In vitro metabolism of 2,2',3,4',5,5',6-heptachlorobiphenyl(CB187) with liver microsomes of rats, hamsters and guinea pigs.

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Introduction

PCB congeners possess extremely high lipophilicity and biological stability, and as a result they are not easily eliminated from the body once ingested. In particular, not only 2,4,5-trichloro-substituted but also 6 or more chlorine-substituted PCBs such as 2,2',3',4,4',5-hexa-chlorobiphenyl (hexaCB) (CB138), 2,2',4,4',5,5'-hexaCB (CB153), 2,2',3,4,4',5,5'-heptachloro-biphenyl (heptaCB) (CB180) and 2,2',3,4',5,5',6-heptaCB (CB187) have been detected in blood and adipose tissues of mammals and human mother's milk at higher concentration[1-3]. In addition, the 4-hydroxy (OH)-metabolite of CB187 has been reported to be present in human blood at the highest concentration of that derived from other PCB congeners[4,5]. Although CB187, a tri-*ortho*-PCB, is one of the minor component in the commercial PCB preparations such as Clophen, Aroclor and Kanechlor, the toxic equivalency factor (TEF) which is used for dioxin-like PCB congeners including coplanar-PCBs and mono-*ortho*-PCBs to assess the potency of the toxicity has not been set up for di- and tri-*ortho*-PCB congeners. These facts indicate that 4-OH-PCB187 become more persistent and more important toxicologically than the parent CB187. However, there is little report about biotransformation *in vivo* or *in vitro* of CB187 in animals. Therefore, we examined CB187 metabolism by liver microsomes of rats, hamsters and guinea pigs.

Materials and Methods

CB187 was synthesized by the method of Cadogan[6] using 1,2,4,5-tetrachlorobenzene and 2,4,5-trichloroaniline as starting materials. 4-Methoxy (MeO)-CB187 and 5'-MeO-2,2',3,4',5,6-hexaCB (CB146) were also synthesized by the method of Cadogan[6]. 4'-MeO-2,2',3,3',5,5',6-heptaCB (CB178) was got by the diazo-coupling reaction with 1,2,4,5-tetrachloroaniline and 2,3,6-trichlorophenol[7] and subsequent methylation by diazomethane. Finally, they were purified by silica gel 60 column chromatography, TLC (silica gel $60F_{254}$) and reverse-phase HPLC. All other chemicals used were of the highest quality commercially available.

Liver microsomes from male Wistar rats (5 weeks old), male Golden Syrian hamsters (body weight about 90 g) and male Hartley guinea pigs (body weight about 250 g) were prepared the next day after the last i.p. injection of P450 inducers, PB and MC, at a dose of 80 mg/kg/day and 20 mg/kg/day for 3 days, respectively. In the *in vitro* study, CB187 was incubated at 37ºC with 1 mg

protein of animal liver microsomes, 0.33 mM NADPH-generating system, 6 mM $MgCl_2$ and 100 mM HEPES buffer (pH 7.4) in a total volume of 1 ml. After incubation for 60 min, unchanged CB187 and its metabolites were extracted three times with chloroform-methanol (2:1, v/v) and *n*-hexane. The pooled organic layer was evaporated to dryness, methylated with diazomethane and applied to a gas chromatograph HP5890 Series II equipped with ECD under the conditions as follows: DB-1 capillary column(30 m x 0.25 mm, 0.25 μm thickness); carrier gas, N_2 (1ml/min); column temp., 230ºC; injection port temp., 250ºC; detector temp., 250ºC. Analysis of CB187 and its metabolites in GC/MS was performed using Agilent 5973 inert MSD under the conditions as follows: HP-5 fused capillary column (60 m x 0.25 mm, 0.25 μm thickness); carrier gas, He (1ml/min); column temp., 70ºC(1.5min)-20ºC/min-230ºC(0.5min)-4ºC/min-280ºC(5min); injection port temp., 250ºC; detector temp., 230ºC

Results

When PCB187 was incubated for 60 min with guinea pig liver microsomes, three metabolites named tentatively M-1, M-2 and M-3 were observed at retention times of 17.57 min, 21.49 min and 22.51 min in GC/ECD, respectively as shown in Figure 1. The order of the amount of metabolites formed was M-2=M-1 M-3. Both M-1 and M-2 were increased to 3-fold of untreated by PB treatment but were decreased to less than 50% of untreated by MC-treatment. In contrast, M-3 was induced to about 2-fold by MC treatment despite the concentration was rather low. In rats, M-2 and M-3 were formed as a major and a minor metabolite, respectively, only in PB-treated rats but not in untreated and MC-treated rats. In hamsters, PB-treatment resulted in the formation of only M-2. To get some information about chemical structure of the metabolites, the large scale incubation (100ml) with liver microsomes of PB-treated guinea pigs was conducted at 37ºC for 60 min and the metabolites were extracted, methylated by diazomethane and applied to GC/MS. Table 1 shows the mass spectral data and retention times of three methylated metabolites. The methylated derivatives of M-2 and M-3 had the same molecular weight of 422 and the potent fragment ion of M^+-15 (m/z 407) indicating monomethoxylated compounds at 4- or 4'-position. Moreover, their retention times in GC/MS were completely agreed with synthetic 4'-MeO-CB178 and 4-MeO-CB187, assuming that M-2 and M-3 were 4'-OH-CB178 and 4-OH-CB187, respectively. On the other hand, the methylated M-1 had a molecular weight of 388, which suggested that M-1 is a monohydroxylated and dechlorinated metabolite. The precise chemical structure of M-1 is now unclear. The methylated derivative of M-1 was not 5'-MeO-CB147 because they showed different retention times in GC/MS (Table 1).

