THE DISTRIBUTION OF METABOLITES OF 2,2',3,4,4',5',6-HEPTACHLOROBIPHENYL (CB183) IN RATS AND GUINEA PIGS

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Abstract

The *in vivo* metabolism of 2,2',3,4,4',5',6-heptachlorobiphenyl (CB183) was studied in rats and guinea pigs. Similarly to the *in vitro* metabolism, 3'-hydroxy (OH)-CB183 and 5-OH-CB183, were found as major metabolites in the serum and the feces of both species (serum < feces). Phenobarbital (PB)-pretreatment of animals increased the production of both metabolites, whereas 3-methylcholanthrene (MC)-pretreatment suppressed the CB183 metabolism in rats and guinea pigs. Our previous study reported that 5-methoxy (MeO)-CB183 had the same retention time as 4-MeO-2,2',3,4',5,5',6-heptachlorobiphenyl (CB187) on DB-1 capillary column. However, the use of SP-2330 capillary column resulted in an excellent separation of 5-MeO-CB183 and 4-MeO-CB187. From these results, it is concluded that 4-OH-CB187 could not be produced from CB183 in rats and guinea pigs.

Introduction

4-OH-metabolites of PCB congeners have been detected in human blood at higher concentrations[1-3] and have been shown to possess various toxicological activities to disturb homeostasis of thyroid hormone[4] and vitamin A in animal blood, to behave as an estrogen or antiestrogen,[5] to inhibit estrogen sulfotransferase,[6] to decrease cell-cell communication in gap junction[7] and also to act as an agonist for thyroid hormone receptor.[8] Among them, 4-OH-CB187 which is a PCB metabolite with the highest concentration in human blood, is thought to be presumably from CB187 or CB183, a minor component in PCB preparations.

Recently, we have demonstrated by the *in vitro* and *in vivo* studies that 4-OH-CB187 could be produced from CB187 in rats and guinea pigs and was exclusively detected in the blood but not in the feces.[9, 10] Moreover, we have reported that CB183 was metabolized to two OH-metabolites by liver microsomes of rats and guinea pigs.[11] From the data of GC-MS, one metabolite (M-1) was determined to be 3'-OH-CB183 but the methylated derivative of another one (M-2) looked like 4-MeO-CB187 or 5-MeO-CB183 in terms of retention time in GC. Therefore, to clarify whether 4-OH-CB187 is formed from CB183 or not, we examined the *in vivo* metabolism of CB183 in rats and guinea pigs. We report here that 4-OH-CB187 is not a metabolite of CB183 in both species.

Materials and Methods

CB183, 4-MeO-CB187 and 5-MeO-CB183 were synthesized by the method of Cadogan.[12] 3'-OH-CB183 was synthesized by the method of Hutzinger et al.[13] and methylated by diazomethane. Twelve male Wistar rats (body wt. about 200 g) and twelve male Hartley guinea pigs (body wt. about 300 g) were divided into untreated, PB- and MC-treated groups and administered PB and MC ip at a dose of 80 and 20 mg/kg/day for two days, respectively. Two days after the last injection of PB and MC, CB183 was injected ip at a single dose of 80 μmol/kg. Animals were sacrificed 4 days after administration of CB183 and blood was isolated. The feces were pooled during the experiment. Dry powdered feces were extracted with acetone-*n*-hexane (2:1, v/v) for 24 h in a Soxhlet apparatus. The serum (0.5 ml) was acidified with 0.5 M sulfuric acid (0.25 ml) and then extracted with chloroform-methanol (2:1, v/v) and *n*-hexane. The extracts were methylated with diazomethane and applied to GC-ECD (HP5890 Series II). CB183 and its metabolites

were quantified by a calibration curve of authentic CB183 for GC peak area. The GC conditions were as follows: column, DB-1 (30 m x 0.25 mm, 0.25 μm thickness) or SP-2330 (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.

