2,2',3',4',5,5'-hexaCB, showed no

Table 1. Effects of Administration of 2,3',4',5-tetraCB, 2,2',3',4',5- and 2,2',4',5,5'-pentaCBs, 2,2'3',4',5,5'-hexaCB and their 3- and 4-$MeSO_2$-PCBs on the Contents of Cytochromes and the Activities of Drug-Metabolizing Enzymes of Liver Microsomes in Rats

Compound	Dose (μmol/kg)	Cytochrome P-450 [a]	Cytochrome b_5 [a]	Aminopyrine *N*-demethylase [b]	Aniline hydroxylase [c]	7-Ethoxy-coumarin *O*-deethylase [d]	Benzo[a]-pyrene hydroxylase [e]
Control		0.98 ± 0.01	0.49 ± 0.01	83.6 ± 1.7	21.4 ± 0.6	1.8 ± 0.0	120.2 ± 4.4
2,3',4',5-tetraCB	342	1.75 ± 0.06 *	0.68 ± 0.01 *	154.3 ± 2.8 *	21.7 ± 0.8	5.3 ± 0.2 *	441.1 ± 9.5 *
3-$MeSO_2$-2,3',4',5-tetraCB	10	1.81 ± 0.04 *	0.61 ± 0.01 *	161.4 ± 4.6 *	21.3 ± 0.7	4.4 ± 0.2 *	320.8 ± 15.3 *
4-$MeSO_2$-2,3',4',5-tetraCB	10	0.94 ± 0.02	0.53 ± 0.01	69.0 ± 1.4 *	21.2 ± 0.8	1.8 ± 0.1	117.2 ± 7.8
2,2',3',4',5-pentaCB	342	1.46 ± 0.04 *	0.62 ± 0.01 *	157.1 ± 2.9 *	20.5 ± 0.6	3.1 ± 0.1 *	283.9 ± 14.3 *
3-$MeSO_2$-2,2',3',4',5-pentaCB	0.5	1.30 ± 0.03 *	0.59 ± 0.01 *	149.7 ± 6.2 *	20.0 ± 0.4	2.5 ± 0.1 *	252.7 ± 11.6 *
4-$MeSO_2$-2,2',3',4',5-pentaCB	0.5	0.98 ± 0.01	0.51 ± 0.01	87.4 ± 2.0	18.3 ± 0.6 *	1.6 ± 0.1	138.9 ± 3.6 *
2,2',4',5,5'-pentaCB	342	1.75 ± 0.03 *	0.59 ± 0.02 *	161.6 ± 3.6 *	20.8 ± 0.8	3.8 ± 0.3 *	287.6 ± 10.2 *
3-$MeSO_2$-2,2',4',5,5'-pentaCB	0.5	1.50 ± 0.05 *	0.60 ± 0.00 *	151.4 ± 3.8 *	22.8 ± 0.5	3.4 ± 0.2 *	260.1 ± 8.7 *
4-$MeSO_2$-2,2',4',5,5'-pentaCB	0.5	1.01 ± 0.01	0.51 ± 0.01	83.3 ± 3.5	20.3 ± 0.6	2.2 ± 0.2	106.8 ± 5.3
2,2',3',4',5,5'-hexaCB	342	1.33 ± 0.02 *	0.57 ± 0.01 *	142.4 ± 2.8 *	18.5 ± 0.4 *	2.7 ± 0.2 *	251.2 ± 11.5 *
3-$MeSO_2$-2,2',3',4',5,5'-hexaCB	2	1.68 ± 0.04 *	0.59 ± 0.02 *	164.0 ± 4.5 *	20.9 ± 0.2	3.3 ± 0.1 *	308.1 ± 11.3 *
4-$MeSO_2$-2,2',3',4',5,5'-hexaCB	2	1.10 ± 0.04 *	0.56 ± 0.02 *	93.5 ± 5.1	19.6 ± 0.7	1.8 ± 0.1	129.5 ± 8.2

Rats were given i.p. PCBs or their methyl sulfone derivatives and killed 96 hr after the administration.
Results are expressed as the mean ± S.E. for 4-8 animals.
a) nmol/mg protein, b) nmol HCHO/mg protein/20 min, c) nmol *p*-aminophenol/mg protein/20 min,
d) nmol 7-hydroxycoumarin/mg protein/min, e) pmol 3-hydroxybenzo[a]pyrene/mg protein/min.
* Significantly different from the control, $P<0.05$.

significant increasing effect on the contents of cytochromes P-450 and b_5, and the activities of aminopyrine *N*-demethylase, aniline hydroxylase, 7-ethoxycoumarin *O*-deethylase and benzo[a]pyrene hydroxylase with a few exceptions(Table 1).

In conclusion, these results suggest that 3-$MeSO_2$-PCB congeners studied have strong PB-type inductive effects on hepatic microsomal drug-metabolizing enzymes, while 4-$MeSO_2$-PCB congeners studied have little effect.

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