METABOLISM OF 2,2',3,4',5,5'-HEXACHLOROBIPHENYL (CB146) BY LIVER MICROSOMES FROM RATS, HAMSTERS AND GUINEA PIGS

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Introduction

Some hydroxy (OH)-metabolites of various PCB congeners such as 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl (CB187), 4-OH-2,2',3,4',5,5'-hexachlorobiphenyl (hexaCB) (CB146), 4-OH-2,3,3',4',5-pentachlorobiphenyl (CB107), 3'-OH-2,2',3,4,4',5'-hexaCB (CB138) and 3-OH-2,2',4,4',5,5'-hexaCB (CB153) have been found in human blood at higher concentrations.[1-5] Recent studies have shown that such OH-metabolites possess various toxicological activities to disturb homeostasis of thyroid hormone and vitamin A in animal blood, to behave as an estrogen or antiestrogen and to inhibit estrogen sulfotransferase.[6-8] However, which PCB congener is the mother compound of the OH-metabolites remains obscure. These OH-metabolites is thought to be formed via a direct hydroxylation or via an epoxide formation and subsequent NIH-shift of chlorine. Recently, we have demonstrated that 4-OH-CB187 could be produced from CB187 but not from 2,2',3,4,4',5',6-heptachloro-biphenyl (CB183) in rats and guinea pigs.[9, 10]

On the other hand, 4-OH-CB146 is also considered to be produced from CB146 via a direct hydroxylation, and from CB153 and CB138 via a 3,4- or 4,5-epoxide formation and NIH-shift of chlorine. Mimura et al. reported that the concentration of CB146, CB138 and CB153 in the causal oil of Yusho contaminated with Kanechlor 400 was 2.49 ppm, 19.81 ppm and 13.20 ppm, respectively.[11] Our previous studies on the metabolism of CB138 and CB153 in guinea pigs did not find 4-OH-CB146 as a metabolite of both CB138 and CB153.[12,13] Therefore, we examined the *in vitro* metabolism of CB146 by liver microsomes of rats, guinea pigs and hamsters and the effects of cytochrome P450 (P450) inducers, phenobarbital (PB) and 3-methylcholanthrene (MC) were also investigated to get some information on P450 enzymes responsible for CB146 metabolism.

Materials and Methods

CB146, 6-MeO-CB146, 3'-MeO-CB146, 4-MeO-CB146, 4'-MeO-CB133 and 3-MeO-CB153 were synthesized by the method of Cadogan.[14] The chemical purities of these compounds were >99% as determined by GC. Male Wistar rats (body weight about 200 g), male Hartley guinea pigs (body weight about 300 g) and male Golden Syrian hamsters (body weight about 90 g) were divided into untreated, PB-treated and MC-treated groups (4 animals each) and administered with PB and MC ip at a dose of 80 and 20 mg/kg/day for three days, respectively. Animal liver microsomes were prepared the next day after the last injection of PB and MC. In the *in vitro* study, 40 μM CB146 was incubated at 37ºC for 60 min with NADPH-generating system (0.33 mM NADP, 5 mM glucose-6-phosphate and 0.2 unit of glucose-6-phosphate dehydrogenase), 6 mM $MgCl_2$, 100 mM HEPES buffer (pH 7.4) and 1 mg protein of animal liver microsomes in a total volume of 1 ml. After incubation, unchanged CB146 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 (HP5890 Series II). The CB146 metabolites were quantified by a calibration curve of authentic CB146 for GC peak area. The GC-ECD conditions were as follows: column, DB-1 (30 m x 0.25 mm, 0.25 μm thickness) capillary column; 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

