Metabolism of 2,2',5,5'-Tetrachlorobiphenyl by Rabbit Liver Microsomes

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Introduction

2,2',5,5'-Tetrachlorobiphenyl (TCB), a major component of Kanechlor 400, possesses phenobarbital(PB)-type inducing ability of liver microsomal drug metabolizing enzymes [1] and is metabolized mainly to 3-hydroxy-2,2',5,5'-TCB and to methylsulphone-metabolites as minor metabolites [2]. We have demonstrated the regioselectivity in the hydroxylation of 2,2',5,5'-TCB by cytochrome P450 (P450) from rats, guinea pigs and hamsters. Namely, rat CYP2B1, hamster P450HPB-1 and guinea pig P450GP-1(CYP2B18) catalyze only 3-hydroxylation [3-5], but hamster CYP2A8 catalyzes 4-hydroxylation [6]. On the other hand, although lots of P450 isoforms have been purified from rabbit liver, their catalytic activity for the hydroxylation of PCB has not been elucidated yet

Gardner *et al.* found a small amount of *trans*-3,4-dihydro-3,4-dihydroxy-2,2',5,5'-TCB in addition to 3- and 4-hydroxy-2,2',5,5'-TCB in rabbit urine [7]. This was the first report suggesting the formation of 3,4-epoxide as an intermediate in PCB metabolism. However, we have never detected any urinary metabolites of TCB including 2,2',5,5'-TCB, 3,3',4,4'-TCB and 2,3',4',5-TCB in rats and hamsters. In addition, Sundstrom *et al.* detected a few unique metabolites of 2,2',4,4',5,5'-hexachlorobiphenyl in rabbits feces [8]. These facts indicate that the rabbit might have unique enzyme systems in PCB metabolism. Therefore, we examined the metabolism of 2,2',5,5'-TCB with rabbit liver microsomes to determine which isoform of P450 is most important.

Materials and Methods

Seven male Japanese white rabbits (body wt. about 4 kg) were used. Three of them were pretreated with PB dissolved with a drinking water at a concentration of 0.1% (w/v) for 2 weeks *ad. libitum*. Rabbits were killed under anesthesia by injection of pentobarbital and livers were removed Liver microsomes were prepared as described previously [1]. An isoform of PB-

inducible rabbit P450, CYP2B4, was purified with a specific content of 10.3 nmol/mg protein according to the method as described elsewhere [5]. Antiserum against CYP2B4 were prepared in three guinea pigs.

The metabolism of 2,2',5,5'-TCB with rabbit liver microsomes was conducted as reported previously [9]. Both 3- and 4-hydroxy-metabolites were analyzed as trimethylsilylated derivatives using a gas chromatograph HP5890 Series II equipped with an electron capture detector under the conditions as follows: column, DB-1 capillary column (15 m x 0.25 mm i.d., 0.33 μm thickness); carrier gas, N_2 (1 ml/min); column temp., 200℃; injection port temp., 250℃; detector temp., 250℃. Inhibition study of microsomal metabolism of 2,2',5,5'-TCB with guinea pig antiserum raised against CYP2B4 was performed as reported previously [4-6].

Results

Table 1 shows the activities of 3- and 4-hydroxylations by liver microsomes of untreated and PB-treated animals. In untreated rabbits, liver microsomes formed both 3- and 4-hydroxy-metabolites at a equal rate. Pretreatment of PB, a typical P450 inducer, accelerated not only 3-hydroxylation but also 4-hydroxylation of 2,2',5,5'-TCB in rabbits. These results suggest that the metabolizing enzyme of 2,2',5,5'-TCB in rabbits are just like that in hamsters rather than rats.

Table 1. Metabolism of 2,2',5,5'-TCB with Liver Microsomes of Untreated and PB-Treated Rabbits, Hamsters, Rats and Guinea Pigs

Animal	Treatment	Metabolite formed (pmol/min/mg protein)	
		3-OH	4-OH
Rabbit[1)]	None	9.8 ± 1.7 (100)	8.5 ± 1.7 (100)
	PB	23.8 ± 10.2 (243)	20.5 ± 4.4 (241)
Hamster[2)]	None	6.3 ± 0.5 (100)	5.6 ± 0.3 (100)
	PB	20.1 ± 0.2 (319)	11.1 ± 0.3 (198)
Rat[2)]	None	N.D.	N.D.
	PB	324.0 ± 15.4	N.D.
Guinea pig[2)]	None	8.4 ± 0.9 (100)	N.D.
	PB	19.3 ± 2.2 (230)	1.1 ± 0.1

N.D., not detected. PB, phenobarbital.

1) Each value represents the mean ± S.D. of three or four animals.

2) The data was cited from the reference [9]. Each value represents the mean ± S.D. of three determinations and those in parentheses are the relative ratio to the control.

and guinea pigs and that a constitutive and PB-inducible P450 isoform is responsible for the microsomal 2,2',5,5'-TCB metabolism in rabbits.