Results and Discussion

Previously, we reported that 4-OH-CB187 can be produced from CB183 in the *in vitro* study using animal liver microsomes because the retention times of 4-MeO-CB187 and a CB183 metabolite (M-2), completely agreed in DB-1 capillary column (30 m length).[11] However, when SP-2330 capillary column (30 m length) was used, a candidate of CB183 metabolite (5-MeO-CB183) and 4-MeO-CB187 could be separated with the retention times of 23.69 min and 24.13 min, respectively (Fig. 1). As a result of reexamination of all metabolites found in the *in vitro* study and in this study, no 4-OH-CB187 was found in CB183 metabolism (data not shown).

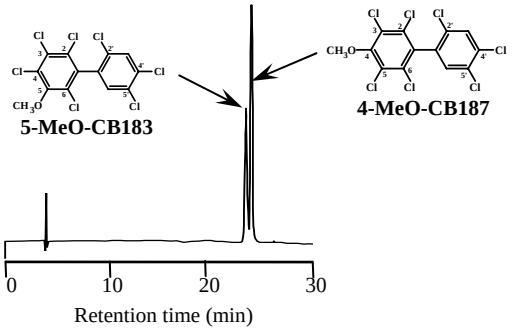

Fig. 1 Separation of two synthetic standards, 4-MeO-CB187 and 5-MeO-CB183, by GC-ECD with SP-2330 capillary column

Table 1 shows the concentrations of unchanged CB183 and two metabolites, 3'-OH-CB183 (M-1) and 5-OH-CB183 (M-2), in the serum of rats and guinea pigs 4 days after exposure to CB183. In untreated rats, in addition to CB183 (6.29 nmol/ml of serum), M-1 and M-2 were detected at the concentrations of 0.23 and 0.37 nmol/ml of serum, respectively. In untreated guinea pigs, the concentrations of CB183, M-1 and M-2 were 4.74, 1.00 and 0.17 nmol/ml of serum. M-1 was about 6 times higher level than M-2. Similarly to our *in vitro* study, [11] PB-treatment increased both M-1 and M-2 to 1.5- to 1.7-fold of untreated in rats and only M-2 to 1.8-fold of untreated in guinea pigs. On the other hand, MC-treatment decreased both metabolites to less than 60 % of untreated ones in both species. These results suggest that PB-treatment is more effective to accelerate CB183 metabolism in rats and guinea pigs.

Fecal excretion of CB183, M-1 and M-2 in rats and guinea pigs are shown in Table 2. In addition to unchanged CB183, both M-1 and M-2 were observed in 4-days feces at much higher concentration than those in the serum of both animal species. M-1 and M-2 were 5.7 and 3.4 nmol/g of dry feces in untreated rats and also 4.1 and 0.8 nmol/g of dry feces in untreated guinea pigs, respectively. PB-treatment accelerated fecal excretions of M-1 and M-2 to about 2 to 3-fold of untreated rats and increased them slightly in guinea pigs. MC-treatment decreased both metabolites in rat feces and M-2 in guinea pig feces in the similar manner to those in the serum. Our previous study[10] showed that, in guinea pigs dosed with CB187, 4-OH-CB187 was distributed exclusively to the blood but was not found in the feces at all. In this study, we observed relatively high amount of M-2 (5-MeO-CB183) in the feces of guinea pigs and rats. Thus, the result that M-2 showed a different distribution pattern from 4-OH-CB187 supported the fact that M-2 is not 4-OH-CB187.

