METABOLISM OF 2,2',3,4',5',6-HEXACHLOROBIPHENYL (CB149) BY LIVER MICROSOMES FROM RATS, HAMSTERS, GUINEA PIGS AND HUMANS

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Abstract

The *in vitro* metabolism of 2,2',3,4',5',6-hexachlorobiphenyl (hexaCB)(CB149) by liver microsomes of rats, hamsters, guinea pigs and humans was compared. In all species, 5-hydroxy (OH)-CB149 was a major metabolite and both 4-OH- and 4,5-diOH-CB149 were minor metabolites. The order of total metabolites formed in untreated animals including humans was hamsters = guinea pigs > rats > humans. Phenobarbital (PB) treatment resulted in a remarkable increase of both 5-OH- and 4,5-diOH-CB149 in rats and hamsters and a slight increase of those in guinea pigs. These results suggest that PB-inducible cytochrome P450 (P450) enzymes play an important role in CB149 metabolism.

Introduction

Among various PCB congeners, 2,4,5-trichloro-substituted PCBs including 2,2',4,4',5,5'-hexaCB (CB153), 2,2',3',4,4',5-hexaCB (CB138) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (heptaCB)(CB180) have been found at higher concentrations in human tissues such as blood, liver and adipose tissues.[1-5] In addition, some OH-metabolites such as 4-OH-2,2',3,4',5,5',6-heptaCB (CB187), 4-OH-2,2',3,4',5,5'-hexaCB (CB146), 4-OH-2,3,3',4',5-pentachlorobiphenyl (pentaCB)(CB107), 3-OH-CB138 and 3-OH-CB153 have also been detected at relatively high level in blood and liver. Moreover, we have reported a catechol metabolite, 3',4'-diOH-2,2',4,5,5'-pentaCB (CB101) present exclusively in the serum of animals given CB101.[6,7] Recently, Haraguchi has observed 4,5-diOH-CB149 present in human blood at high level in a similar manner to 4-OH-CB187.[8] Some catechol metabolites of PCB congeners have been suggested estrogenic.[9] Therefore, in this study, to elucidate the metabolic pathways from CB149 to 4,5-diOH-CB149 and P450 isoforms responsible for CB149 metabolism, we examined the *in vitro* metabolism of CB149 using liver microsomes of rats, guinea pigs, hamsters and humans, and the effect of P450 inducers, PB and 3-methylcholanthrene (MC), on CB149 metabolism in animals.

Materials and Methods

CB149 and its metabolites (4-MeO-CB149, 5-MeO-CB149 and 4,5-diMeO-CB149) were synthesized by the method of Cadogan.[10] The chemical purities of these compounds were >99% as determined by GC. Liver microsomes from male Wistar rats (body weight about 200 g), male Golden syrian hamsters (body weight about 90 g) and male Hartley guinea pigs (body weight about 290 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. Individual human liver microsomes prepared from 9 male and 8 female Caucasians were purchased from BD-Gentest Biosciences (MA, USA). 40 μM CB149 was incubated at 37ºC for 30-60 min with 0.33 mM NADPH-generating system, 6 mM $MgCl_2$, 100 mM HEPES buffer (pH 7.4) and 0.5 mg protein of human liver microsomes in a total volume of 0.5 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 pooled organic layer was evaporated to dryness, methylated with diazomethane and applied to GC-ECD and GC-MS. The CB149 metabolites were quantified by a calibration curve of authentic CB149 for GC peak area. The GC-ECD conditions were as follows: column, DB-1 capillary column (30 m x 0.25 mm, 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 were as follows: column, DB-1 capillary column (30 m x 0.25 mm, 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

In GC-ECD, three metabolites, namely the methylated derivatives of M-1, M-2 and M-3, were detected at the retention times of 17.4 min, 18.2 min and 19.6 min, respectively, in the *in vitro* system using rat liver microsomes (Fig. 1). By comparison of mass fragmentation pattern and retention time in GC-MS, three metabolites were determined as 5-OH-CB149 (M-1), 4-OH-CB149 (M-2) and 4,5-diOH-CB149 (M-3) as shown in Table 1. In untreated animals, only 5-OH-CB149 was formed and the activity was 6.3, 14.6 and 14.5 pmol/min/mg protein in rats, hamsters and guinea pigs, respectively (Table 2). Human liver microsomes formed 5-OH-CB149 at a rate of 1.9~2.5 pmol/min/mg protein and also two other metabolites. No significant sex difference was observed in humans. PB treatment increased 5-OH-CB149 dramatically to 253-fold that in untreated rats, to 11-fold that in untreated hamsters and also to 2.4-fold that in untreated guinea pigs. Moreover, PB treatment showed a significant increase of 4,5-diOH-CB149 in three animal species and of 4-OH-CB149 in rats and hamsters. In contrast, MC treatment decreased 5-OH-CB149 to less than 70% that of untreated hamsters and guinea pigs, whereas it resulted in a significant increase of 4-OH-CB149 only in hamsters.

