THE PARTICIPATION OF RAT CYP3A ENZYMES IN THE METABOLISM OF 2,2',4,5,5'-PENTACHLOROBIPHENYL (CB101)

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Introduction

2,2',4',5,5'-pentachlorobiphenyl (CB101), a major component of PCB preparations such as Aroclor and Kanechlor, is known to be metabolized to 3'-hydroxy (OH)-, 4'-OH-, 3-OH- and 3',4'-diOH-CB101 and also 3'- and 4'-methylsulfone-CB101 in animals (Fig.1)[1]. In addition, we showed using rat liver microsomes that pretreatment of phenobarbital (PB) to rats accelerated the formation of 3'-OH- and 3',4'-diOH-CB101 (Fig. 2 and 3)[2]. These facts suggest that PB-inducible isoforms of cytochrome P450 (P450), CYP2B enzymes, are involved in the formation of both metabolites. 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 liver[3]. Recently, we have elucidated the involvement of CYP3A enzymes in 2,2',3',4,5,6'-hexachlorobiphenyl (CB149) metabolism using dexamethasone (DEX) and ketoconazole (KCZ) which is a typical inducer and a potent inhibitor of CYP3A enzymes, respectively[4]. In this study, we examined the participation of CYP3A enzymes in the metabolism of CB101 as well as CB149.

Materials and methods

CB101 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. CB101 (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 CB101 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. CB101 metabolites were quantified by a calibration curve of authentic CB101 for the peak area in GC. The conditions of GC-ECD (HP5890 Series II) were as follows: 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: 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 CB101 metabolism, KCZ dissolved in 5 µl of dimethylsulfoxide (DMSO) was added to the incubation mixture at final concentrations of 2.5 and 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 was started by addition of CB101 and conducted at 37ºC for 60 min. The analyses of CB101 metabolites were done as described above. The western blotting of rat liver microsomes was conducted according to the method of Guengerich *et al.*[6] The staining of CYP3A and CYP2B proteins was performed using rabbit antibodies against rat CYP3A1 and CYP2B1, the peroxidase-conjugated goat antibody against rabbit IgG and the immunostaining kit produced by Konica (Tokyo, Japan).

Results and Discussion

GC-ECD chromatograms of the methylated derivatives of CB101 metabolites formed by liver microsomes of untreated, PB-treated and DEX-treated rats are shown in Fig. 2. Similarly to our previous report,[2] liver microsomes of PB-treated rats had the highest activity to form 3'-OH- and 3',4'-OH-CB101. Liver microsomes of DEX-treated rats produced 3'-OH-CB101 as a major metabolite and 3',4'-diOH-CB101 as a minor metabolite. Next, we compared the activity to form 3-OH-, 3'-OH-, 4'-OH- and 3', 4'-diOH-CB101 in liver microsomes of

untreated, PB-treated and DEX-treated rats (Fig. 3). In untreated microsomes, 3'-OH- and 4'-OH-CB101 were formed with the activity of 2.1 and 3.8 pmol/min/mg protein, respectively. In PB-treated microsomes, a remarkable increase of 3'-OH-CB101 (138-fold of untreated microsomes) was observed and 3',4'-diOH-CB101 appeared with the activity of 24 pmol/min/mg protein. In DEX-treated microsomes, 3'-OH-CB101 and 3',4'-diOH-CB101 were formed at rates of 39 and 4 pmol/min/mg protein, respectively, but both 3-OH and 4'-OH-CB101 were not almost found. These results suggest that CYP3A enzymes catalyze the 3'-hydroxylation of CB101 and additional 4'-hydroxylation, but at a much slower rate than CYP2B enzymes do.

