Metabolism of 2,3',4',5-Tetrachlorobiphenyl by Cytochrome P450 from Rats, Guinea Pigs and Hamsters

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1. Introduction

Polychlorinated biphenyls (PCBs) are known as worldwide environmental pollutants.[1] In animals, the first event of biotransformation of PCBs is the oxygenation of aromatic rings by liver microsomal cytochrome P450(P450). Several studies demonstrated that two P450 subfamilies, namely CYP1A and CYP2B, are responsible for the hydroxylation of PCBs.[1-6] For example, among three symmetric tetrachlorobiphenyls (TCBs) such as 3,3',4,4'-, 3,3',5,5'- and 2,2',5,5'-TCB, the former two TCBs are metabolized by rat CYP1A1,[3,4] but the last one by rat CYP2B1[3] and hamster P450HPB-1.[5] Furthermore, it has been reported that 2,2',4,4',5,5'-hexachlorobiphenyl, a highly persistent PCB congener in adipose tissue and blood of human, is hydroxylated by dog CYP2B11,[2,6] guinea pig P450GP-1[6] and human CYP2B6.[6]

2,3',4',5-TCB, a structurally asymmetric TCB, has been known as a major component of Kanechlor 400. However, there is little information on the metabolism of 2,3',4',5-TCB. Recently, Matsusue *et al.* showed that similarly to 2,2',5,5'-TCB, the major metabolic pathway of 2,3',4',5-TCB in rats is the 3-hydroxylation, which is catalyzed by CYP2B1.[7] In the present paper, we studied species differences in the metabolism of 2,3',4',5-TCB with liver microsomes and purified P450 isoforms of rats, guinea pigs and hamsters.

2. Materials and Methods

Phenobarbital (PB) and 3-methylcholanthrene (MC) were injected i.p. to male Wistar rats, male Hartley guinea pigs and male Golden syrian hamsters at a dose of 80 mg/kg/day for 3 days and 20 mg/kg/day for 3 days, respectively. These animals were killed 24 h after the last injection of each inducer. 3,3',4,4',5-Pentachlorobiphenyl (PenCB) was also used as another MC-type inducer and injected i.p. to rats and hamsters at a single dose of 5 mg/kg and to guinea pigs at a single dose of 0.1 mg/kg. Animals were killed 5 days after the injection. Prior to sacrifice, animals were starved for 12 h.

Two forms of rat P450, CYP1A1 and CYP2B1, were purified by the method of Harada and Omura.[8] Three forms of hamster P450, HPB-1, CYP1A2 and CYP2A8 were purified as described elsewhere.[5,9] Guinea pig P450GP-1 was purified as reported by Oguri *et al.*[10]

The metabolism of 2,3',4',5-TCB with liver microsomes and a reconstituted system containing purified P450 was conducted as reported previously.[2,3] Analysis of TCB metabolites was performed 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. x 0.33 μm thickness); carrier gas, N_2(1 ml/min); column temp., 200°C; injection port temp., 250°C; detector temp., 250°C.

3. Results

Microsomal Metabolism of 2,3',4',5-TCB: To gain some information about the P450 isoforms catalyzing the hydroxylation of 2,3',4',5-TCB, effect of P450 inducers on the metabolism of the TCB was examined using liver microsomes of untreated and P450 inducer-treated rats, guinea pigs and hamsters (Table 1). In untreated animals, a marked species difference was observed for the hydroxylation activity of 2,3',4',5-TCB. In guinea pigs, only 3-hydroxy-2,3',4',5-TCB was formed at a rate of 26.2 pmol/min/mg protein, but in rats, no metabolite was formed. Interestingly, hamster liver microsomes formed both 3-hydroxy- and 4-hydroxy-2,3',4',5-TCB at rates of 5.9 and 46.8 pmol/min/mg protein, respectively. The order of over all yield of metabolites was hamsters > guinea pigs > rats. Among P450 inducers, PB accelerated the 3-hydroxylation of 2,3',4',5-TCB in all the species with the order of rats >> hamsters > guinea pigs. On the other hand, the treatment with MC or PenCB increased the activity for 4-hydroxylation in rats and hamsters but not in guinea pigs.

Table 1. Metabolism of 2,3',4',5-TCB with Liver Microsomes of Untreated and P450 Inducers-Treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	Metabolite formed (pmol/min/mg protein)	
		3-OH	4-OH
Rat	None	ND	ND
	PB	489.1 ± 12.7	ND
	MC	ND	15.2 ± 9.7
	3,3',4,4',5-PenCB	ND	25.6 ± 0.2
Guinea pig	None	26.2 ± 2.5 (1.0)	ND
	PB	127.3 ± 6.8 (4.9)	ND
	MC	18.4 ± 1.5 (0.7)	ND
	3,3',4,4',5-PenCB	8.5 ± 0.6 (0.3)	ND
Hamster	None	5.9 ± 1.4 (1.0)	46.8 ± 2.1 (1.0)
	PB	40.7 ± 2.1 (6.9)	59.0 ± 2.3 (1.3)
	MC	ND	96.4 ± 9.6 (2.1)
	3,3',4,4',5-PenCB	ND	81.6 ± 17.4 (1.7)

ND, not detected. Each value represents the mean ± S.D. of three determinations and those in parentheses are the relative ratio to the control.

