THE S-OXIDATION OF PCB METHYL SULFOXIDE TO PCB METHYL SULFONE WITH ANIMAL LIVER MICROSOMES AND HUMAN CYTOCHROME P450

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Abstract
We investigated the S-oxidation of 3- and 4-MeSO-2,2',5,5'-tetrachlorobiphenyl (CB52) to their $MeSO_2$-CB52, to clarify the enzymes which are concerned in the S-oxidation of MeSO-PCBs to $MeSO_2$-PCBs, using animal liver microsomes such as rats, guinea pigs and mice, human cytochrome P450 (P450) and human flavin-containing monooxygenase (FMO). 3- and 4-$MeSO_2$-CB52 were formed by untreated liver microsomes of animals with high activity for the S-oxidation. PB pretreatment increased the production of these $MeSO_2$-CB52 metabolites in rats and mice. CYP2B6, CYP2C9*1 and CYP2C19 were concerned in the S-oxidation step of MeSO-CB52 to $MeSO_2$-CB52 with high catalyzing activity, while CYP1A1, CYP1A2, CYP2A6 and CYP3A4 catalyzed the S-oxidation with low catalyzing activity. In contrast, FMO isoforms did not form any amounts of $MeSO_2$-CB52. These results demonstrate that P450 isoforms, especially, CYP2B6, CYP2C9*1 and CYP2C19 which are inducible by Phenobarbital type P450 inducer, participate in the S-oxidation of MeSO-PCBs to $MeSO_2$-PCBs. These findings also suggest that FMO isoforms are not involved in the S-oxidation of MeSO-PCBs to $MeSO_2$-PCBs.

Introduction
The methylsulfonyl PCBs ($MeSO_2$-PCBs), one of the PCB metabolites, are persistent environmental contaminants as well as PCBs. The $MeSO_2$-PCBs were first identified in the blubber from Baltic seal [1] and also were detected as metabolites in the feces of mice administered with 2,2',5,5'-tetrachlorobiphenyl (CB52) [2]. Nowadays, $MeSO_2$-PCBs can be found in the various tissues such as liver and lung of wild mammals, e.g. seals, whales and polar bears, including humans. The chemical structures of main retainable $MeSO_2$-PCBs in biota are substituted with $MeSO_2$-group at 3- or 4-positions of 2,5-dichloro- or 2,5,6-trichloro-subsutituted type PCBs. For example, 3- and 4-$MeSO_2$-CB52 are typical congeners retainable in biota.
Certain $MeSO_2$-PCBs, especially 3-$MeSO_2$-2,5-dichloro-substituted type PCBs, show high accumulating properties in the liver of rats and more potent Phenobarbital (PB)-like inducing abilities of hepatic drug-metabolizing enzymes than their parent PCBs in rats [3]. $MeSO_2$-PCBs were also reported to inhibit the cell-cell communication in rat liver cells [4]. Recently, some 3- and 4-$MeSO_2$-PCBs are known to decrease the level of serum thyroid hormone [5]. Therefore, certain $MeSO_2$-PCBs can be considered to be one of the endocrine disrupters.
The formation mechanism of $MeSO_2$-PCBs is suggested by Bakke *et al.* as follows [6]. PCBs undergo cytochrome P450-mediated metabolism forming an arene oxide intermediate, which results in both polychlorobiphenylols and glutathione conjugates in the liver. The glutathione conjugates excreted to the intestinal tract are transformed to thiols after several degradation steps by a series of enzymatic reaction. Then, thiols are transformed to methylthio-PCBs (MeS-PCBs) by S-methylation in small intestine. After reabsorption of MeS-PCBs in the liver, MeS-PCBs are oxidized to form PCB methylsulfoxide (MeSO-PCBs) and $MeSO_2$-PCBs by subsequent S-oxidation. In general, two enzymes, cytochrome P450 (P450) and flavin-containing monooxygenase (FMO) are involved in the S-oxidation of S-containing xenobiotics. From the examination using MeS-2,3',4',5-tetraCB (CB70), Koga *et al*. have reported that P450 is more responsible for this consecutive S-oxidation than FMO [7]. However, it is difficult to evaluate the enzymes or individual P450 isoforms which participate in the each S-oxidation step of MeS→MeSO and MeSO→$MeSO_2$, because both MeSO-CB70 and $MeSO_2$-CB70 are formed from MeS-CB70 at once in their study.
In the present study, therefore, we examined the enzymes which were concerned in the S-oxidation of MeSO-PCBs to $MeSO_2$-PCBs step. 3- and 4-MeSO-CB52 were used for this purpose and were incubated with animal liver microsomes such as rat, guinea pig and mice, human P450 and human FMO isoforms. We also

studied the effects of inducers of P450 such as PB and 3-methylchoranthrene (3-MC).

