THE *S*-OXIDATION OF METHYLTHIO-2,3',4',5-TETRACHLORO-BIPHENYL WITH RAT LIVER MICROSOMES

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Introduction

Recently, the toxicological aspects of the sulfur (S)-containing metabolites of polychlorinated biphenyls (PCBs) as well as their hydroxylated metabolites have attracted considerable attention. Kato et al. have shown that certain 3-methysulfone ($MeSO_2$)-PCBs possess more potent phenobarbital (PB)-like inducing ability of liver microsomal monooxygenases than their parent PCBs[1)] and that some $MeSO_2$-PCBs decrease the level of thyroid hormone in blood[2)].

The formation of $MeSO_2$-PCBs from their parent PCBs are thought to proceed via a series of enzymatic reactions such as the monooxygenation of PCBs and glutathione conjugation in the liver, the C-S cleavage and S-methylation in the small intestine and subsequent S-oxidation in the liver[3-5)]. However, the enzyme systems catalyzing the S-oxidation of methylthio (MeS)-PCB to $MeSO_2$-metabolite remained to be elucidated. So far, two enzymes, cytochrome P450 (P450)[6)] and flavin-containing monooxygenase (FMO)[7)], have been known as an oxidizing enzyme for the S atom in various S-containing compounds. Therefore, this study was planned to determine which enzyme is more important, P450 and FMO, in the S-oxidation of PCBs and we described the effects of various inducers and inhibitors of P450 and FMO on the formation of MeSO- and $MeSO_2$-metabolites from 3- and 4-MeS-2,3',4',5-tetrachlorobiphenyl (CB70) by rat liver microsomes.

Materials and Methods

3- and 4-MeS-CB70 were synthesized from 2,5-dichlorothioanisole and 3,4-dichloro-aniline and both 3- and 4-$MeSO_2$-CB70 were prepared as described elsewhere[8)].

Nine male rats (body wt. about 150 g) were used and divided to three groups, untreated, PB- and 3-methylcholanthrene (MC)-pretreated groups. PB and MC were dissolved in saline and corn oil, and injected intraperitoneally at a dose of 80 and 20 mg/kg/day for 3 days, respectively. Rats were killed the next day after the last injection of each P450 inducer and their livers were removed. Liver microsomes were prepared by a conventional centrifugation method.

The S-oxidation of 3- and 4-MeS-CB70 was assayed for 1 hr at 37°C using an incubation system consisting of MeS-CB70, NADPH-generating system and rat liver microsomes under aerobic conditions. After extraction with organic solvents such as chloroform-methanol (2:1) and n-hexane, MeSO- and $MeSO_2$-metabolites were analyzed using a gas chromatograph HP5890 Series II equipped with an electron capture detector

(ECD) under the conditions as follows: column, DB-1 capillary column (30 m x 0.25 mm i.d., 0.33 μm thickness); carrier gas, N_2 (1 ml/min); column temp., 230 °C; injection port temp., 250 °C; detector temp., 250 °C.

Results

Effect of P450 inducers on the S-oxidation of MeS-CB70: When the mixture of 3- and 4- MeS-CB70 was incubated with NADPH and rat liver microsomes at 37 °C for 60 min, four products were found in ECD/GC (Fig. 1). From their mass spectral data, they corresponded to two metabolites each and were found to be 3- and 4-MeSO-, 3- and 4-$MeSO_2$-CB70 with the retention times of 31.71 min, 33.08 min, 36.04 min and 38.64 min, respectively.

