INVOLVEMENT OF RAT CYP3A ENZYMES IN THE METABOLISM OF 2,2',3,4',5',6-HEXACHLOROBIPHENYL (CB149)

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Introduction

Metabolism of PCB congeners has been known to proceed primarily via a hydroxylation by liver microsomal cytochrome P450 (P450) such as CYP1A, CYP2B and CYP2A enzymes[1,2]. However, in general, 2,4,5-trichloro- and more than six chlorines-substituted PCB congeners have been thought not to be metabolized easily in animals. Recently, we have shown using animal liver microsomes that 2,2',3,4',5',6-hexachlorobiphenyl (CB149) was easily metabolized to 5-hydroxy (OH)- and 4,5-diOH-CB149 as major metabolites and a minor metabolite (4-OH-CB149) and that the pretreatment of phenobarbital (PB) accelerated the formation of two major metabolites (Fig. 1)[3]. These facts suggest that PB-inducible P450, CYP2B enzymes, are responsible for the formation of both 5-OH- and 4,5-diOH-CB149.

On the other hand, PB has been known to induce not only CYP2B enzymes but also CYP3A enzymes which contribute to the metabolism of many drugs in human[4]. In this study, to elucidate an involvement of CYP3A enzymes in PCB metabolism, we examined the effects of a CYP3A inducer, dexamethasone (DEX)[4], and a famous CYP3A inhibitor, ketoconazole (KCZ)[4], on CB149 metabolism.

Materials and methods

CB149 and its three metabolites were synthesized by the method of Cadogan[5]. The chemical purities of these compounds were >99% as determined by GC. Liver microsomes from male Wistar rats (body weight about 200 g) were prepared the next day after the last ip injection of P450 inducers, DEX and PB, at a dose of 100 and 80 mg/kg/day for three days, respectively. CB149 (40 µM) was incubated at 37ºC for 20 min with 0.33 mM NADPH-generating system, 6 mM $MgCl_2$, 100 mM HEPES buffer (pH 7.4) and 1 mg protein of rat liver microsomes in a total volume of 1 ml. After incubation, unchanged CB149 and its metabolites were extracted three times with the mixture of 1 ml of chloroform-methanol (2:1, v/v) and 3 ml of *n*-hexane. The organic layer was pooled and evaporated to dryness. The residue was methylated with diazomethane and applied to GC-ECD and GC-MS. CB149 metabolites were quantified by a calibration curve of authentic CB149 for GC peak area. The conditions of GC-ECD (HP5890 Series II) were: column, DB-1 capillary column (30 m x 0.25 mm i.d., 0.25 µm thickness); carrier gas, N_2 (1 ml/min); column temp., 230ºC; injection port temp., 250ºC; detector temp., 250ºC. The conditions of GC-MS (Shimadzu QP2010) were as follows: column, DB-1 capillary column (30 m x 0.25 mm i.d., 0.25 µm thickness); carrier gas, He (1 ml/min); oven temp., 70ºC (1.5 min) - 20ºC/min - 230ºC (0.5 min) - 4ºC/min - 280ºC (5 min); injection port temp., 250ºC; detector temp., 230ºC.

For the inhibition study on CB149 metabolism, KCZ dissolved in 5 µl of dimethylsulfoxide (DMSO) was added to the incubation mixture at a final concentration of 2.5 µM or 25 µM. KCZ was pre-incubated at 37ºC for 5 min with liver microsomes of DEX-treated rats, HEPES buffer (pH 7.4), $MgCl_2$ and NADPH-generating system. The incubation started after addition of CB149 and was conducted at 37ºC for 60 min. The analyses of CB149 metabolites were done as described above.

Rat liver microsomes were separated by SDS-PAGE on a 9% polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane according to the method of Guengerich *et al.*[6] and then CYP3A and CYP2B proteins were stained using rabbit antibodies against rat CYP3A1 and CYP2B1, peroxidase-conjugated goat antibody against rabbit IgG and Konica immunostain kit.

