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Guinea Pig Liver Cytochrome P450 Responsible for 3-Hydroxylation of 2,5,2',5'-Tetrachlorobiphenyl

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2,5,2',5'-Tetrachlorobiphenyl (TCB) is one of the major components of PCB preparation, Kanechlor 400, which caused a mass food poisoning called Yusho in Japan in 1968 (Kuratsune 1996) and has phenobarbital (PB)-type inducing ability of rat liver microsomal enzymes (Yoshimura et al. 1979). The main metabolic pathway of this PCB isomer is 3-hydroxylation of aromatic ring catalyzed by cytochrome P450 (P450). So far, two groups of P450 superfamily, namely CYP1A and CYP2B subfamily, have been known to be involved in the hydroxylation of PCBs in mammals (Koga and Yoshimura 1996). In particular, the 3-hydroxylation of 2,5,2',5'-TCB is catalyzed by some P450 isoforms belonging to CYP2B subfamily, for example, CYP2B1 and 2B2 in rat liver and P450HPB-1 in hamster liver (Ishida et al. 1990; Koga et al. 1995a).

The guinea pig has been known to be the most sensitive animal for the toxicity of PCBs and their related compounds such as polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) (Kociba and Cabey 1980). On the other hand, there is little information on metabolism of these polychlorinated compounds in the guinea pig. Our recent study using some TCB isomers showed that guinea pig liver microsomes can metabolize 2,5,2',5'-TCB primarily to 3-hydroxy-2,5,2',5'-TCB at a comparatively high rate, but not coplanar TCBs such as 3,4,3',4'- and 3,5,3',5'-TCB, and that the 3-hydroxylation activity of 2,5,2',5'-TCB was stimulated to about 3-fold by PB-treatment (Koga et al. 1995b). More recently, we reported for the first time that guinea pig liver P450 (P450$_{GP-1}$) and human liver P450 (CYP2B6) can catalyze the 3-hydroxylation of a persistent PCB congener, 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) with much less activity than dog P450 (CYP2B11) (Ariyoshi et al. 1995) and that the guinea pig has the unique P450-dependent monooxygenase systems, which hydrolyzes 2,4,5,2',4',5'-HCB via both a 2,3- and a 3,4-arene oxides (Ariyoshi et al. 1997). In this study, we examined the involvement of a PB-inducible guinea pig P450 (P450GP-1) in 2,5,2',5'-TCB metabolism.

MATERIALS AND METHODS

2,5,2',5'-TCB and 4-hydroxy-2,5,2',5'-TCB were synthesized as described previously. (Koga et al. 1995b). 3-Hydroxy-2,5,2',5'-TCB was purified from the

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feces of rats treated intraperitoneally with 2,5,2',5'-TCB at a single dose of 200 mg/kg according to the method of Hanioka et al. (1991).

For purification of P450GP-1, ten Hartley male guinea pigs (body wt 280-300 g, 5 wk old) were used. PB was injected intraperitoneally at a single dose of 80 mg/kg/d for 3 days. The isolation of P450GP-1 from PB-treated guinea pigs was performed by the methods of Koga et al. (1995a) except that the third step using DE-52 column was omitted because the use of the column lowered the recovery of P450 considerably. Final preparation of P450GP-1 had a specific content of 15.2 nmol/mg protein and a 1.6% yield. The sequence of 20 amino-terminal amino acid residues of P450GP-1 was identical with that of $P450_{GP-1}$, a PB-inducible P450 isoform reported previously (Oguri et al. 1991). Rabbit antiserum against P450GP-1 was prepared as described elsewhere (Koga et al. 1995a).

The activity for 3- and 4-hydroxylations of 2,5,2',5'-TCB by purified P-450 was assayed as described elsewhere (Ishida et al. 1991). After incubation for 20 min at 37°C, the metabolite(s) and unchanged substrate were extracted twice with a mixture of 7 mL of chloroform-methanol (2:1) and 14 mL of *n*-hexane and once with 20 mL of *n*-hexane, trimethylsilylated with 40 µL of *N*,*O*-bis-(trimethylsilyl)acetamide and applied to gas chromatography under the conditions as reported previously (Koga et al. 1995a).