Discussion

In this study, we demonstrated that CB149 was transformed to 5-OH-CB149 as a major metabolite and to both 4-OH- and 4,5-diOH-CB149 as minor metabolites in all species and that the formation of 5-OH- and 4,5-diOH-CB149 are markedly increased by PB-treatment. The postulated metabolic pathways are illustrated in Fig. 2. These findings suggest that PB-inducible P450 isoforms such as rat CYP2B1[11], hamster CYP2B[12] and guinea pig CYP2B18[13] and human CYP2B6[14] catalyze the 5- and 4-hydroxylation of CB149. The finding that human liver microsomes formed 4-OH-CB149 at lower level may suggest an involvement of human CYP2A6 as reported in CB101 metabolism.[15] On the other hand, the order of the amount of 4,5-diOH-CB149 formed in animals was rats >> hamsters > guinea pigs. When the incubation time was prolonged from 30 min to 60 min, the remarkable increase of 4,5-diOH-CB149 accompanied by the remarkable decrease of 5-OH-CB149 was observed in PB-treated rats (data not shown). These results indicate that CYP2B enzymes play an important role in two oxidative steps from CB149 to 4,5-diOH-CB149 via 5-OH-CB149. Comparing our results on CB101 metabolism,[16] total amount of CB149 metabolites was much higher than that of CB101 metabolites, suggesting that the chlorine atom at 2-position of CB149 is very important in the metabolism of PCBs having the adjacent hydrogens at *meta-para* position because it might modurate both the rate of the 5- or 4-oxidation by PB-inducible CYP2B enzymes and an involvement of CYP2B or CYP2A enzymes.

Acknowledgments

The work was partially supported by the Health and Labour Sciences Research Grants (N. K.) from Ministry of Health, Labour and Welfare of Japan, and the Grant-in-Aid for Scientific Research (B) (no. 20404006; K. H.) and for Scientific Research (C) (no. 20510070; Y. K.) from Japan Society for the Promotion of Science.

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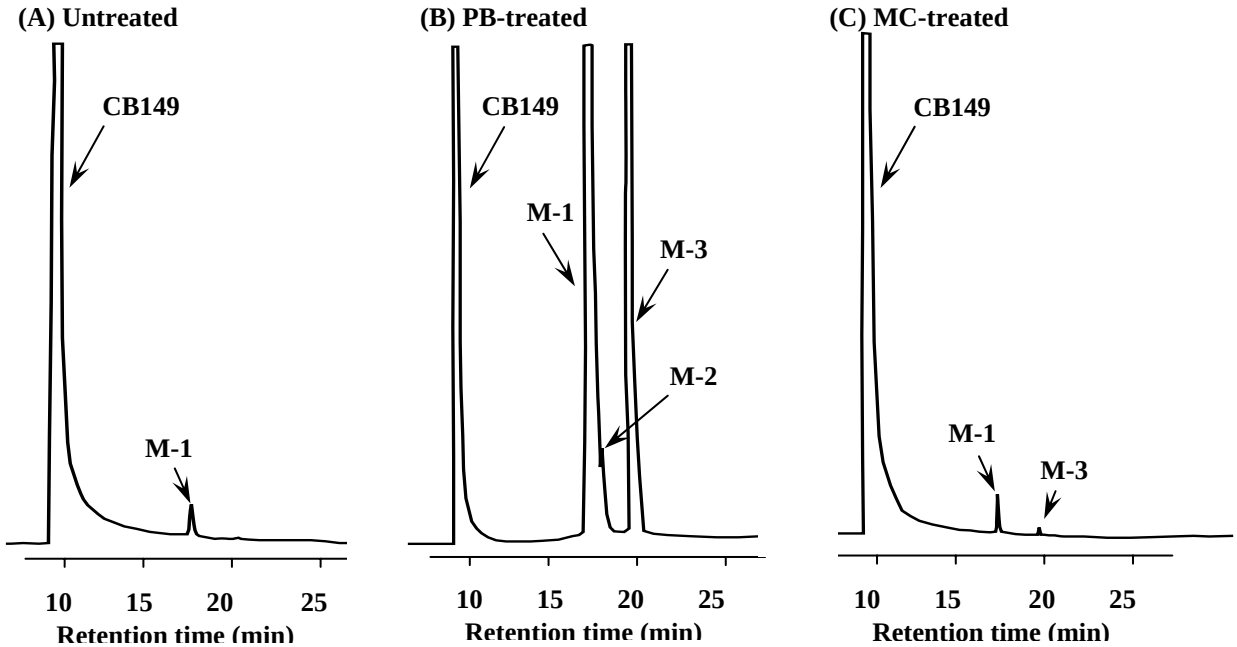

Fig. 1 Gas chromatography of the methylated derivatives of CB149 metabolites formed by liver microsomes of untreated, PB-treated and MC-treated rats.