Metabolism of 2,3',4',5-TCB by Purified P450: Several isoforms of P450 were purified from three

species and their catalytic activities for the 3- and 4-hydroxylation of 2,3',4',5-TCB were examined using a reconstituted system containing each P450 isoform, rat NADPH-P450 reductase, dilauroylphosphatidylcholine, and NADPH-generating system. As shown in Table 2, the regioselectivity was clearly observed in the 3- and 4-hydroxylations of 2,3',4',5-TCB by each P450 isoform. Namely, rat CYP2B1, guinea pig P450GP-1 and hamster HPB-1 which are induced by PB showed the activity only for the 3-hydroxylation of the TCB, whereas rat CYP1A1 and hamster CYP2A8 catalyzed only 4-hydroxylation. Although hamster CYP1A2 showed both activities, the activity for 4-hydroxylation was about 4 times higher than that for 3-hydroxylation. Among these isoforms, rat CYP1A1 and CYP2B1catalyzed 3- and 4-hydroxylation of 2,3',4'-5-TCB, respectively, at much higher rates than each isoform from guinea pigs and hamsters.

Table 2. Metabolism of 2,3',4',5-TCB by Cytochrome P450 isoforms from Rats, Guinea pigs and Hamsters

Metabolite	Rat		Guinea pig	Hamster		
	1A1[a]	2B1	GP-1[b]	HPB-1	1A2	2A8
	(pmol/min/nmol P450)					
3-OH	ND	217	28	27	4	ND
4-OH	84	ND	ND	ND	17	7

N.D., not detected. [a] Data were taken from the reference7). [b] The rabbit cytochrome b5 (0.1 nmol) was added in the incubation system.

4. Discussion

In *in vitro* system using liver microsomes and purified P450 isoforms from rats, guinea pigs and hamsters, 2,3',4',5-TCB were metabolized to give 3-hydroxy- and 4-hydroxy-2,3',4',5-TCB, and the formation ratio of each metabolite varied from one species to another. The species difference in the metabolism of 2,3',4',5-TCB with liver microsomes could be explained by the study using purified P450 isoforms. The postulated metabolic pathways of 2,3',4',5-TCB by P450 isoforms are shown in Fig. 1. In common to three species, a PB-inducible P450 isoform (CYP2B) is most important for 3-hydroxylation of 2,3',4',5-TCB, whereas a MC-inducible P450 isoform (CYP1A and 2A) is mainly involved in the 4-hydroxylation.

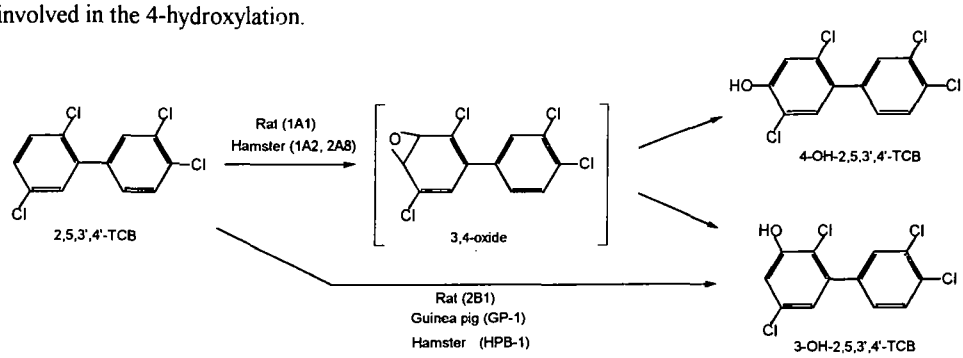

Fig. 1. Postulated Metabolic Pathways of 2,3',4',5-TCB by Cytochrome P450

It has been reported that 2,2',5,5'- and 3,3',4,4'-TCB are metabolized by P450 isoforms belonging to CYP2B and CYP1A subfamilies, respectively[12]. In the present study, the pattern of metabolism of 2,3',4',5-TCB in animals was very similar to that of 2,2',5,5'-TCB. However, some difference were observed between the metabolism *in vitro* of 2,3',4',5-TCB and 2,2',5,5'-TCB. First, rat CYP1A1 had the 4-hydroxylation activity for 2,3',4',5-TCB but not for 2,2',5,5'-TCB[3]. Second, in hamsters, although 2,2',5,5'-TCB was hydroxylated only by CYP2A8 (data not shown), 2,3',4',5-TCB was metabolized by not only CYP2A8 but also CYP1A2. These results indicate the difference in the substrate specificity of the MC-inducible P450 isoforms for both TCBs.

5. References

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