For the inhibition study of microsomal metabolism of 2,5,2',5'-TCB with antiserum against P450GP-1, the assay system containing 160 µ*M* 2,5,2',5'-TCB dissolved in 20 µL of dimethylsulfoxide, 6 m*M* $MgCl_2$, 100 m*M* *N*-hydroxyethylpiperazine-*N*'-2-ethanesulfate buffer (pH 7.4), NADPH-generating system (0.33 m*M* NADP, 8 m*M* glucose-6-phosphate, 1 units of glucose-6-phosphate dehydrogenase) and 1 mg protein of liver microsomes in a final volume of 1 mL was used. The microsomes were preincubated with antiserum against P450GP-1 at room temperature for 30 min. The incubation was initiated by addition of NADPH-generating system and conducted for 20 min at 37°C.

NADPH-P450 reductase were purified from liver microsomes of PB-treated male Wistar rats by the method of Yasukochi and Masters (1976). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting of microsomal proteins were conducted essentially according to the methods of Laemmli (1971) and Guengerich et al. (1982). Konica immunostaining kit (Konica Co., Japan) was used to stain P450GP-1 by hydroperoxide-horse radish peroxidase reaction.

RESULTS AND DISCUSSION

Table 1 shows the catalytic activities of P450GP-1 toward 2,5,2',5'-TCB in a reconstituted system. P450GP-1 catalyzed the 3-hydroxylation with a turnover rate of 15.3 nmol/mm/nmol P450 in the absence of rat cytochrome b_5 but not the

4-hydroxylation. Addition of cytochrome b_5 accelerated 3.4-fold of 3-hydroxylation of 2,5,2',5'-TCB by P450GP-1. However, the activity of P450GP-1 was only one tenth of that of rat CYP2B1 (Ishida et al. 1991). Such a stimulating effect of cytochrome b_5 is observed in the metabolisms of 2,4,5,2',4',5'-HCB by dog CYP2B11 (Duignan et al. 1987) and of 2,5,2',5'- and 2,5,3',4'-TCB by rat CYP2B1 (Matsusue et al. 1996). These results suggest that P450GP-1 is responsible for the 3-hydroxylation of 2,5,2',5'-TCB in guinea pig liver and that cytochrome b_5 is needed for the maximal activity of 3-hydroxylation.

Table 1. Metabolism of 2,5,2',5'-TCB by purified P450GP-1

Reaction	Metabolite formed (pmol/min/nmol P450)	
	– b_5	+ b_5
3-Hydroxylation	15.3 (1.0)	51.6 (3.4)
4-Hydroxylation	N.D.	N.D.

N.D., not detected. b_5, cytochrome b_5.
Values are means of two determinations and those in parentheses are the ratio relative to the system in the absence of cytochrome b_5.

Using rabbit antiserum against P450GP-1, we tried to detect P450GP-1 in liver microsomes of untreated and of three typical P450 inducers-treated guinea pigs (Fig. 1). In liver microsomes of untreated guinea pigs, P450GP-1 was detected at a significant high concentration. This fact suggests that this P450 isoform is constitutive in guinea pig liver. Moreover, P450GP-1 was markedly increased by PB-treatment, but was decreased by 3-methylcholanthrene (MC)- and 3,4,5,3',4'-pentachlorobiphenyl-treatments. These results were well associated with the induction profile of the activity of 2,5,2',5'-TCB 3-hydroxylation with liver microsomes of P450 inducers-treated guinea pigs in our previous studies (Koga et al. 1995b). In addition, P450GP-1 showed the crossreactivity with antibody against rat CYP2B1 (data not shown).

To clarify the contribution of P450GP-1 on 2,5,2',5'-TCB metabolism in guinea pig liver, antiserum against P450GP-1 was added to the incubation mixtures including liver microsomes of untreated and PB-treated guinea pigs (Fig. 2). Addition of antiserum (300 μL=22.5 mg protein/mg microsomal protein) resulted in almost complete inhibition of 3-hydroxylation of 2,5,2',5'-TCB in untreated guinea pigs and about 90% inhibition of that in PB-treated guinea pigs. These results confirm that P450GP-1 plays a major role in the 3-hydroxylation of 2,5,2',5'-TCB in the liver of untreated and PB-treated guinea pigs. Previously, we reported that 3-hydroxy-2,5,2',5'-TCB is a detoxicated metabolite on the basis of the biological

