METABOLISM OF 2,3',4,5,5'- PENTACHLOROBIPHENYL (CB120) BY LIVER MICROSOMES OF RATS AND GUINEA PIGS

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Introduction

Some hydroxylated (OH) polychlorinated biphenyls (PCBs) such as 4-OH-CB187, 4-OH-CB146, 4-OH-CB107, 3-OH-CB138, 3-OH-CB153 and 4'-OH-CB120 have been detected at relatively high level following PCB congeners such as CB153, CB138 and CB180 in human blood, liver and adipose tissues.[1-8] Recently, 4'-OH-CB120 has also been detected in the blood of patients 37 years after PCB poisoning (Yusho) in Japan at higher level than healthy volunteers.[9] The OH-metabolite is thought to be derived from CB118 and CB120. Actually, Haraguchi *et al*. found 4'-OH-CB120 in the blood of rats dosed CB118, suggesting that 4'-OH-CB120 was formed from CB118 via a 4',5'-epoxide formation and the NIH-shift of chlorine in 4'-position.[10] On the other hand, there is no report on whether 4'-OH-CB120 could be formed from CB120 via a direct hydroxylation or not. Therefore, we examined the *in vitro* metabolisms of CB120 by liver microsomes of rats and guinea pigs and the effect of cytochrome P450 (P450) inducers, phenobarbital (PB) and 3-methylcholanthrene (MC) on CB120 metabolism in both animals.

Materials and methods

CB120 and its metabolites were synthesized by the method of Hutzinger *et al.*[11] Liver microsomes from male Wistar rats (body weight about 250 g) and male Hartley guinea pigs (body weight about 320 g) were prepared the next day after the last ip injection of P450 inducers, PB and MC, at a dose of 80 and 20 mg/kg/day for three days, respectively. 40 µM CB120 was incubated at 37ºC for 60 min with 0.33 mM NADPH-generating system, 6 mM $MgCl_2$, 100 mM HEPES buffer (pH 7.4) and also 1 mg protein of animal liver microsomes in a total volume of 1 ml. After incubation, unchanged CB120 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. CB120 metabolites were quantified by a calibration curve of authentic CB120 for GC peak area. The conditions of GC-ECD (HP5890 Series II) used 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. For determination of molecular weights of CB120 metabolites, GC-MS (Shimadzu QP2010) was used. The conditions 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.

Results

GC-ECD chromatograms of the methylated derivatives of CB120 metabolites formed by liver microsomes of PB-treated rats and guinea pigs were shown in Fig. 1. M-1 was detected at a retention time of 13.46 min in PB-treated rats. In contrast, another metabolite, M-2, was found at a retention time of 13.16 min in PB-treated guinea pigs. Next, we compared the activity to form M-1 and M-2 in liver microsomes of rats and guinea pigs (Table 1). In rats, M-1 was formed by liver microsomes of both PB- and MC-treated animals with the activity of 1.07 and 0.87 pmol/min/mg protein, respectively. In guinea pigs, M-2 was formed by liver microsomes of PB-treated animals and the activity was 1.00 pmol/min/mg protein. Liver microsomes of untreated rats and guinea pigs had no activiy to form a metabolite.

In order to get some information on the chemical structures of M-1 and M-2, a large scale incubation (100 ml) was carried out using liver microsomes of PB-treated rats and guinea pigs. GC-MS data obtained was shown in Table 2. The methylated M-1 and M-2 had a molecular weight of 354, indicating that both metabolites were methoxy (MeO)-pentachlorobiphenyl. The methylated M-1 also showed an intense fragment ion of $[M^+-15]$, suggesting that the MeO-group of M-1 was substituted at 4- or 4'-position. In addition, its mass spectral data and retention time completely agreed with those of authentic 4'-MeO-CB120. In contrast, the methylated M-2 agreed with 3-MeO-CB120 in regard to the mass fragmentation and retention time. From these results, M-1 and M-2 were determined to be 4'-OH-CB120 and 3-OH-CB120, respectively.

Discussion

In this study, we demonstrated that with a very slow rate, CB120 was metabolized to 4'-OH-CB120 (M-1) in the *in vitro* system using liver microsomes of PB- and MC-treated rats but not of guinea pigs. In general, it is known that PB-inducible P450 isoforms (rat CYP2B1,[12] guinea pig CYP2B18[13]) catalyze the 3-hydroxylation of PCBs, while MC-inducible P450 isoforms (rat CYP1A1,[12,14] hamster and human CYP2A enzymes[15,16]) mainly catalyze the 4- or 4'-hydroxylation of PCBs. The result obtained in this study suggests that both CYP2B1 and CYP1A1 are responsible for the formation of 4'-OH-CB120 in rat liver and also CYP2B1 was somewhat more active for CB120 than CYP1A1. On the other hand, liver microsomes of PB-treated guinea pigs produced 3-OH-CB120, suggesting that guinea pig CYP2B18 is most important in the 3-hydroxylation of CB120 in guinea pig liver. The postulated metabolic pathways of CB120 in rats and guinea pigs are illustrated in Fig. 2.

Previously, Kuroki and Masuda reported that the blood level of CB118 and CB105 in Yusho patients was much lower than that in healthy volunteers.[17] Moreover, Mimura *et al*. showed that the concentration of CB118 was 7.1 ~ 8.8% of total PCBs in the causal oil of Yusho, whereas no CB120 was found.[18] Considering these facts, it could be concluded that as well as 4-OH-CB107, almost all of 4'-OH-CB120 in human serum is produced from CB118 not from CB120.