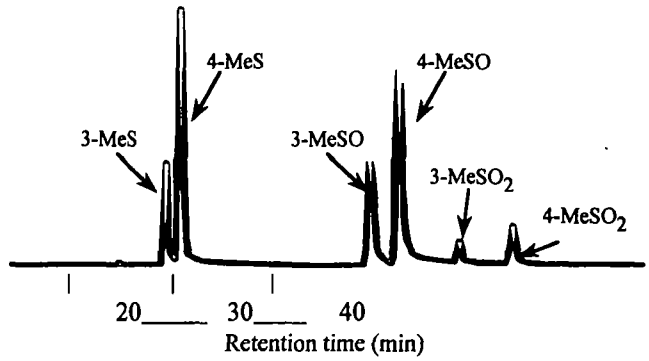


Fig. 1. Gas chromatogram of S-containing metabolites of 3- and 4-MeS-CB70 formed by rat liver microsomes

Table 1 showed the effect of two typical P450 inducers, PB and MC, on the formation of S-containing metabolites from 3- and 4-MeS-CB70. Although we reported previously that the liver microsomes of untreated rats showed no catalytic activity for the hydroxylation of some tetrachlorobiphenyls (TCBs) such as 3,3',4,4'-TCB (CB77), 2,2',5,5'-TCB (CB52) and CB70[9-11], much higher activity for the formation of both MeSO- and $MeSO_2$-CB70 from MeS-CB70 was observed in untreated microsomes. Both PB and MC increased the sum of MeSO- and $MeSO_2$-metabolites formed to about 2-fold of control. Especially, PB treatment accelerated the S-oxidation step from 3-MeSO-CB70 to 3-$MeSO_2$-metabolite, whereas MC increased the S-oxidation of 4-MeSO-CB70 to 4-$MeSO_2$-metabolite. In addition, the S-oxidation from 3- and 4-MeS-CB70 to their $MeSO_2$-metabolites proceeded more rapidly at pH 7.4 which is optimal for P450 than at pH 8.3 which is the optimal pH for FMO (data not shown).

Effect of inhibitors of P450 and FMO on formation of $MeSO_2$-CB70: Two potent P450 inhibitors, SKF-525A and N-benzylimidazole, at a concentration of 0.1 and 1.0 mM, respectively, almost completely inhibited the formation of 3- and 4-$MeSO_2$-metabolite from 3- and 4-MeS- CB70 (data not shown). Liver FMO is completely inactivated by the incubation at 50 °C for 1 min[12]. As a result, preincubation of rat liver microsomes at 50 °C for 1 min resulted in only

Table 1 Effect of P450 inducers on the S-oxidation of 3- and 4-MeS-CB70 with rat liver microsomes

Treatment	Metabolite formed (pmol/min/mg protein)			
	MeSO	$MeSO_2$	Total	$MeSO_2$/MeSO
3-MeS-CB70				
Control	46.2 (1.0)	30.8 (1.0)	77.0 (1.0)	0.67
PB	73.4 (1.6)	69.4 (2.3)	142.8 (1.9)	0.95
MC	147.0 (3.2)	47.6 (1.5)	194.6 (2.5)	0.32
4-MeS-CB70				
Control	72.3 (1.0)	57.4 (1.0)	129.7 (1.0)	0.79
PB	102.8 (1.4)	93.8 (1.6)	196.6 (1.5)	0.91
MC	89.9 (1.2)	138.4 (2.4)	228.3 (1.8)	1.54

Each value represents mean of two determinations and values in parentheses are the relative ratio to control microsomes.

about10% inhibition of the formation of $MeSO_2$-metabolite (data not shown). Addition of methimazole, a famous inhibitor of FMO, at a concentration of 0.45 mM showed only 30% inhibition of the formation of $MeSO_2$-metabolite. These results suggest that FMO involved in the S-oxidation of MeS-CB70 to much less extent than P450 did.

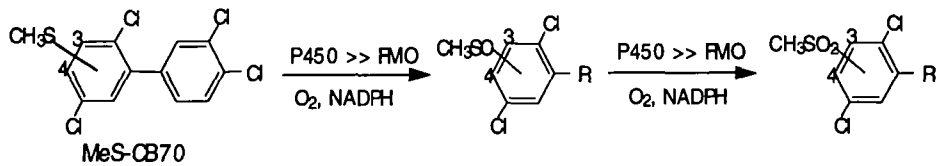
Effect of antiserum against rat CYP1A1 on formation of $MeSO_2$-CB70: After 30 min- preincubation of MC-microsomes with antiserum against rat CYP1A1 at room temperature, the S-oxidation of MeS-CB70 was initiated by addition of NADPH. Addition of antiserum (300 μl) resulted in about 50% and 70% inhibition of control serum in the formation of 3- and 4-$MeSO_2$-metabolites, respectively.