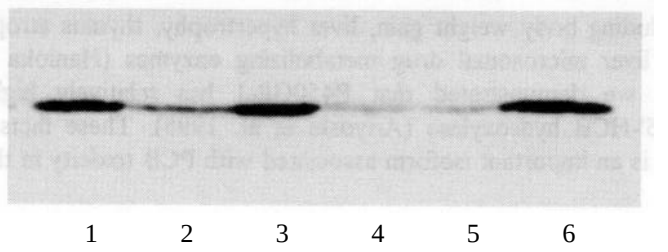

Figure 1. Immunoblot analysis of liver microsomes from untreated and P450 inducer-treated guinea pigs with antiserum against P450GP-1. Lanes 1 and 6 contain purified P450GP-1 (0.5 μg protein). Lanes 2, 3, 4 and 5 contain the liver microsomes (10 μg protein each) from untreated, phenobarbital-treated, 3-methylcholanthrene-treated and 3,4,5,3',4'-pentachlorobiphenyl-treated guinea pigs, respectively.

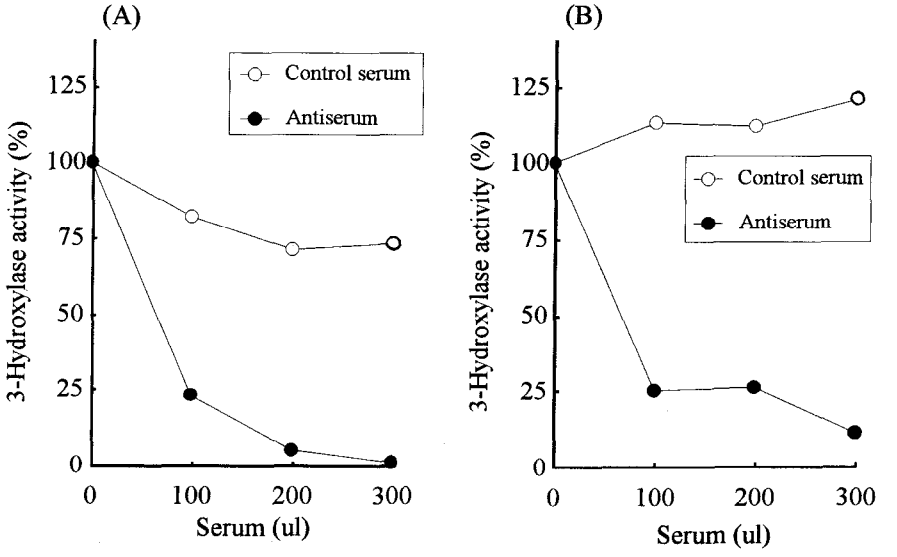

Figure 2. Effect of antiserum against P450GP-1 on 2,5,2',5'-TCB metabolism with liver microsomes from untreated (A) and phenobarbital-treated (B) guinea pigs. Open and closed circles indicated control serum and antiserum raised against P450GP-1.
Each point represents the mean of duplicate determinations.

effects including body weight gain, liver hypertrophy, thymus atrophy, inductive effects of liver microsomal drug metabolizing enzymes (Hanioka et al. 1991). Moreover, we demonstrated that P450GP-1 has relatively high activity of 2,4,5,2',4',5'-HCB hydroxylase (Ariyoshi et al. 1995). These facts suggest that P450GP-1 is an important isoform associated with PCB toxicity in the guinea pig.

As mentioned above, several planar halogenated compounds with the MC-type inducibility are highly toxic to the guinea pig. Although Abe and Watanabe (1982) purified three isoforms of P450 from liver microsomes of MC-treated guinea pigs, the catalytic activity towards coplanar PCB such as 3,4,3',4'- and 3,5,3',5'-TCB has not been elucidated yet. The guinea pig P450 isoforms belonging to CYP1A subfamily which can metabolize these coplanar PCB isomers appear to be induced to a much less extent than those of other animals by MC-type P450 inducers (Yoshimura et al. 1981; Huang and Gibson 1991). This might be one of reasons why the guinea pig is more sensitive for high toxic coplanar compounds such as PCBs, PCDFs and PCDDs.

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