Results and Discussion

GC-ECD chromatograms of the methylated derivatives of CB149 metabolites formed by liver microsomes of untreated, DEX-treated and PB-treated rats were shown in Fig. 2. Similarly to our previous report[3], liver microsomes of PB-treated rats had the highest activity to form 5-OH- and 4,5-diOH-CB149. Liver microsomes of DEX-treated rats produced 5-OH-CB149 as a major metabolite and two minor metabolites (4-OH- and 4,5-diOH-CB149). Next, we compared the activity to form 5-OH-, 4-OH- and 4,5-diOH-CB149 in liver microsomes of untreated, DEX-treated and PB-treated rats (Fig. 3). In untreated microsomes, a metabolite (5-OH-CB149) was formed with the activity of 11 pmol/min/mg protein. In PB-treated microsomes, a remarkable increase of 5-OH-CB149 (145-fold of untreated microsomes) and 4,5-diOH-CB149 was observed. In DEX-treated microsomes, 5-OH-CB149 was formed at a rate of 237 pmol/min/mg protein (22-fold of untreated microsomes) and 4,5-diOH-CB145 was also formed at a rate of less than 12 pmol/min/mg protein. These results suggest that CYP3A enzymes catalyze the 5-hydroxylation of CB149 at much slower rate than that of CYP2B enzymes. In addition, the activity of CYP3A enzymes from 5-OH-CB149 to 4,5-diOH-CB149 seems to be much less than that of CYP2B enzymes.

The inhibitory effect of a typical CYP3A inhibitor, KCZ, on the formation of 5-OH- and 4,5-diOH-CB149 by liver microsomes of DEX-treated rats was examined. As shown in Table 1, addition of 25 μM KCZ to the incubation mixture inhibited the formation of 5-OH-CB149 and 4,5-diOH-CB149 to 48% and 20% of that of DMSO only, respectively, supporting that CYP3A enzymes are involved in two oxidative steps from CB149 to 4,5-diOH-CB149 via 5-OH-CB149.

Immunostaining of rat liver microsomes by antibodies against CYP3A1 and CYP2B1 showed the result that in DEX-treated microsomes, a marked increase of CYP3A protein band was observed but no CYP2B protein band were found (data not shown). Thinking together with these results, it is suggested that CYP3A enzymes play a major role in CB149 metabolism by liver microsomes of DEX-treated rats.

In this study, we demonstrated that CYP3A enzymes take part in PCB metabolism as well as CYP1A, CYP2B and CYP2A enzymes. On the other hand, in human liver, a CYP3A enzyme, especially CYP3A4, is known to be the most highly abundant P450 isoform[7]. This fact suggests that CYP3A enzymes responsible for CB149 metabolism may be more important in human than in rats. Mimura *et al.* reported that CB149 in the causal oil of Yusho comprised 0.5 ~ 0.7% of total PCB and that CB149 levels in the blood and breast milk from two Yusho patients were very low (0.01 ~ 0.05 ppb)[8]. Moreover, Todaka *et al.* showed that no CB149 was detected in the blood of Yusho patients and healthy volunteers[9], suggesting that CB149 could be metabolized and excreted easily in human. We observed the production of 4,5-diOH-CB149 by liver microsomes of PB- and DEX-treated rats. Garner *et al.* reported that certain PCB catechols have estrogenic activity in the *in vitro* system using cultured HeLa cells[10]. To estimate the PCB toxicity for human, further studies on PCB metabolism in human is needed.

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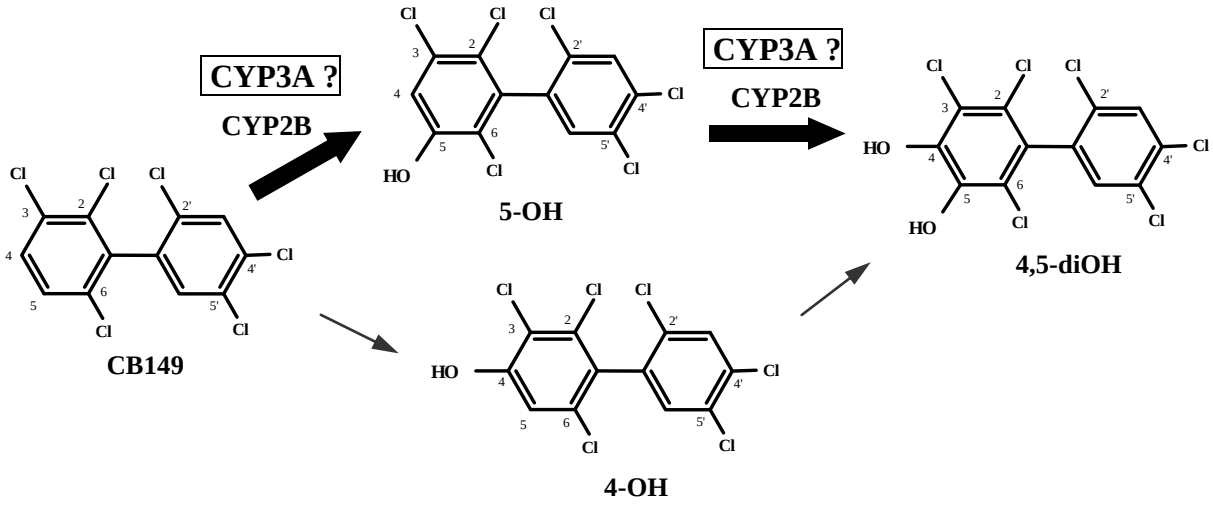