When CB146 was incubated with NADPH and guinea pig liver microsomes for 60 min at 37ºC, the methylated derivatives of three metabolites, tentatively designated as M-1, M-2 and M-3, were detected at the retention times of 16.11 min, 16.75 min and 17.23 min in GC-ECD (Fig. 1). From the data of GC-MS, the methylated derivatives of M-1, M-2 and M-3 had the same molecular weight of 388, indicating that three metabolites were all mono-methoxy-hexaCB. In addition, the comparison of mass fragmentation patterns and retention times with synthetic samples evaluated that M-1 and M-2 were assumed to be 3'-OH- and 4-OH-CB146, respectively (Table 1). At present, the chemical structure of M-3 remains to be determined. As shown in Table 2, rats liver microsomes showed the highest activity to form 4-OH-CB146 (M-2) of all untreated animals used here and the activity was 24.1 pmol/hr/mg protein in rats and 1.4 pmol/hr/mg protein in guinea pigs. Hamsters had no activity. In addition, PB-treatment increased the activity to 5-fold of that in untreated guinea pigs but only to 1.2-fold of that in untreated rats. The formation of 3'-OH-CB146 (M-1) was markedly increased by PB-treatment in three animals and the order of the activity was guinea pigs > rats > hamsters. On the other hand, MC-treatment involved in a 50% decrease of 4-OH-CB146 in untreated rats and in 3.9-fold increase of M-3 in untreated guinea pigs.

Discussion

In this study, we demonstrated that 4-OH-CB146 could be produced from CB146 by liver microsomes of guinea pigs and rats, and that the metabolic profiles of CB146 were very similar to those of CB187 with respect to the species differences and the effect of P450 inducers.[14] The postulated metabolic pathways of CB146 in animal livers are illustrated in Fig. 2. These findings suggest that PB-inducible P450 isoforms such as guinea pig CYP2B18, rat CYP2B1 and hamster CYP2B[15] are involved in the formation of 3'-OH-CB146 and 4-OH-CB146. On the other hand, M-3, a metabolite specific in guinea pigs, appears to be formed by a MC-inducible guinea pig CYP1A enzyme. The fact that MC-treatment did not increase M-3 in rats and hamsters might indicate the difference in the catalytic activity of CYP1A enzymes.

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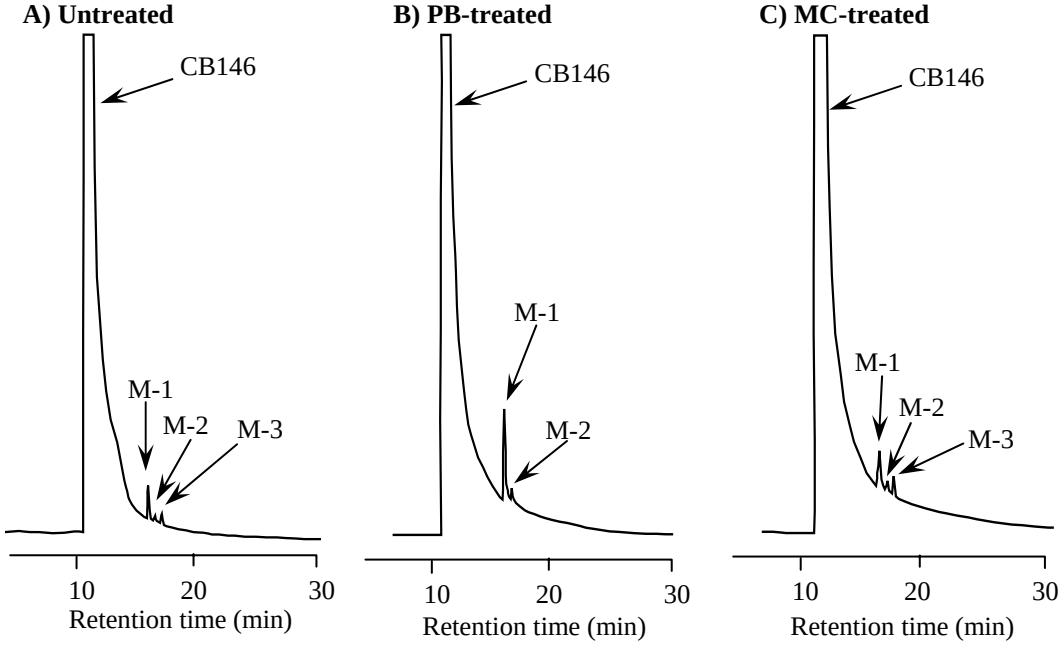

Fig. 1 GC-ECD chromatograms of the methylated derivatives of CB146 metabolites formed by liver microsomes of untreated (A), PB-treated (B) and MC-treated (C) guinea pigs.

