IN VITRO METABOLISM OF 2,2',3,4,4',5',6-HEPTACHLOROBIPHENYL (CB183) WITH LIVER MICROSOMES FROM RATS, GUINEA PIGS AND HAMSTERS

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Introduction

4-Hydroxy (OH)-metabolites of various PCB congeners containing 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl (heptaCB) (CB187), 4-OH-2,2',3,4',5,5'-hexachlorobiphenyl (hexaCB) (CB146), 4-OH-2,3,3',4',5-pentachlorobiphenyl (pentaCB) (CB107), 4-OH-2,2,3,3',4',5,5'-heptaCB (CB172) and 4-OH-2,2',3,3',4',5-hexaCB (CB130) have been reported to be retained in human blood at higher concentrations.[1-3] Recent studies have demonstrated that the 4-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.[4-6]

4-OH-CB187 is identified as the major metabolite with the highest concentration among 4-OH-metabolites and is presumably from CB187 and/or 2,2',3,4,4',5',6-heptaCB (CB183). Recently, we have shown that CB187 was metabolized to three OH-metabolites, 4'-OH-2,2',3,5,5',6-hexaCB (CB151), 4'-OH-2,2',3,3',5,5',6-heptaCB (CB178) and 4-OH-CB187 by a phenobarbital (PB)-inducible animal cytochrome P450 (P450) and that the order of total activity to produce the metabolites was guinea pigs > rats > hamsters.[7] Moreover, we have demonstrated that on day 4 following an ip administration of CB187 to guinea pigs, 4-OH-CB187 was exclusively present in the blood, whereas 4'-OH-CB178, a major metabolite, was distributed to the blood and the feces (blood < feces).[8] Malmberg *et al.* have also reported that after an iv injection of 4-OH-metabolite, 4-OH-CB187 in rat blood was retained for about 4 times longer time (half life: 15 days) than 4-OH-CB107, a major metabolite of 2,3',4,4',5-pentaCB (CB118).[9] Such a difference in the distribution of 4-OH-metabolites to the blood is thought to depend upon the potency of affinity for transthyretin, a protein transporting thyroid hormone and vitamin A in the blood.[4]

On the other hand, 4-OH-CB187 could be produced from CB183 via a 4,5-epoxide formation and NIH-shift of chlorine. CB183 is a minor component in PCB preparations[10] and the blood level of CB183 is one fourth that of CB187 in pregnant women.[3] That may be the reason why there is no report available on the metabolism of CB183 in animals. Therefore, we examined the *in vitro* metabolism of CB183 by liver microsomes of rats, guinea pigs and hamsters and the effects of P450 inducers, PB and 3-methylcholanthrene (MC) were also investigated to get some information on P450 enzymes responsible for CB183 metabolism.

Materials and Methods

CB183 was synthesized by the method of Cadogan[11] using 1,2,3,5-tetrachlorobenzene and 2,4,5-trichloroaniline as starting materials. 4-Methoxy (MeO)-CB187 and 5-MeO-CB183 were also synthesized by the method of Cadogan.[11] 3'-OH-CB183 and 4'-OH-2,2',3,3',4,5',6-heptaCB (CB175) were isolated by silica gel column chromatography and preparative reverse-phase HPLC after a diazo-coupling reaction with 2,3,4,6-tetrachloroaniline and 2,3,6-trichlorophenol. The methylation was conducted by addition of diazomethane. All other chemicals used were of the highest quality commercially available.

Liver microsomes from male Wistar rats (body weight about 180 g), male Hartley guinea pigs (body weight about 290 g) and male Golden syrian hamsters (body weight about 90 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. In the *in vitro* study, 40 μM CB183 was incubated at 37ºC for 60 min 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, unchanged CB183 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 CB183 metabolites were quantified by a calibration curve of authentic CB183 for GC peak area. The conditions 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. GC-MS was performed using Shimadzu GCMS-QP2010 under the following conditions: DB-1 fused capillary column (30 m x 0.25 mm id, 0.25 μm thickness); carrier gas, He (1 ml/min); column temp., 70ºC (1.5 min) - 20ºC/min - 230ºC (0.5 min) - 4ºC/min - 280ºC (5 min); injection port temp., 250ºC; detector temp., 230ºC.

