Metabolism of Tetrachlorobiphenyls by Chinese Hamster Liver Microsomes

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Introduction

The biotransformation of polychlorinated biphenyls, world-wide environmental pollutants, is initiated mainly by an oxygenation of aromatic ring catalyzed by liver cyochrome P450(P450) [1]. A series of our studies using some tetrachlorobiphenyls (TCBs), major components of Kanechlor 400 caused Yusho poisoning in Japan in 1968, demonstrated that the hydroxylating ability of liver microsomes from rats, guinea pigs and hamsters for some TCBs could be explained by the catalytic activity of each P450 isoform. Namely, phenobarbital (PB)-inducible P450 isoforms such as rat CYP2B1, Golden syrian (GS) hamster P450HPB-1 and guinea pig P450GP-1(CYP2B18) catalyze 3-hydroxylation for 2,2',5,5'- and 2,3',4',5-TCB. Moreover, 3-methylchlanthrene (MC)-inducible isoforms such as rat CYP1A1, GS hamster CYP2A8 and GS hamster CYP1A2 catalyze 4-hydroxylation for 2,2',5,5'- or 2,3',4',5-TCB [2-7]. In addition, rat CYP1A1 shows the catalytic activity for 4-and 5-hydroxylation of 3,3',4,4'-TCB [2] and for 4-hydroxylation of 3,3',5,5'-TCB [8].

Recently, Fukuhara *et al.* have reported that Chinese (CH) hamsters differ from GS hamsters in terms of susceptibility of monooxygenase activities to P450 inducers, PB and MC [9]. For example, aflatoxin B_1 activation and 7-ethoxycoumarin *O*-deethylase in CH hamsters are makedly accelerated by PB-treatment, whereas in GS hamsters by MC-treatment. Therefore, in this study we compared the metabolism of three TCBs, 2,2',5,5'-, 2,3',4',5- and 3,3',4,4'-TCB, by liver microsomes of CH hamsters with those of GS hamsters.

Materials and Methods

Twelve male CH hamsters (body wt. about 20 g) were used and divided to three groups, untreated, PB- and 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 2 days, respectively. CH hamsters were killed 2 days after the last injection of each P450 inducer and their livers were removed. Liver microsomes were prepared as described previously [10].

The metabolism of TCB with hamster liver microsomes was conducted as reported previously [10]. Hydroxy-metabolites were analyzed as trimethylsilylated (for 2,2',5,5'- and 3,3',4,4'-TCB) or methylated (for 2,3',4',5-TCB) 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℃. The total P450 contents and the activities of 7-

ethoxyresorufin (7-ER) *O*-deethylase and coumarin 7-hydroxylase were measured by the methods of Omura and Sato [11], Prough *et al*. [12] and Yamazaki *et al*. [13], respectively.

Results

Liver monooxygenase activities: Table 1 shows the effect of P450 inducers on total P450 contents and the activities for 7-ER *O*-deethylation and coumarin 7-hydroxylation in liver microsomes of CH and GS hamsters. In CH hamsters, total P450 content was increased 3-fold of control by treatment of PB or MC. In common with both strains, 7-ER *O*-deethylase activity was remarkably elevated by MC-treatment. In addition, a slight and significant increase of the activity was observed in PB-treated CH hamsters. On the other hands, a marked difference was found in the induction mode of coumarin 7-hydroxylase activity between CH and GS hamsters. The activity was accelerated to 4.7-fold of control in PB-treated CH hamsters, but only a slight increase of the activity was shown in PB-treated GS hamsters. MC-treatment resulted in a significant decrease of the activity in both strains. These results indicate that the 7-ER *O*-deethylation and coumarin 7-hydroxylation in CH hamsters are primarily catalyzed by a MC- and PB-inducible P450 isoform, respectively.

Table 1. Effect of Cytochrome P450 Inducers on Liver Microsomal Monooxygenases in Chinese and Golden Syrian Hamsters

Treatment	P450 content (nmol/mg protein)	7-ER *O*-deethylase (nmol/min/mg protein)	Coumarin 7-hydroxylase (pmol/min/mg protein)
Chinese hamsters[1)]			
None	0.486 ± 0.027 (1.00)	0.063 ± 0.009 (1.00)	416.5 ± 73.7 (1.00)
PB	1.459 ± 0.339** (3.00)	0.124 ± 0.024* (1.99)	1964.9 ± 479.5** (4.72)
MC	1.435 ± 0.153** (2.95)	0.572 ± 0.185* (9.13)	100.8 ± 27.5** (0.24)
Golden syrian hamsters[2)]			
None	1.025 (1.00)	0.179 (1.00)	255.3 (1.00)
PB	1.502 (1.47)	0.114 (0.64)	341.2 (1.34)
MC	1.917 (1.87)	2.865 (16.0)	129.4 (0.51)

* Significantly different from the control, $p<0.05$.