Fig. 1 Postulated metabolic pathways of CB149 in rat liver

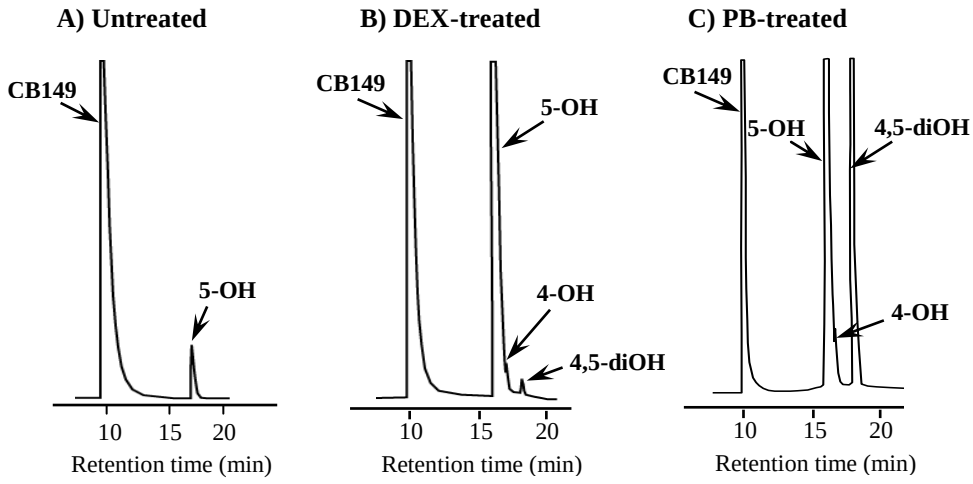

Fig. 2 GC-ECD chromatograms of the methylated derivatives of CB149 metabolites formed by liver microsomes of untreated (A), DEX-treated (B) and PB-treated (C) rats

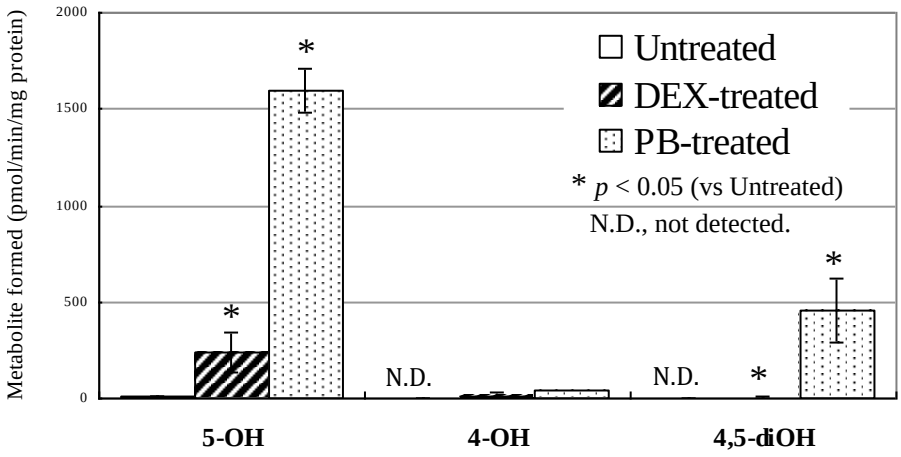

Fig. 3 Effects of cytochrome P450 inducers on CB149 metabolism by rat liver microsomes

Each bar represents mean ± S.D. of four animals.

Table 1 Effect of ketoconazole, a CYP3A inhibitor, on CB149 metabolism by liver microsomes of DEX-treated rats

	Metabolite formed (pmol/min/mg protein)		
Addition	5-OH	4-OH	4,5-diOH
DMSO only	119.7 ± 26.0 (100)	N.D.	10.63 ± 1.06 (100)
2.5 μM KCZ	103.4 ± 10.4 (86)	N.D.	3.53 ± 0.97* (33)
25 μM KCZ	57.2 ± 10.9* (48)	N.D.	2.11 ± 1.80* (20)

N.D., not detected.
Ketoconazole (KCZ) was dissolved with 5 μl of dimethylsulfoxide (DMSO).
Values are mean ± S.D. of four determinations.
* Significantly different from DMSO only.