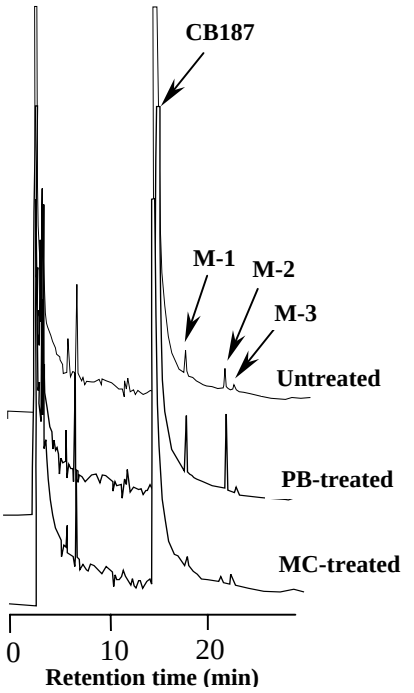

Figure 1: Gas chromatograms of the methylated derivatives of CB187 metabolites formed by guinea pig liver microsomes

Table 1: Mass spectral data and retention times of methylated derivatives of three CB187 metabolites and synthetic compounds in GC/MS.

Compound	Molecular weight	Mass spectral data						Retention time(min)
		$[M^+]$	$[M^+-15]$	$[M^+-35]$	$[M^+-43]$	$[M^+-70]$	$[M^+-113]$	
CB187	392	100	-	26	-	84	-	14.573
M-1	388	100	5	-	36	8	43	15.667
M-2	422	100	26	-	31	-	55	16.628
M-3	422	100	26	-	40	-	58	16.884
5'-MeO-CB147	388	100	20	-	26	7	50	15.285
4'-MeO-CB178	422	100	30	-	38	-	57	16.628
4-MeO-CB187	422	100	15	-	46	-	54	16.884

Discussion

In this study, we found that CB187 could be easily metabolized to three metabolites by guinea pig liver microsomes and they corresponded to a monohydroxy-hexaCB (M-1) and two monohydroxy-heptaCB (M-2 and M-3) from the GC/MS data. By comparison with synthetic standards, M-2 and M-3 were determined to be 4'-OH-CB178 and 4-OH-CB187, respectively. The postulated metabolic pathways were shown in Figure 2. Our recent studies[8-10] demonstrated that 2,4,5-trichloro-substituted PCB congeners such as CB118, CB138 and CB153 were metabolized to their 2-hydroxy metabolites as a major metabolite in guinea pig liver and the 2-hydroxylation was catalyzed by PB-inducible cytochrome P450 such as guinea pig CYP2B18 and rat CYP2B1, suggesting that the metabolite might be formed via a 2,3-epoxide formation and subsequent NIH-shift of a chlorine at 2-position to 3-position on 2,4,5-trichloro-substituted benzene ring. In this study, CB187 metabolism was similarly accelerated by PB treatment in common to three animals, whereas the metabolites formed was not a 2-hydroxy-metabolite but 4- or 4'-hydroxy-metabolites. Although the reason why the metabolic profile of CB187 was different from that of CB118, CB138 and CB153 is unclear at present, it would be considered that CB187, a tri-*ortho*-PCB, is more bulky than mono- or di-*ortho*-PCB such as CB118, CB138 and CB153. These results suggest that the substrate specificity of CYP2B enzyme for 2,4,5-trichloro-substituted PCB congeners could be influenced by the degree of an angle where two benzene rings in a PCB molecule rotate each other.

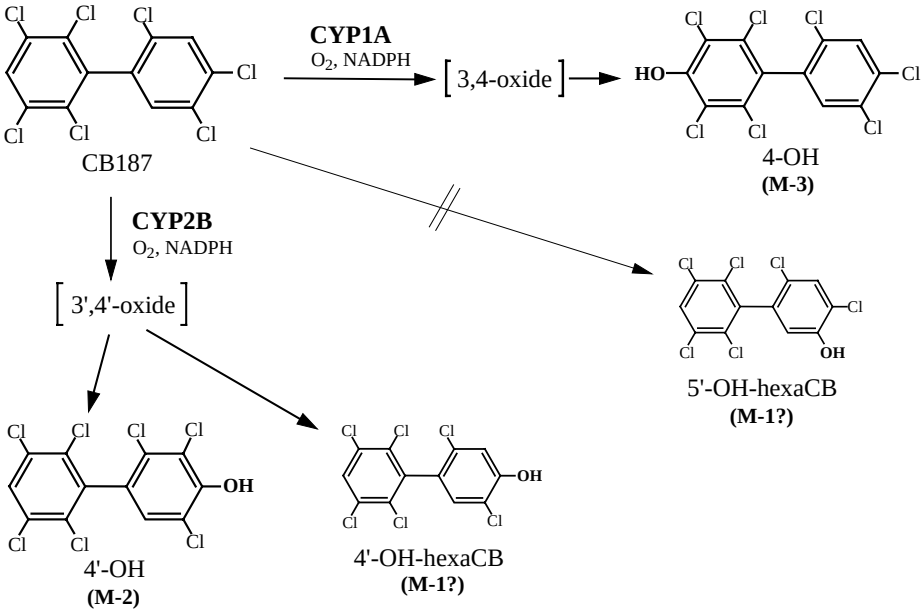

Figure 2: Postulated pathways of PCB187 in animals.

As mentioned above, 4-OH-CB187 is a most abundant metabolite of all hydroxylated metabolites in human blood, whereas it was not a major metabolite formed by animal liver microsomes used in the present study. The fact suggests that the amount of 4-OH-CB187 formed in animal liver is small, while it would be distributed preferably to blood and retained there selectively during life span of animals.

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