Results

CB183 was incubated for 60 min at 37ºC with animal liver microsomes and NADPH-generating system under aerobic conditions. As shown in Figure 1, CB183 was metabolized to two metabolites (named M-1 and M-2 tentatively) by PB-treated microsomes in all species much more effectively than untreated and MC-treated microsomes. The retention time of the methylated M-1 and M-2 in GC-ECD was 25.21 min and 25.63 min, respectively. Although no metabolite was detected in untreated rat microsomes, PB treatment to rats resulted in a remarkable increase of M-1 and M-2. The production rate of M-1 and M-2 was 62 and 158 pmol/hr/mg protein, respectively. In guinea pigs, M-1 was increased to 4.5-fold of untreated ones by PB treatment. In hamsters, PB treatment showed an appearance of M-1 and an increase of M-2 (4.8-fold of untreated).

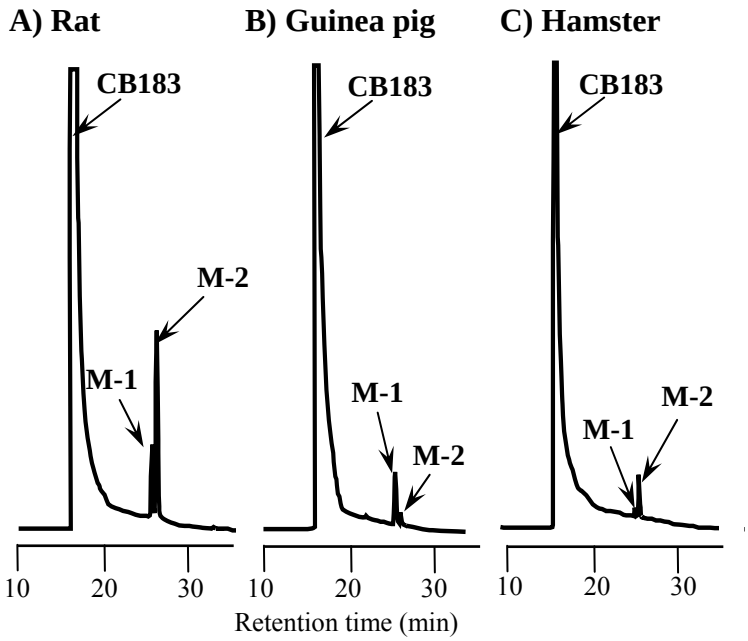

Figure 1 GC-ECD chromatograms of the methylated CB183 metabolites formed by liver microsomes of PB-treated rats (A), guinea pigs (B) and hamsters (C).

Table 1 shows the mass spectral data and retention times of the methylated M-1 and M-2. The methylated CB183 metabolites had the same molecular weight of 422, suggesting that they are monohydroxy-metabolites of CB183. The methylated M-2 showed the potent fragment ion of M^+-15 (m/z 407). This finding indicates that M-2 is 4- or 4'-OH-metabolite of CB183. In fact, the retention time of the methylated M-2 in GC-ECD and GC-MS were completely agreed with that of authentic 4-MeO-CB187. From these results, M-2 was determined to be 4-OH-CB187. In PB-treated microsomes, the order of the amount of

4-OH-CB187 was rat > hamster > guinea pig (Figure 1). On the other hand, the methylated M-1 had the fragment ion of M^+-43 (m/z 379) but not of M^+-15 (m/z 407), which suggests that the methoxy group in M-1 is substituted on a *meta* (3,3',5 or 5')-positions. When compared with 5- and 3'-MeO-CB183 synthesized as described in Materials and Methods, the methylated M-1 agreed with 3'-MeO-CB183 with respect to the retention time in GC-ECD and mass fragmentation in GC-MS.

Table 1 Mass spectral data and retention times of methylated derivatives of two CB183 metabolites and synthetic compounds in GC-MS.