** Significantly different from the control, $p<0.01$.

1) Each value represents the mean ± S.D. of three or four animals and those in parentheses are the relative ratio to the control. 2) The data were cited from the reference [10].

TCB metabolism: In 2,2',5,5'-TCB metabolism, all CH hamster microsomes produced a metabolite with a retention time at 7.10 min (data not shown), which corresponds to a trimethylsilylated derivative of 4-hydroxy-2,2',5,5'-TCB by comparison with the authentic sample. The formation of the metabolite was increased about 4-fold by PB-treatment. Similarly, CH

hamster liver microsomes converted 2,3',4',5-TCB into its 4-hydroxy-metabolite and PB- treatment resulted in about 6-fold stimulation of the control. For a coplanar PCB, 3,3',4,4'-TCB, all CH hamster liver microsomes showed no hydroxylase activity (data not shown), although MC-treated GS hamsters metabolized it mainly to 4-hydroxy-metabolite [10].

Western blotting and immunostaining using rabbit antisera against three GS hamster P450, HPB-1, CYP1A2 and CYP2A8, gave us an information that a P450 isoform immunologically similar to CYP2A8 was found only in liver microsomes of MC-treated CH hamsters but no protein band in those of PB-treated ones (data not shown).

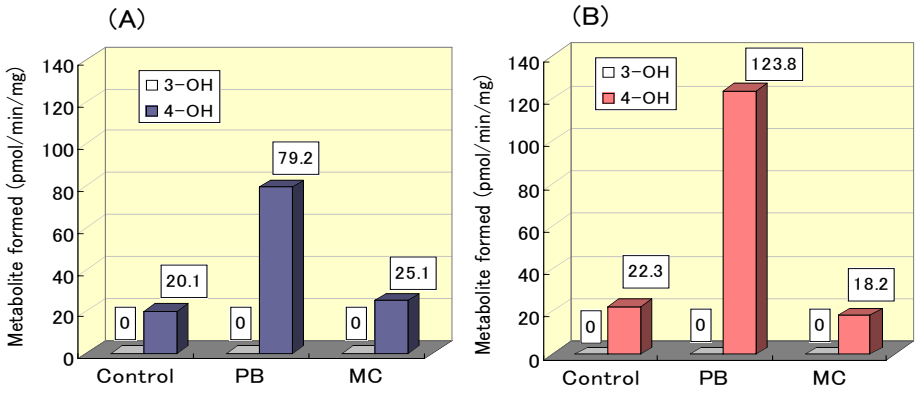

Fig. 2 Effects of P450 Inducers on the Metabolism of 2,2',5,5'-TCB (A) and 2,3',4',5-TCB (B)

Discussion

In GS hamsters, a PB-inducible P450 isoform (HPB-1) is most important for 3-hydroxylation of 2,2',5,5'- and 2,3',4',5-TCB, whereas one or two MC-inducible isoforms (CYP2A8 and CYP1A2) are mainly involved in the 4-hydroxylation. Very interestingly, CH hamster liver microsomes had only 4-hydroxylase activity for 2,2',5,5'- and 2,3',4',5-TCB but not 3-hydroxylase one (Fig.3). Moreover, the 4-hydroxylase activity was increased by PB treatment. These results suggest the existence of a very unique P450 isoform in CH hamster liver. In addition, the induction profile of the 4-hydroxylation activity for 2,2',5,5'- and 2,3',4',5-TCB by P450 inducers was almost agreed with that of coumarin 7-hydroxylase. Very recently, Fukuhara *et al.* have demonstrated

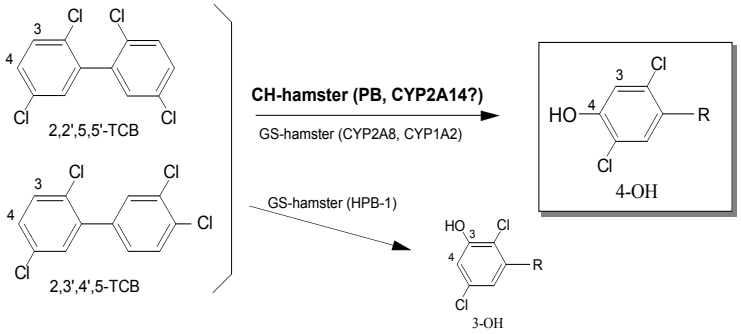

Fig. 3. Postulated Pathways of 2,2',5,5'- and 2,3',4',5-TCB in Chinese and Golden Syrian Hamster Liver

purification, characterization, and cDNA cloning of a major PB-inducible P450 isoform, called CYP2A14, in CH hamster liver [14]. This P450 isoform has high catalytic activity for 7-ethoxycoumarin *O*-deethylation and aflatoxin B_1 activation as well as coumarin 7-hydroxylation. These facts indicate that the 4-hydroxylase of 2,2',5,5'- and 2,3',4',5-TCB in CH hamster liver might be identical with CYP2A14.

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