Materials and Methods

Chemicals: A mixture of 3- and 4-MeSO-CB52, 99% pure, was synthesized from the mixture of 3-, 4- and 6-MeS-CB52, which was prepared by Cadogan reaction from 2,5-dichloroaniline and 2,5-dichloroanisole, with H_2O_2 at room temperature. 6-MeSO-CB52 formed together was removed from 3- and 4-MeSO-CB52 by silica gel column chromatography. The composition ratio of 3- and 4-MeSO-CB52 in the mixture was 2:5 by gas chromatography with electron capture detection. 3- and 4-$MeSO_2$-CB52 were synthesized as described previously [8].

Animal treatments: Nine male Wistar rats (6 weeks old, body wt. about 160 g), fifteen male BALB/cA Jcl mice (8 weeks old, body wt. about 28 g) and nine male Herthley guinea pigs (body wt. about 250 g) were used and animals were divided into three groups, untreated, PB- and 3-MC-pretreated groups. PB and 3-MC were dissolved in saline and corn oil, and injected intraperitoneally at a dose of 100 and 20 mg/kg/day for 3 days, respectively. Animals were sacrificed 24 hours after the last injection of each P450 inducer and their livers were removed. Liver microsomes (protein content; 13.6~18.2 mg protein/mL) were prepared by a conventional centrifugation method.

Human P450 and human FMO: CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1 and CYP3A4 (cytochrome P450 content; 39~200 pmole/mg protein), which are expressed from an each transfected P450 cDNA in the cell line, were purchased from BD Gentest. FMO1, FMO3 and FMO5 (protein content; 5.0 mg/mL), which are expressed from each human FMO cDNA using a baculovirus expression system, were purchased from BD Gentest.

Metabolism of 3- and 4-MeSO-CB52; The mixture of 3- and 4-MeSO-CB52 dissolved in DMSO (final conc. 10 μM) was incubated for 5~20 min. at 37℃ with NADPH-generating system, $MgCl_2$ and animal liver microsomes, human P450 or human FMO in 100 mM HEPES buffer (pH 7.4) under aerobic conditions (total volume; 1.0 mL). After the extraction with dichloromethane and dehydration with sodium sulfate (anhydrous), the extracts were re-dissolved in *n*-hexane and were analyzed for unchanged MeSO-CB52 and $MeSO_2$-CB52 metabolites on GC (electron capture detector: ECD) and GC/MS.

Instruments; GC analyses were performed on a GC-17A (Shimadzu) equipped with an ECD under the conditions as follows: column, DB-5 fused silica capillary column (30 m×0.25 mm i.d.); carrier gas, N_2; temperature program, 70℃(2 min)-20℃/min-220℃(1min)-3℃/min-280℃(20 min); injection port temp., 250℃;detector temp., 330℃. GC/MS was carried out on a JMS-AX505W (JEOL) apparatus in EI mode.. carrier gas, helium; column conditions and temperature program are analogous to those described for GC. The quantification of the $MeSO_2$-CB52 metabolites was performed on a GC (ECD) using the calibration curves of 3- and 4-$MeSO_2$-CB70.

Results and Discussion

S-oxidation with animal liver microsomes: 3- and 4-$MeSO_2$-CB52 were formed from the mixture of 3- and 4-MeSO-CB52 by incubation with untreated microsomes of rats, guinea pigs and mice. The turnover rates of 3- and 4-$MeSO_2$-CB52 in untreated microsomes were 76 ~ 90 and 139 ~ 216 pmol/min/mg protein, respectively, showing high activity for the S-oxidation of MeSO-PCBs to $MeSO_2$-PCBs and no marked differences of $MeSO_2$-CB52 formation among animals tested (Table 1). However, differences of stereo-specific formation of 3- and 4-$MeSO_2$-CB52 by P450 inducers were observed among animal species. In rats, 4-$MeSO_2$-CB52 was increased by PB treatment, being 1.8-fold of control. PB treatment also increased the formation of both 3- and 4-$MeSO_2$-CB52, 1.6- and 2.2-fold of control, respectively, in mice, while no effects of P450 inducers were observed in guinea pigs.

S-oxidation with human P450 and human FMO: P450 control microsomes did not give any amounts of 3- and 4-$MeSO_2$-CB52, but, among the ten P450 isoforms studied, CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9*1, CYP2C19 and CYP3A4 showed the S-oxidation ability of 3- and 4-MeSO-CB52 to their $MeSO_2$-CB52 with significant difference in magnitude of the activity (Fig. 1). Especially, CYP2B6, CYP2C9*1 and CYP2C19 exhibited significantly high activity for the S-oxidation of MeSO-CB52 to $MeSO_2$-CB52. CYP1A1, CYP1A2, CYP2A6 and CYP3A4 showed slight catalyzing activity for this S-oxidation. In addition, isomer specific formation of $MeSO_2$-CB52 was observed in some P450 isoforms. For example, CYP2C9*1 selectively showed much higher catalyzing activity for the formation of 4-$MeSO_2$-CB52. On the contrary, CYP2B6 and CYP1A2

Table 1 Effect of P450 inducers on the S-oxidation of 3-and 4-MeSO-CB52 with liver microsomes of rats , guinea pigs and mice