Discussion

We have demonstrated in this study that the treatment of PB and MC increased the S-oxidation activity of 3- and 4-MeS-CB70 to their MeSO- and $MeSO_2$-metabolites and two P450 inhibitors strongly inhibited the S-oxidation. These facts suggest that P450 rather than FMO mainly involved in two S-oxidation steps from MeS-CB70 to $MeSO_2$-CB70 (Fig. 2). The S-oxidation activity for MeS-CB70 in the liver of untreated rats was much higher than the activity for hydroxylation of CB70 as reported previously[11]. If MeS-PCBs are reabsorbed from the small intestine and redistributed to the liver, it would be more easily oxidized to their MeSO- and $MeSO_2$-PCBs.

Our previous study reported that the relationship between the structure of PCB and the catalytic activity of P450 isoforms can be seen in the hydroxylation of PCB[9,11]. For example, a MC-inducible P450, CYP1A1, hydroxylates selectively the coplanar PCBs such as CB77 and CB80 (3,3',5,5'-TCB), whereas a PB-inducible P450, CYP2B1, mainly metabolizes non-coplanar PCB such as CB52 and CB70. In this study, a MC-inducible isoform in rat liver appears to prefer 4-MeS-CB70 to 3-MeS-CB70 in the S-oxidation. In contrast, a PB-inducible isoform likes 3-MeS-CB70. Although the immunochemical study using antiserum against rat CYP1A1 indicated that MC-inducible isoforms of P450, CYP1A1 and/or CYP1A2, are important as an S-oxidase of MeS-PCB in MC-microsomes,

it is evident that another isoforms of P450 also have the S-oxidation activity to some extent.



MeS-CB70

Fig. 2. Postulated metabolic pathways of MeS-CB70 in rat liver

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References

1. Kato, Y., Haraguchi, K., Kazashima, M., Yamada, S. and Kimura, R. (1995) Chem.-Biol. Interaction 95, 257-268.
2. Kato, Y., Haraguchi, K., Shibahara, T., Masuda, Y. and Kimura, R. (1998) Arch. Toxicol. 72, 541-544.
3. Bakke, J. E., Bergman, A. and Larsen, G. L. (1982) Science 217, 645-647.
4. Bakke, J. E., Feil, V. J. and Bergman, A. (1983) Xenobiotica 13, 555-564.
5. Bakke, J. E., Bergman, A. L., Brandt, I., Darnerud, P. O. and Struble, C. (1983) Xenobiotica 13, 597-605.
6. Kashiyama, E., Yokoi, T., Odomi, M., Funae, Y., Inoue, K. and Kamataki, T. (1997) Drug Metab. Dispos. 6, 716-724.
7. Cashman, J. R. (1995) Chem. Res. Toxicol. 8, 165-181.
8. Haraguchi, K., Kuroki, H. and Masuda Y. (1987) J. Agric. Food Chem. 35, 178-182.
9. Ishida, C., Koga, N., Hanioka, N., Saeki, H. K. and Yoshimura, H. (1991) J. Pharmacobio-Dyn. 14, 276-284.
10. Koga N., Kikuichi-Nishimura N. and Yoshimura H. (1995) *Biol. Pharm. Bull.* 18, 705-710.
11. Koga N., Kikuichi N., Kanamaru T., Kuroki H., Matsusue K., Ishida C., Ariyoshi N., Oguri K. and Yoshimura H. (1998) Chemosphere 37, 1895-1904.
12. Grothusen, A., Hardt, J., Brautigam, L., Lang, D. and Bocker, R. (1996) Arch. Toxicol. 71, 64-71.