In addition to 3- and 4-hydroxy-metabolites, two metabolites (M-3, M-4) were formed by rabbit liver microsomes (data not shown). The trimethysilylated derivative of M-3 detected close behind 4-hydroxy-metabolite was also observed with hamster liver microsomes and its gas chromatography-mass spectrometry revealed that M-3 was monohydroxy-TCB [9], whereas the trimethysilylated derivative of M-4 was retained in the column for a longer time than others. The precise structures of M-3 and M-4 remain to be elucidated at present.

By western blotting and immunostaining using guinea pig antiserum against CYP2B4, we tried to detect CYP2B4 in liver microsomes of untreated and PB-treated rabbits. In untreated rabbits, CYP2B4 was detected at a low but significant concentration. Moreover, CYP2B4 protein was markedly increased by PB-treatment (data not shown). These facts indicate that CYP2B4 is a constitutive isoform in rabbit liver and that the increase of CYP2B4 protein is well associated with the induction profile of the activities for 3- and 4-hydroxylations of 2,2',5,5'-TCB as shown in Table 1.

To clarify the contribution of CYP2B4 on 2,2',5,5'-TCB metabolism in rabbit liver, antiserum against CYP2B4 was added to the incubation mixtures containing liver microsomes of PB-treated rabbits (Fig. 1). Addition of antiserum resulted in about 90% inhibition of both 3- and 4-hydroxylations of 2,2',5,5'-TCB in PB-treated rabbits.

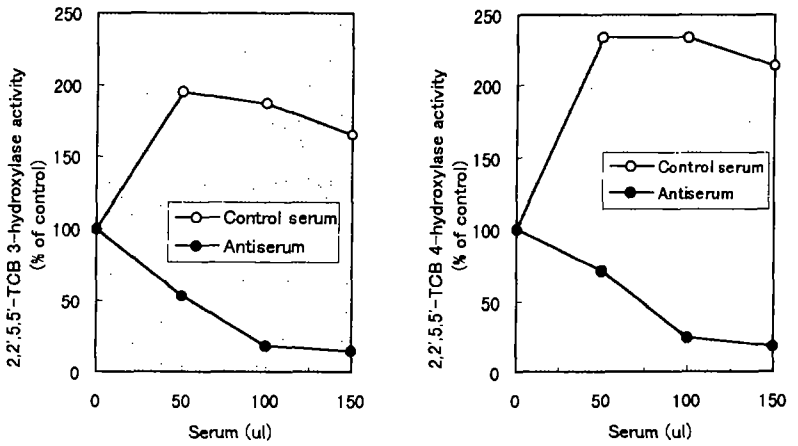

Fig. 1. Effect of Antiserum against Rabbit CYP2B4 on 2,2',5,5'-TCB Metabolism with Liver Microsomes from PB-treated Rabbits.

Discussion

In *in vitro* system using rabbit liver microsomes, 2,2',5,5'-TCB was metabolized to give 3-hydroxy- and 4-hydroxy-2,2',5,5'-TCB to an equal extent, indicating that the rabbit is similar to the hamster rather than the rat and the guinea pig in respect to the metabolic pattern of 2,2',5,5'-TCB. The postulated metabolic pathways of 2,2',5,5'-TCB in rabbit liver are shown in Fig. 2. Generally, a PB-inducible P450 isoform (CYP2B) is most important for 3-hydroxylation of

2,2',5,5'-TCB, whereas a MC-inducible P450 isoform (CYP1A and 2A) is mainly involved in the 4-hydroxylation. However, the facts that both 3- and 4-hydroxylase activities in rabbit liver are induced by PB pretreatement and inhibited almost completely by addition of antiserum against CYP2B4 strongly suggest that one enzyme, namely CYP2B4, is associated with both activities. Thus, it is probable that the metabolism of 2,2',5,5'-TCB by CYP2B4 proceeds via a 3,4-arene oxide and subsequent isomerization to 3- and 4-hydroxy-metabolites.

Rabbit liver microsomes produced two another metabolites, M-3 and M-4, which were also inhibited by antiserum against CYP2B4. This observation suggests not a little involvement of CYP2B4 in the formation of M-3 and M-4.

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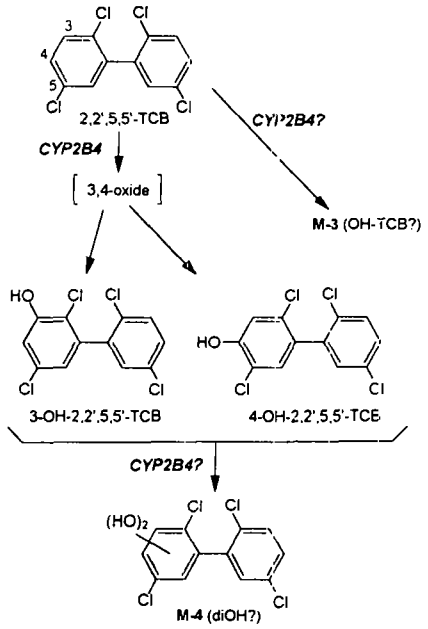

Fig. 2. Postulated Metabolic Pathways of 2,2',5,5'-TCB in Rabbit Liver

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