Liver Microsomal Metabolism of Three Tetrachlorobiphenyl Isomers: Species Difference between Rats, Guinea Pigs and Hamsters

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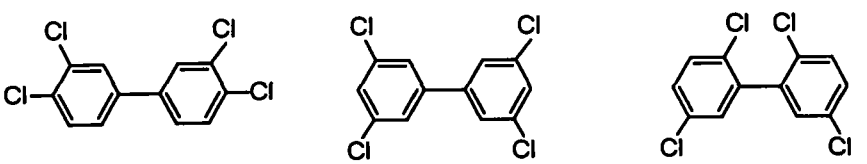
1. Introduction

Tetrachlorobiphenyls(TCBs) are the major components of polychlorinated biphenyl(PCB) preparations such as Kanechlor 400, Aroclor 1248 and Clophen A40 including 3,3',4,4'-TCB and 2,2',5,5'-TCB. The former is a prototype of highly toxic coplanar PCBs having the 3-methylcholanthrene(MC)-type inducing ability of liver enzymes and the latter shows low toxicity and the phenobarbital(PB)-type induction of liver enzymes[1].

In general, PCBs are metabolized mainly through hydroxylation by liver microsomal monooxygenase systems containing cytochrome P450(P450). Our recent studies using male Wistar rats have revealed that 3,3',4,4'-TCB was metabolized to 4- and 5-hydroxylated metabolites by P4501A1, a major form induced by MC treatment and 2,2',5,5'-TCB was converted to 3-hydroxy-2,2',5,5'-TCB by P4502B1 and P4502B2, major forms inducible by PB treatment[2]. Moreover, 3,3',5,5'-TCB, which is present in low to non-detectable levels in PCB preparations and in the global ecosystem, was hydroxylated to 4-hydroxy-3,3',5,5'-TCB by P4501A1[3].

It is known that the guinea pig is highly sensitive to PCBs and related compounds such as polychlorinated dibenzofurans(PCDFs) and dibenzo-*p*-dioxins(PCDDs), whereas the hamster is the most insensitive[4,5]. Although a number of studies have been reported about the mechanism of species difference in the responsiveness for these compounds, little information about the metabolism of PCBs in guinea pigs and hamsters is available so far. Therefore, we chose three symmetric TCB isomers, 3,3',4,4'-TCB, 2,2',5,5'-TCB and 3,3',5,5'-TCB as shown in Fig. 1 to compare their metabolisms with liver microsomes of rats, guinea pigs and hamsters.



3,3',4,4'-TCB　　3,3',5,5'-TCB　　2,2',5,5'-TCB

Fig. 1. Chemical Structures of Three Tetrachlorobiphenyl Isomers Used in This Study

2. Methods and Materials

Three TCB isomers and their hydroxylated metabolites were synthesized as reported previously[2,3,6]. PB and MC at a dose of 80 mg/kg/day for 3 days and 20 mg/kg/day for 3 days, respectively, were injected i.p. to male Wistar rats, male Hartley guinea pigs and male Golden syrian hamsters. These animals were killed 24 h after the last injection of each inducer. 3,4,5,3',4'-Pentachlorobiphenyl(PenCB) was 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. Liver microsomes were prepared as described previously[7].

The microsomal metabolism of TCB isomers was conducted as reported elsewhere[2,3]. For analysis of TCB metabolites, gas chromatography 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., 210 ℃ (for 3,3',4,4'- and 3,3',5,5'-TCB) or 200 ℃ (for 2,2',5,5'-TCB); injection port temp., 250℃; detector temp., 250℃.

3. Results

1) Microsomal monooxygenase activity(data not shown): The P450-dependent activities of benzphetamine(BZ) *N*-demethylase and 7-ethoxyresorufin(7-ER) *O*-deethylase which is known to be a typical activity catalyzed by P450 1A1 and by P450 2B1 and 2B2 in rats[8], respectively, were measured with liver microsomes of untreated and P450 inducers-treated rats, guinea pigs and hamsters. In all species, BZ *N*-demethylase activity was about 2 or 3 times that of control after PB treatment. 7-ER *O*-deethylase activity was markedly induced by treatment of MC and PenCB in rats and hamsters, whereas the activity was only slightly increased in guinea pigs.

2) 3,3',4,4'-TCB metabolism(Fig. 2): In agreement with the previous report[2], liver microsomes from MC- and PenCB-treated rats metabolized 3,3',4,4'-TCB effectively to 4-hydroxy-3,5,3',4'-TCB

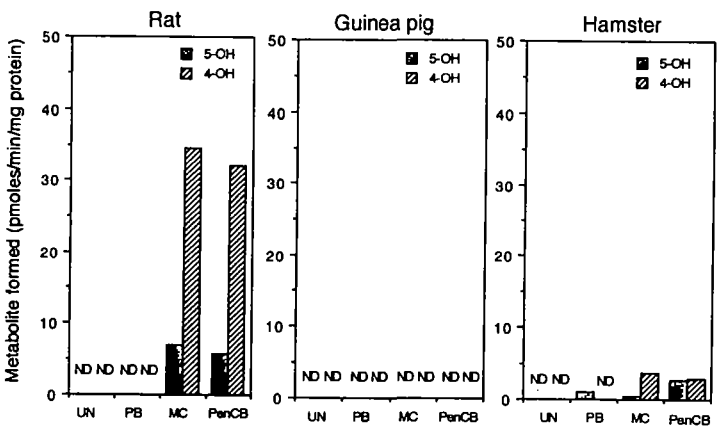


Fig. 2. Metabolism of 3,3',4,4'-TCB by Liver Microsomes from Untreated and Various Inducers-treated Rats, Guinea Pigs and Hamsters.

and 5-hydroxy-3,3',4,4'-TCB. Amount of the 4-hydroxylated metabolite formed is about 5 times that of the 5-hydroxylated one. In hamsters, both hydroxylation activities were also observed in liver microsomes from MC- and PenCB-treated animals, but the 4-hydroxylase activity in either MC- or PenCB-microsomes was about 10% of that of rat. In contrast to this, all microsomes from guinea pigs showed no hydroxylase activity for 3,3',4,4'-TCB.