The postulated pathways in rats and guinea pigs are shown in Fig. 2. We found two metabolites (M-1 and M-2) in the serum and feces of rats and guinea pigs and the chemical structures of M-1 and M-2 were finally determined to 3'-OH-CB183 and 5-OH-CB183, respectively. It is apparent that both metabolites are produced by a direct hydroxylation mechanism by PB-inducible cytochrome P450 isoforms such as rat CYP2B1 and guinea pig CYP2B18.[9, 11]

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Table 1 Concentration of CB183 and its metabolites in the serum of untreated, PB-treated and MC-treated rats and guinea pigs on day 4 after injection of CB183

Treatment	Concentration (nmol/ml of serum)		
	CB183	M-1	M-2
Rat			
Untreated	6.29 ± 3.99	0.23 ± 0.18	0.37 ± 0.38
	(100)	(100)	(100)
PB-treated	5.76 ± 1.40	0.39 ± 0.18	0.57 ± 0.31
	(92)	(170)	(154)
MC-treated	5.12 ± 2.13	0.13 ± 0.11	0.21 ± 0.16
	(81)	(57)	(57)
Guinea pig			
Untreated	4.74 ± 1.33	1.00 ± 0.41	0.17 ± 0.08
	(100)	(100)	(100)
PB-treated	3.80 ± 1.17	0.52 ± 0.30	0.30 ± 0.14
	(80)	(52)	(176)
MC-treated	5.23 ± 0.97	0.55 ± 0.28	0.04 ± 0.08
	(110)	(55)	(24)

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

* Significantly different from untreated animals, $p<0.05$.

Table 2 Fecal excretion of CB183 and its metabolites in untreated, PB-treated and MC-treated rats and guinea pigs during the exposure of CB183

	Treatment	Concentration (nmol/g of dry feces) CB183	M-1	M-2
Rat				
	Untreated	27.11 ± 11.19 (100)	5.66 ± 4.56 (100)	3.39 ± 3.08 (100)
	PB-treated	65.02 ± 9.35* (240)	10.87 ± 3.05 (192)	11.41 ± 2.28* (337)
	MC-treated	60.67 ± 31.30 (224)	2.33 ± 0.58 (39)	1.91 ± 0.37 (56)
Guinea pig				
	Untreated	18.38 ± 1.23 (100)	4.11 ± 0.59 (100)	0.81 ± 0.12 (100)
	PB-treated	11.52 ± 4.41* (63)	5.01 ± 1.85 (122)	1.07 ± 0.47 (132)
	MC-treated	8.45 ± 2.02* (46)	3.95 ± 2.65 (96)	0.57 ± 0.08* (70)

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

* Significantly different from untreated animals, $p<0.05$.

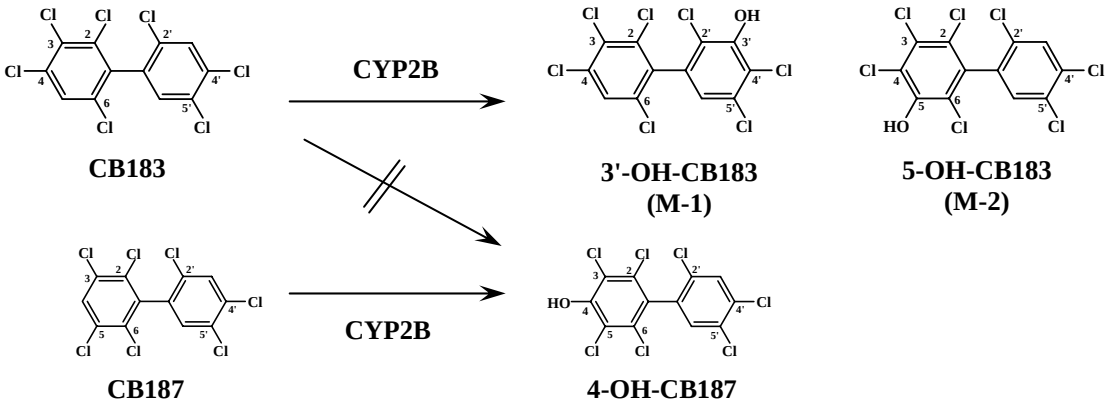

Fig. 2 Postulated metabolic pathways of CB183 in animals