Evidence that 3-Methylsulfonyl Metabolite of 2,2',4,5,5'-pentachlorobiphenyl is Causative Substance of Induction of Hepatic Microsomal Drug-Metabolizing Enzymes by the Parent Compound in Rats

Yoshihisa Kato[A], Koichi Haraguchi[B], Koichi Tomiyasu[A], Hiroyuki Saito[A], Yoshito Masuda[B] and Ryohei Kimura[A]

[A]School of Pharmaceutical Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422, Japan

[B]Daiichi College of Pharmaceutical Sciences, 22-1, Tamagawa-cho, Minami-ku, Fukuoka 815, Japan

1. Introduction

Methylsulfonyl ($MeSO_2$) derivatives of polychlorinated biphenyls (PCBs) have been identified in different tissues of mammals from the Canadian and Swedish environment[1,2] and of humans from the Japanese and Swedish environment.[3, 4] In the previous presentation, we showed that 3-$MeSO_2$ metabolites derived from nine PCB congeners were phenobarbital (PB)-type inducers of microsomal drug-metabolizing enzymes, and 3-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl (3-$MeSO_2$-2,2',4',5,5'-pentaCB) was an especially potent inducer.

In our preceding paper,[5] we reported that the administration of dichlorophenyl methyl sulfones, metabolites of *m*-dichlorobenzene (*m*-DCB), resulted in an effective induction of hepatic microsomal drug-metabolizing enzymes in rats. We also showed that the inducing effect of *m*-DCB on hepatic microsomal drug-metabolizing enzymes was not attributable to the action of *m*-DCB per se but to that of its $MeSO_2$ metabolites.[6]

The present study was designed to investigate the effect of 3-methyl sulfone derived from 2,2',4,5,5'-pentaCB on the drug-metabolizing enzyme induction by the parent compound.

2. Materials and methods

Chemicals. 2,2',4,5,5'-pentaCB (IU-101), 2,3',4,4',5-pentaCB (IU-118) and 3,3',4,4'-tetrachlorobiphenyl (3,3',4,4'-tetraCB) (IU-77) were synthesized according to the Cadogan coupling reactions.[7] The $MeSO_2$-PCBs were prepared as described elsewhere.[8] The purity of these compounds was > 99% when analyzed by gas chromatography (GC). 3-Hydroxybenzo[*a*]pyrene was the kind gift of Prof. Nadao Kinoshita of Kyushu University, Japan. The microsomal P450 standards and antibodies against purified cytochrome P450s were obtained from Daiichi Pure Chemicals Co. Ltd. (Tokyo, Japan). Other chemicals were obtained as commercial reagent grade.

Animal treatments. The rats used and their treatments were described in the previous presentation.

For the bile duct-cannulated treatment, the common bile duct was cannulated *in situ* with polyethylene tubing (PE-10, Clay Adams) under ether anesthesia, and the rats were held as described by Aimoto *et al.*[9] For the antibiotic treatment, rats were given an oral does of 25 mg/kg of kanamycin sulfate and of lincomycin hydrochloride twice daily for 9 days.

Biochemical analyses. Microsomes were prepared according to the procedure described previously.[10] 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.[5]

Cytochromes P450 and b_5 contents were estimated according to the method of Omura and Sato.[12, 13] Benzo[*a*]pyrene hydroxylase activity was determined by the method of Nebert and Gelboin.[14] The immunoblotting and immunochemical quantitation were performed as described by Imaoka *et al.*[15]

Determination of $MeSO_2$-PCBs in liver. Preparation of GC samples from the liver was carried out by the method of Bergman *et al.*[16] 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 ℃. Carrier gas was nitrogen at a flow rate of 50 ml/min.

3. Results and discussion

The ability of 3-$MeSO_2$-2,2',4',5,5'-pentaCB, which was one of the major $MeSO_2$ metabolites of PCBs present in human milk and seal blubber,[1, 4] at a dose of 2 μmol/kg to increase the content of cytochrome P450 and the activities of aminopyrine *N*-demethylase and aniline hydroxylase was higher than those of parent compound (2,2',4,5,5'-pentaCB), mono-*ortho* coplanar PCB (2,3',4,4',5-pentaCB) and coplanar PCB (3,3',4,4'-tetraCB) at a dose of 342, 80 and 80 μmol/kg, respectively.

CYP2B1 and CYP2B2 were induced 4-9-fold of control by 3-$MeSO_2$-2,2',4',5,5'-pentaCB. CYP3A2 and CYP2C6 were induced 1.5 and 3-fold with this methyl sulfone, respectively. None of these forms was induced by the treatment with 4-$MeSO_2$-isomers. 3-$MeSO_2$- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs had no inducing effect on CYP1A1 and CYP1A2.