3) 2,2',5,5'-TCB metabolism(Fig. 3): In rats, the 3-hydroxylation is the main pathway in 2,2',5,5'-TCB metabolism[2]. In common with three animals, the 3-hydroxylation activity for 2,2',5,5'-TCB was stimulated markedly by PB treatment. The rate of 3-hydroxylation in PB-microsomes was 324, 19 and 20 pmol/min/mg protein in rats, guinea pigs and hamsters, respectively. In contrast to rats, the activity was observed at a relatively high level in liver microsomes from untreated guinea pigs and hamsters. In hamsters, 4-hydroxy-2,2',5,5'-TCB in addition to 3-hydroxylated metabolite was

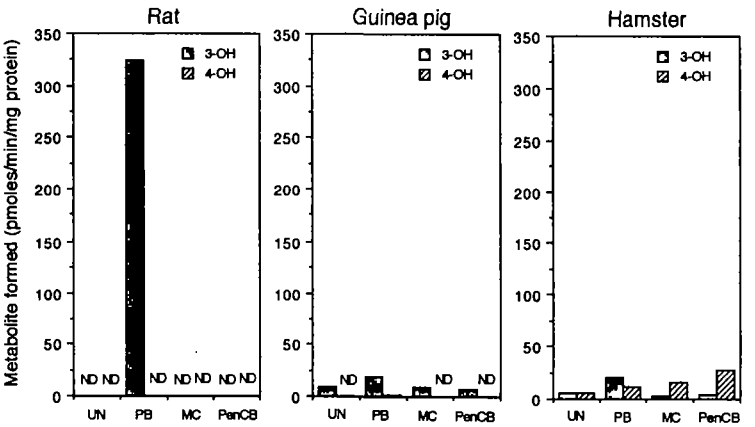


Fig. 3. Metabolism of 2,2',5,5'-TCB by Liver Microsomes from Untreated and Various Inducers-treated Rats, Guinea Pigs and Hamsters.

newly formed by all microsomes and the formation was accelerated to 2.0-, 2.7- and 4.8-fold by pretreatment of PB, MC, PenCB, respectively. In addition, the 4-hydroxylation activity was also detected in PB-treated guinea pigs.

4) 3,3',5,5'-TCB metabolism(Table 1): As reported previously[3], 3,3',5,5'-TCB is metabolized to 4-hydroxy-3,3',5,5'-TCB in liver microsomes only from MC- and PenCB-treated rats. In hamsters, the 4-hydroxylase activity was observed only in MC- and PenCB-microsomes similarly to rats. However, the activity was less than 30% of that of rats. On the other hand, all guinea pig microsomes did not metabolize this TCB.

4. **Discussion**

Here we observed the species difference in the microsomal metabolism of three symmetric TCB isomers in rats, guinea pigs and hamsters. In guinea pigs, 3,3',4,4'-TCB and 3,3',5,5'-TCB which have no *ortho*-chlorine substituent were not metabolized with all microsomes used in this study. In

rats, both TCB isomers are known to be hydroxylated by P450 1A1 [2,3]. The fact that in guinea pig 7-ER *O*-deethylase activity was not induced by MC and PenCB, it is suggested that P450 1A1 or its analogous isoform inducible by MC-type inducers was lacking or in a trace amount in guinea pig liver. In hamsters, P450 1A1 is induced by MC-type inducers in the lung but not in the liver[9]. However, we observed the marked induction of 7-ER *O*-deethylase activity and the hydroxylating activities for both coplanar TCB isomers in MC- and PenCB-microsomes. These results indicate that a MC-inducible P450 isoform other than P450 1A1 is responsible for not only the *O*-deethylation of 7-ER but also the hydroxylation of 3,3',4,4'- and 3,3',5,5'-TCB in hamster liver. Although a few liver microsomal P450 isoforms from MC type inducers-treated hamsters have been reported[10,11], which isoform is active for these reactions is unknown at present.

Table 1. Metabolism of 3,3',5,5'-TCB by Liver Microsomes from Untreated and Inducers-treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	Metabolite formed (pmoles/min/mg protein)
Rat[b]		
	None	N.D.[a]
	PB	N.D.
	MC	45.1 ± 1.6
	PenCB	27.6 ± 0.9
Guinea pig		
	None	N.D.
	PB	N.D.
	MC	N.D.
	PenCB	N.D.
Hamster		
	None	N.D.
	PB	N.D.
	MC	9.1 ± 0.8
	PenCB	10.1 ± 1.4

[a] N.D., not detected. [b] Data were taken from the reference.[3] Each value represents the mean ± S.D. of three determinations.

In agreement with the induction profile of BZ *N*-demethylase activity, the 3-hydroxylation of 2,2',5,5'-TCB was enhanced by PB in three species, suggesting that an isoform of P450 belonging to 2B subfamily may participate in this hydroxylation. In guinea pigs, a PB-inducible form, P450GP-1[12], may be a candidate for 3-hydroxylase of 2,2',5,5'-TCB, but in hamster liver a P450 isoform in 2B subfamily has not been purified and characterized. Interestingly, the high activity of 2,2',5,5'-TCB 4-hydroxylase was found in hamster liver and the activity was accelerated by treatment of PB, MC and PenCB. These results suggest that the different P450 isoforms are involved in the 3- and 4-hydroxylations of 2,2',5,5'-TCB.

5. References

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