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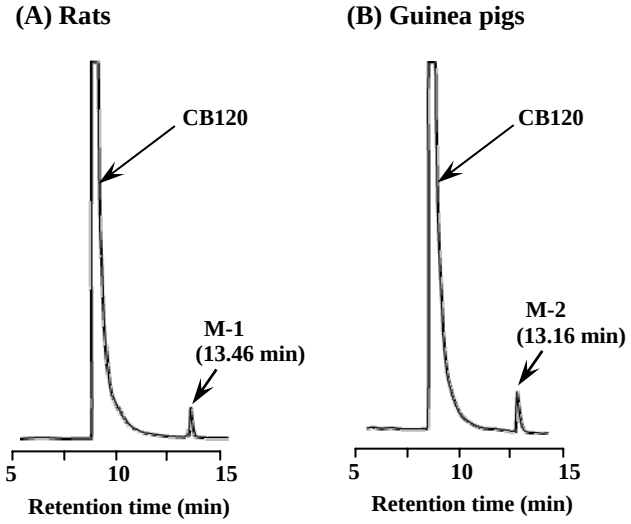

Fig. 1 GC-ECD chromatograms of the methylated derivatives of CB120 metabolites formed by liver microsomes of PB-treated rats (A) and guinea pigs (B)

Table 1 Metabolism of CB120 by liver microsomes of rats and guinea pigs and the effects of P450 inducers on CB120 metabolism

Animal	Metabolite	Metabolite formed (pmol/min/mg protein)		
		Untreated	PB-treated	MC-treated
Rat	M-1	N.D.	1.07 ± 0.13*	0.87 ± 0.19*
	M-2	N.D.	N.D.	N.D.
Guinea pig	M-1	N.D.	N.D.	N.D.
	M-2	N.D.	1.00 ± 0.38*	N.D.

N.D., not detected.
Each value represents the mean ± S.D. of four animals.
* Significantly different from untreated animals (p<0.05).

Table 2 GC-MS data of the methylated derivatives of two CB120 metabolites and their authentic synthetic compounds

Compound	Molecular weight	Mass spectral data (Relative abundance, %)				Retention time (min) in GC-MS
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	$[M^+-50]$	
M-1	354	100	70	29	-	13.32
M-2	354	100	-	34	6	13.14
3-MeO-CB120	354	100	-	44	6	13.14
4-MeO-CB111	354	100	82	42	-	13.26
4'-MeO-CB120	354	100	82	41	-	13.32

-, not detected. CB111, 2,3,3',5,5'-pentachlorobiphenyl; CB120, 2,3',4,5,5'-pentachlorobiphenyl.

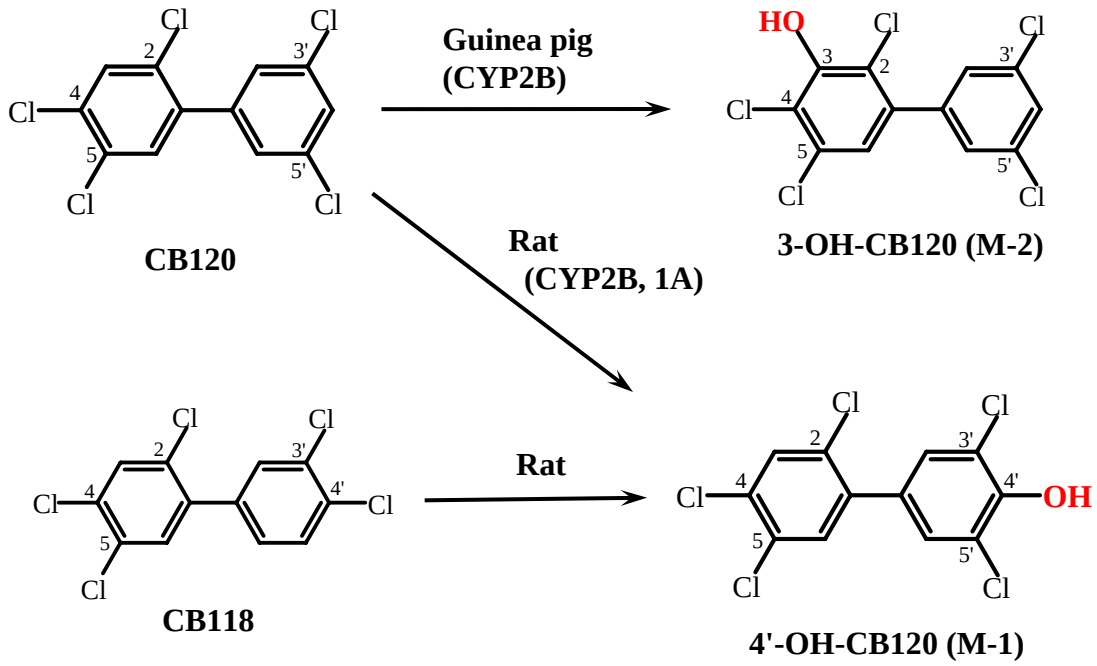

Fig. 2 Postulated metabolic pathways of CB120 and CB118 in animal liver