We investigated the relation between hepatic concentration of 3-$MeSO_2$-2,2',4',5,5'-pentaCB and increasing effect on the drug-metabolizing enzymes after administration of parent compound (2,2',4,5,5'-pentaCB) and its 3-$MeSO_2$ metabolite. The hepatic concentration of 3-$MeSO_2$-2,2',4',5,5'-pentaCB for 6 weeks after administration of 2,2',4,5,5'-pentaCB (342 μmol/kg) was similar to that after the administration of 0.5 μmol/kg of 3-$MeSO_2$-2,2',4',5,5'-pentaCB. A single injection of 2,2',4,5,5'-pentaCB (342 μmol/kg) or 3-$MeSO_2$-2,2',4',5,5'-pentaCB (0.5 μmol/kg) caused the significant increase in the contents of cytochromes P450 and b_5 and the activities of aminopyrine *N*-demetylase and benzo[*a*]pyrene hydroxylase, and these elevated enzyme activities maintained for 6 weeks after. In this case, the extents of increases in the cytochrome contents and the enzyme activities observed for both compounds were almost similar. The inducing effect of both compounds nearly coincided to a time-dependent increase in the hepatic concentration of 3-$MeSO_2$-2,2',4',5,5'-pentaCB. It is strongly suggest that the 3-$MeSO_2$ metabolite contributes to microsomal induction of drug-metabolizing enzymes by 2,2',4,5,5'-pentaCB.

When 2,2',4,5,5'-pentaCB was injected i.p. into bile duct-cannulated rats, no 3- and 4-methyl sulfones were detected in liver. In the antibiotic-treated rats dosed with 2,2',4,5,5'-pentaCB, the metabolite concentrations in the liver markedly decreased (Table 1). These findings suggest that the formation of 3- and 4-methylsulfonyl metabolites from 2,2',4,5,5'-pentaCB depends largely upon the metabolism of some precursor(s) excreted in the bile by intestinal microflora. The increasing effects of 2,2',4,5,5'-pentaCB administration on the content of cytochrome P450 and the activity of aminopyrine metabolizing enzyme in hepatic microsomes were scarcely observed in the bile duct-cannulated rats, in which the drug-metabolizing enzymes were able to be induced by PB treatment. In the antibiotic-treated rats, 2,2',4,5,5'-pentaCB showed no significant effect on the cytochrome P450 content, and less increase in the aminopyrine metabolizing enzyme activity than that in the intact rats (Table 2).

In conclusion, these findings provide an evidence that the induction of drug-metabolizing enzymes by 2,2',4,5,5'-pentaCB is not due to the action of 2,2',4,5,5'-pentaCB but is due to its 3-mehylsulfonyl metabolite.

Table 1. Concentrations of methyl sulfones in liver after administration of 2,2',4,5,5'-pentaCB in bile duct-cannulated and antibiotic-treated rats

	Methyl sulfone concentration (nmol/g)	
Animal	3-$MeSO_2$-2,2',4',5,5'-pentaCB	4-$MeSO_2$-2,2',4',5,5'-pentaCB
Intact	0.30 ± 0.07	0.55 ± 0.16
Bile duct-cannulated	n.d.[a)]	n.d.
Antibiotic-treated	0.12 ± 0.02*	0.27 ± 0.05*

Rats, which previously received the bile duct cannulation or were treated with the antibiotics as described under Materials and methods, were given i.p. 2,2',4,5,5'-pentaCB (342 μmol/kg), and killed 96 hr after the administration. Results are expressed as the mean ± S.E. for 3-5 animals.
a) Not detected.
* $p<0.05$, significantly different from intact group.

Table 2. Effects of 2,2',4,5,5'-pentaCB on the contents of cytochromes and the activities of drug-metabolizing enzymes in liver microsomes of bile duct-cannulated and antibiotic-treated rats

Enzyme	Animal	Control	2,2',4,5,5'-pentaCB	Effect (%)
Cytochrome P450[a)]	Intact	0.90 ± 0.05	1.55 ± 0.18†	+72
	Bile duct-cannulated	0.90 ± 0.10	0.83 ± 0.10	−8
	Antibiotic-treated	1.03 ± 0.11	1.32 ± 0.09	+28
Cytochrome b_5[a)]	Intact	0.48 ± 0.03	0.59 ± 0.03	+23
	Bile duct-cannulated	0.49 ± 0.06	0.43 ± 0.05	−12
	Antibiotic-treated	0.53 ± 0.07	0.67 ± 0.06	+26
Aminopyrine *N*-demethylase[b)]	Intact	96.7 ± 6.1	180.4 ± 16.9†	+87
	Bile duct-cannulated	50.9 ± 3.7*	55.4 ± 6.3	+9
	Antibiotic-treated	90.4 ± 6.0	145.2 ± 2.6†	+61
Aniline hydroxylase[c)]	Intact	19.2 ± 1.5	21.9 ± 0.8	+14
	Bile duct-cannulated	18.2 ± 2.4	14.3 ± 1.9	−21
	Antibiotic-treated	21.2 ± 3.5	27.2 ± 1.7	+28

The experimental conditions were the same as described in the legend to Table 1.
Results are expressed as the mean ± S.E. for 3-5 animals.
a) nmol/mg protein. b) nmol HCHO/mg protein/20 min.
c) nmol *p*-aminophenol/mg protein/20 min.
* $p<0.05$, significantly different from intact control.
† $p<0.05$, significantly different from control.

4. References

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