	Molecular	Mass spectral data					Retention time (min)
Compound	weight	$[M^+]$	$[M^+-15]$	$[M^+-35]$	$[M^+-43]$	$[M^+-50]$	in GC-ECD
CB183	392	100	-	21	-	-	17.23
M-1	422	100	2	-	32	-	25.21
M-2	422	100	26	-	51	-	25.63
3'-MeO-CB183	422	100	4	-	40	-	25.21
4'-MeO-CB172	422	100	26	-	39	-	25.52
4-MeO-CB187	422	100	22	-	47	-	25.63
5-MeO-CB183	422	100	28	-	42	23	25.69

-, not detected.

Discussion

In this study, we demonstrated that the *in vitro* enzyme system using animal liver microsomes metabolized CB183 to two OH-metabolites, 3'-OH-CB183 (M-1) and 4-OH-CB187 (M-2). The postulated pathways are shown in Figure 2. The metabolism of CB183 presumably proceed via a direct hydroxylation at 3'-position and via a 4,5-epoxide formation and subsequent NIH-shift of chlorine. PB treatment increased the activity to metabolize CB183 but MC treatment did not. These results suggest that of P450 isoforms, CYP2B enzymes such as rat CYP2B1, hamster CYP2B and guinea pig CYP2B18 play an important role in CB183 metabolism similarly to CB187 metabolism.

However, there was a big species difference in substrate specificity for CB183 and CB187. In PB-treated animals, the formation rate of 4-OH-CB187 from CB183 was higher in the following order: rats (158 pmol/hr/mg protein) ≫ hamsters (35) ≧ guinea pigs (33). In contrast, the formation rate of 4-OH-CB187 from CB187 was 14 and 10 pmol/hr/mg protein in PB-treated rats and guinea pigs, respectively and the production of 4-OH-CB187 from CB187 was not found in hamsters.[7] Considering from these results, it is apparent that liver microsomes of PB-treated animals metabolize CB183 more rapidly than CB187 to produce 4-OH-CB187. In addition, these results suggest that CB183 is a much better substrate for rat CYP2B1 and hamster CYP2B than guinea pig CYP2B18.

Hovander *et al.* have detected a small amount of 3'-OH-CB183 in addition to 4-OH-CB187 in the pooled plasma sample from Swedish males.[12] In this study, liver microsomes of all species (especially PB-treated) also produced 3'-OH-CB183 and the order of the amount of the metabolite was rats ≧ guinea pigs > hamsters. However, the ratio of 4-OH-CB187 and 3'-OH-CB183 was 2.5 for rats, 4.5 for hamsters and 0.57 for guinea pigs. This fact suggests that the metabolic pattern of CB183 in rats and hamsters is similar to that in human. Further studies on the distribution of CB183 metabolites in animals are needed to evaluate the toxicological significance of 4-OH-CB187.

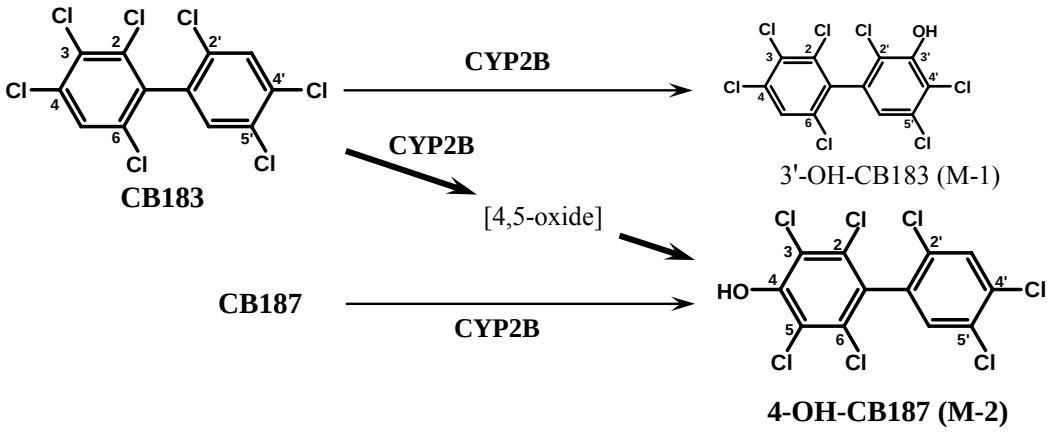

Figure 2 Postulated metabolic pathways of CB183 in animal liver

Acknowledgments
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