Table 1 Mass spectral data and retention times of the methylated derivatives of three CB149 metabolites and its synthetic compounds

Compound	Molecular weight	Mass spectral data (Relative abundance, %)						Retention time (min) in GC-MS
		$[M^+]$	$[M^+-15]$	$[M^+-35]$	$[M^+-43]$	$[M^+-50]$	$[M^+-70]$	
CB149	392	100	-	32	-	-	74	11.95
M-1	388	100	6	-	30	17	-	14.89
M-2	388	100	2	-	33	-	-	15.13
M-3	418	100	29	-	22	-	-	15.61
6-MeO-CB146	388	100	-	-	-	123	29	13.50
5-MeO-CB149	388	100	6	-	30	16	-	14.89
4-MeO-CB149	388	100	4	-	37	-	-	15.13
4,5-diMeO-CB149	418	100	28	-	18	-	-	15.61
3-MeO-CB153	388	100	7	-	45	13	-	16.67

-, not detected.

Table 2 *In vitro* metabolism of CB149 by liver microsomes of rats, hamsters, guinea pigs and humans and the effects of P450 inducers on CB149 metabolism

Treatment	n	Metabolite formed (pmol/min/mg protein) M-1	M-2	M-3
Rat				
Untreated	4	6.3 ± 2.6 (1.0)	N.D.	N.D.
PB-treated	4	1596 ± 116* (253)	36.3 ± 2.9*	452 ± 166*
MC-treated	4	10.3 ± 4.2 (1.6)	N.D.	N.D.
Hamster				
Untreated	4	14.6 ± 1.4 (1.0)	N.D.	N.D.
PB-treated	4	158 ± 23.1*(10.9)	11.4 ± 2.9*	13.3 ± 4.0*
MC-treated	4	9.8 ± 4.8 (0.7)	3.3 ± 1.3*	N.D.
Guinea pig				
Untreated	4	14.5 ± 4.6 (1.0)	N.D.	N.D.
PB-treated	4	34.6 ± 4.5* (2.4)	1.3 ± 1.1	2.8 ± 1.4*
MC-treated	4	6.1 ± 1.6* (0.4)	N.D.	N.D.
Human				
Male	9	2.5 ± 1.8	0.6 ± 0.3	0.2 ± 0.1
Female	8	1.9 ± 1.5	0.6 ± 0.4	0.2 ± 0.2

N.D., not detected.

Each value in animals represents the mean ± S.D. of four animals and those in parentheses are the relative ratio of untreated animals. Each value in humans represents the mean ± S.D. of nine males and eight females.

* Significantly different from untreated animals (p<0.05).

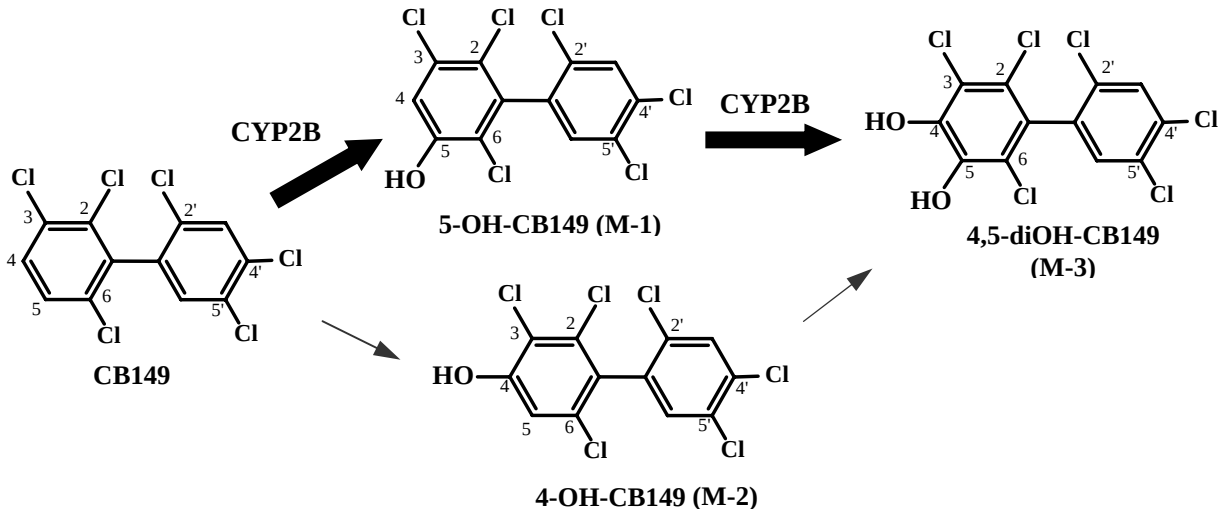

Fig. 2 Postulated metabolic pathways of CB149 in the liver.