Table 1 Mass spectral data and retention times of the methylated derivatives of three CB146 metabolites and its synthetic compounds

Compound	Molecular weight	Mass spectral data (relative abundance, %)					Retention time (min) in GC-ECD
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	$[M^+-50]$	$[M^+-113]$	
M-1	388	100	9	26	10	41	16.11
M-2	388	100	32	22	-	48	16.75
M-3	388	100	N.D.	N.D.	N.D.	N.D.	17.23
6-MeO-CB146	388	100	-	-	134	39	14.14
3'-MeO-CB146	388	100	8	37	12	37	16.11
4'-MeO-CB133	388	100	36	33	-	40	16.27
3-MeO-CB153	388	100	7	45	13	42	16.67
4-MeO-CB146	388	100	44	36	-	46	16.75

-, not detected. N.D., not determined. CB133 (2,2',3,3',5,5'-hexaCB)

Table 2 Effects of P450 inducers on CB146 metabolism with liver microsomes of rats, guinea pigs and hamsters

Treatment	Metabolite formed (pmol/hr/mg protein) M-1	M-2	M-3
Rat			
Untreated	N.D.	24.2 ± 2.3 (1.0)	N.D.
PB-treated	51.7 ± 8.6*	28.0 ± 3.4 (1.2)	N.D.
MC-treated	N.D.	12.7 ± 5.6* (0.5)	N.D.
Guinea pig			
Untreated	28.2 ± 5.4 (1.0)	1.3 ± 1.6 (1.0)	4.7 ± 5.8 (1.0)
PB-treated	117.0 ± 13.2* (4.2)	7.1 ± 1.8* (5.6)	N.D.
MC-treated	26.1 ± 14.6 (0.9)	6.4 ± 5.2 (5.1)	18.2 ± 3.2* (3.9)
Hamster			
Untreated	N.D.	N.D.	N.D.
PB-treated	31.9 ± 5.5*	N.D.	N.D.
MC-treated	N.D.	N.D.	N.D.

N.D., not detected.

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

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

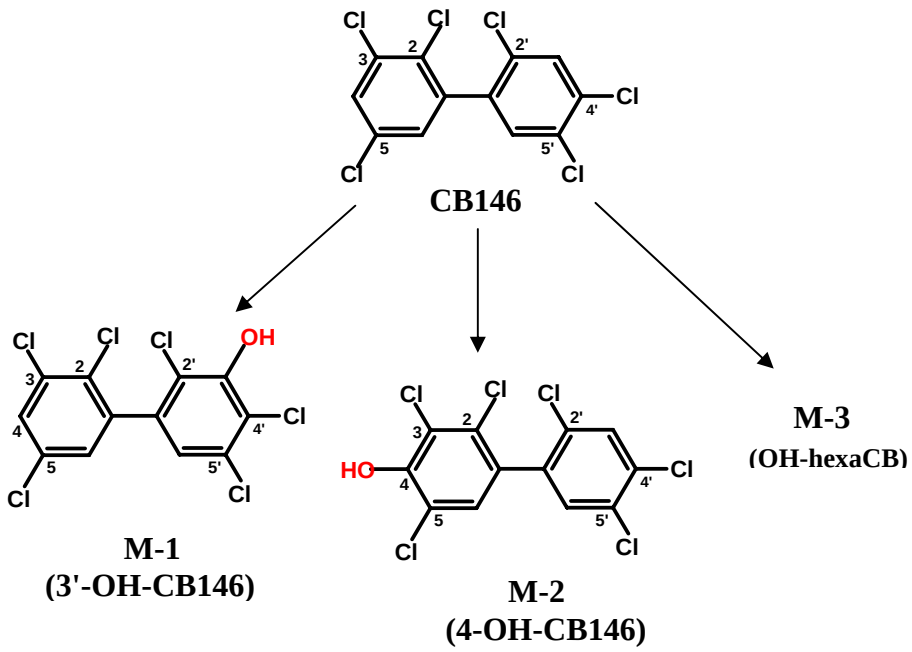

Fig. 2 Postulated metabolic pathways of CB146 in animal liver.