Treatment	metabolite formed (pmol/min/mg protein)			$4\text{-MeSO}_2/3\text{-MeSO}_2$
	3-MeSO_2	4-MeSO_2	Total	
Rats				
Control	89.5±5.8 (1.0)	179.5±24.6 (1.0)	269.1±29.1 (1.0)	2.0
PB	74.2±6.2 (0.8)	331.4±42.1 (1.8)	405.6±45.0 (1.5)	4.5
3-MC	75.3±15.5 (0.8)	135.9±25.1 (0.8)	211.2±40.0 (0.8)	1.8
Guinea pigs				
Control	75.6±7.7 (1.0)	215.7±24.7 (1.0)	291.3±32.3 (1.0)	2.9
PB	88.6±6.8 (1.2)	218.6±20.0 (1.0)	307.2±26.7 (1.1)	2.5
3-MC	68.3±10.2 (0.9)	184.9±29.8 (0.9)	253.2±40.0 (0.9)	2.7
Mice				
Control	89.1±10.7 (1.0)	138.9±11.1 (1.0)	228.1±21.0 (1.0)	1.6
PB	146.6±20.1 (1.6)	310.8±26.0 (2.2)	457.5±45.8 (2.0)	2.1
3-MC	101.8±9.61 (1.1)	168.8±14.8 (1.2)	270.6±23.9 (1.2)	1.5

Each value represents the mean ± S.D.of four determinations and those in parentheses are the relative ratio to the control

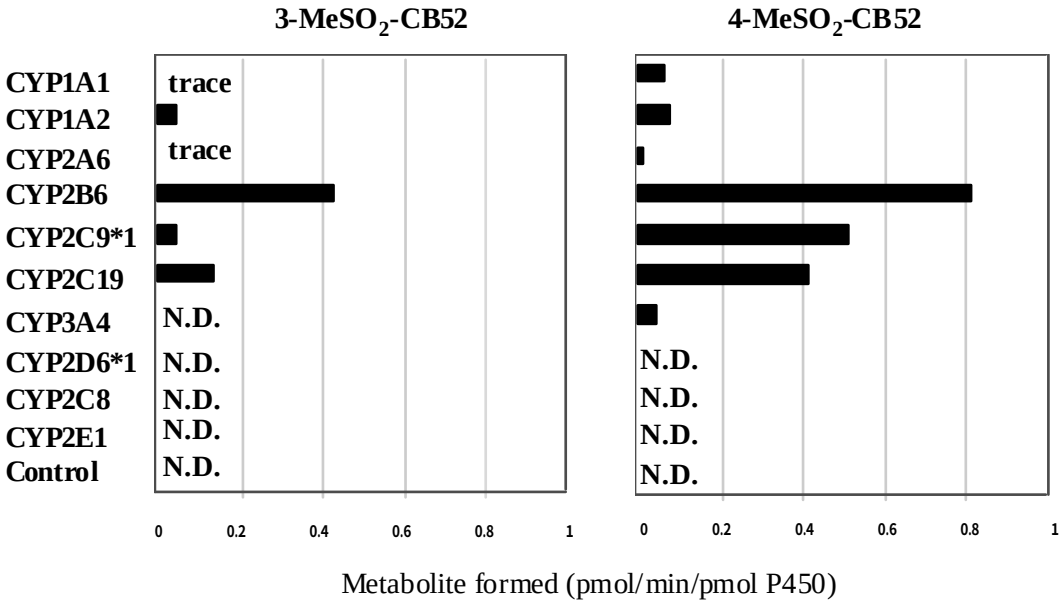

Fig.1 S-oxidation of 3-and 4-MeSO-CB52 with human cytochrome P450(CYP) isoforms. Each value represents mean of two determinations. N.D.; not detected, trace; 0.005 ~ 0.01 pmol/min/pmol P450

showed relatively high ratio of 3-$MeSO_2$-CB52 formation compared to 4-$MeSO_2$-CB52 formation, although the turnover rate of 3-$MeSO_2$-CB52 by CYP1A2 was very small. In contrast, CYP2C8, CYP2D6*1 and CYP2E1 did not show any catalyzing activity for this S-oxidation step (Fig.1). Furthermore, FMO1, FMO3 and FMO5 exhibited no activity for the S-oxidation of MeSO-CB52 to $MeSO_2$-CB52 (data not shown).

In conclusion, these results demonstrate that a lot of P450 isoforms, but not FMO isoforms, are concerned in the S-oxidation step of MeSO-CB52 to $MeSO_2$-CB52. These findings also suggest that CYP2B6, CYP2C9*1 and CYP2C19, which are inducible by PB type inducer, have intense catalyzing activity for this S-oxidation, however, CYP1A1 and CYP1A2, which are inducible by 3-MC type inducer, have slight catalyzing activity. These results quite agree with the findings obtained from the study with animal liver microsomes pretreated by PB and 3-MC in rats and mice. Based on these results, further studies on the S-oxidation step of 3- and 4-MeS-CB52 to 3- and 4-MeSO-CB52 are necessary to evaluate the enzymes which participate in the S-oxidation of a series of S-containing metabolites of PCBs.

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