The effect of KCZ, a specific CYP3A inhibitor, on the formation of 3'-OH-, 4'-OH-CB101 and 3',4'-diOH-CB101 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 3'-OH-CB101 to 48% of that of DMSO only, supporting that CYP3A enzymes are involved in the 3'-hydroxylation of CB101. DMSO has been known to inhibit CYP3A activity[7]. In this study, we observed that addition of DMSO (5 µl) to the incubation system resulted in about 70% inhibition of 3'-OH-CB101 production and a complete inhibition of 3',4'-diOH-CB101 production.

Western blotting of rat liver microsomes by antibodies against rat CYP3A1 and CYP2B1 showed that a marked increase of CYP3A protein was observed in DEX-treated microsomes but no CYP2B protein was found (data not shown). On the other hand, in addition to a wide band of CYP2B enzymes, a faint band of CYP3A enzymes was observed in PB-treated microsomes.

Thinking together with these results, we concluded that CYP3A enzymes play a major role in CB101 metabolism by liver microsomes of DEX-treated rats as well as in CB149 metabolism reported previously[4]. Recently, Erratico *et al*. have reported that rat CYP3A enzymes participate in the metabolism of polybrominated diphenylethers (PBDEs) such as BDE47 and BDE99[8]. It seems that CB101, CB149 and PBDEs which are not planar molecules are good substrates for CYP3A enzymes.

Mimura *et al*. showed that CB101 in the causal oil of Yusho comprised about 4.2% of total PCB and that CB101 level in the blood from two Yusho patients was very low (0.01 ~ 0.02 ppb)[9]. Moreover, Todaka *et al.* reported that no CB101 was detectable in the blood of Yusho patients and healthy volunteers[10]. From these results, it is suggested that CB101 as well as CB149 could be metabolized and excreted easily in human. On the other hand, we have reported the mechanism of CB101-mediated decrease in the thyroxine level of mouse serum[11]. To estimate CB101 toxicity for human, further studies on PCB metabolism in human are needed.

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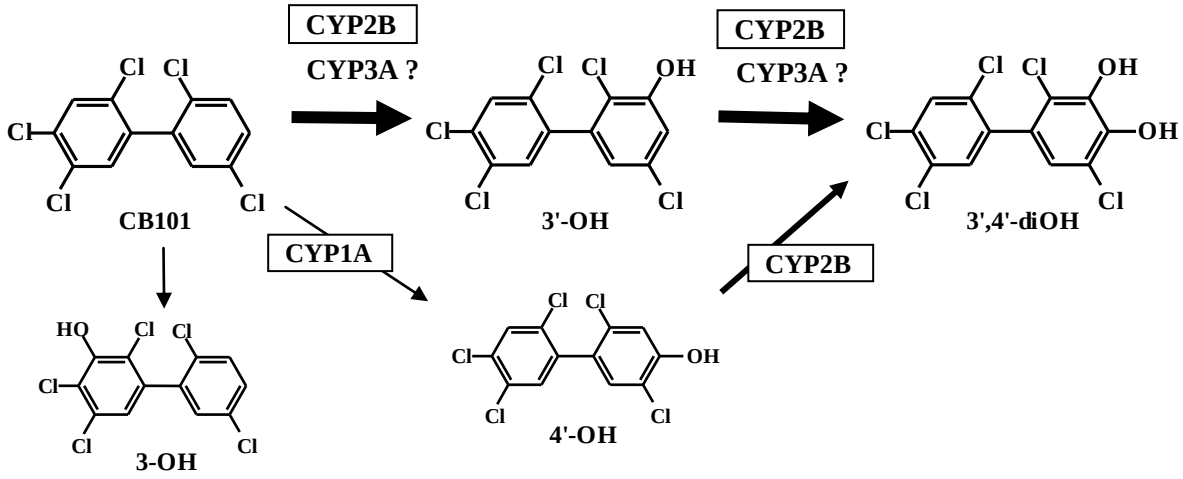

Fig. 1 Postulated metabolic pathways of CB101 in rat liver

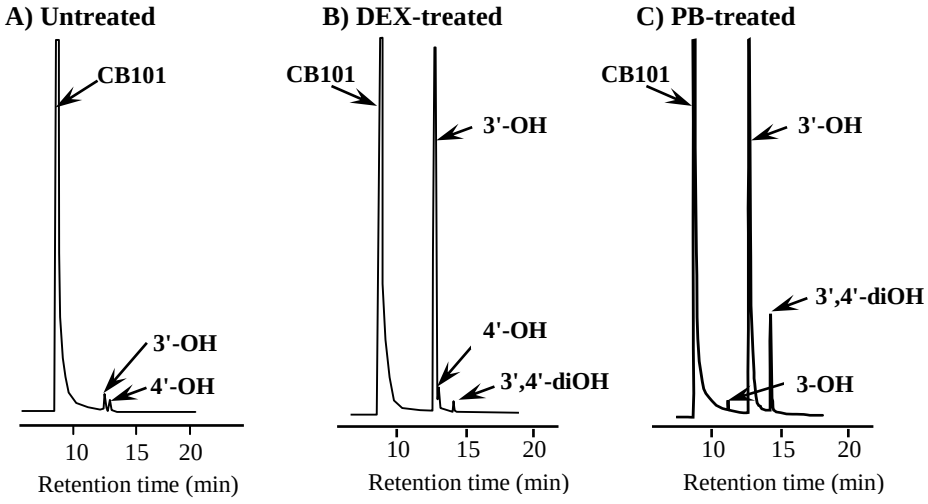

Fig. 2 GC-ECD chromatograms of the methylated derivatives of CB101 metabolites formed by liver microsomes of untreated (A), DEX-treated (B) and PB-treated (C) rats

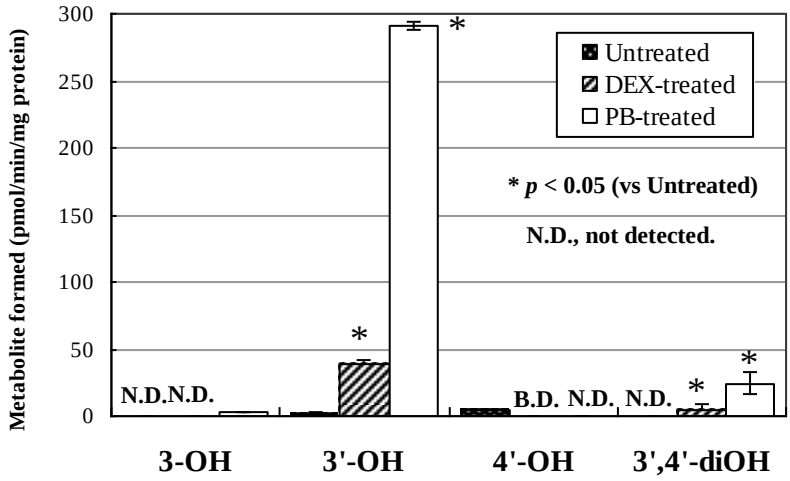

Fig. 3 Effects of cytochrome P450 inducers on CB101 metabolism by rat liver microsomes

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

N.D., not detected. B.D., below detection limit.

Table 1 Effect of a CYP3A inhibitor, KCZ, on CB101 metabolism by liver microsomes of DEX-treated rats

	Metabolite formed (pmol/min/mg protein)		
Addition	3'-OH	4'-OH	3',4'-diOH
DMSO only	12.5 ± 3.3 (100)	N.D.	N.D.
2.5 μM KCZ	11.6 ± 1.9 (86)	N.D.	N.D.
25 μM KCZ	5.1 ± 1.0* (48)	N.D.	N.D.

N.D., not detected.

Ketoconazole (KCZ) was dissolved with 5 μl of dimethylsulfoxide (DMSO).

Values are mean ± S.D. of triplicate determinations.

* Significantly different from DMSO only, $p<0.05$.