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METABOLISM OF 2,2',3,4,4',5,6'-HEPTACHLOROBIPHENYL (CB182) BY RAT, GUINEA PIG AND HUMAN LIVER MICROSOMES

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Introduction

2,4,5-trichloro-substituted PCBs such as CB153, CB138, CB180 and CB187 have been detected in human blood and adipose tissues at much higher concentrations than other PCB congeners (1-5). Particularly, CB187 is a well-known PCB congener because 4-hydroxy (OH)-metabolite of CB187 has been reported to be present persistently in human blood at the highest concentration of other PCB metabolites (1-3,5,6). We previously reported that 4-OH-CB187 was a minor metabolite of CB187 in rats and guinea pigs (7) and, recently, demonstrated that 4-OH-CB187 was detected only in rat and guinea pig serum, but not in their livers and feces during the 4 to 30 days following CB187 injection (8).

On the other hand, the concentration of CB187 in the commertial PCB mixture and in human tissues such as serum and breast milk has been analyzed as the sum of CB187 and CB182, another heptachlorobiphenyl (9-11), because the retention time of CB187 and CB182 in GC was very close each other. In addition, there is little report about in vitro metabolism of CB182 in animals. Therefore, we compared metabolism of CB182 by liver microsomes of rats, guinea pigs and human and also xamined which capillary column, DB-1 or SP-2330 column, is better for separation of both PCBs.

Materials and methods

CB182 and its metabolite were synthesized by the method of Cadogan (12). The chemical purity of the compounds was >98% as determined by GC. Liver microsomes from male Wistar rats (body weight about 200 g) and Hartley guinea pigs (body weight about 300 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. CB182 (40 μM) was incubated at 37ºC for 60 min with 0.33 mM NADPH-generating system, 6 mM $MgCl_2$, 100 mM HEPES buffer (pH 7.4) and 1 mg protein of rat or guinea pig liver microsomes in a total volume of 1 ml. After incubation, unchanged CB182 and its metabolite were extracted three times with the mixture of 1 ml of chloroform-methanol (2:1, v/v) and 3 ml of n-hexane. The organic layer was pooled and evaporated to dryness. The residue was methylated with diazomethane and applied to GC-ECD and GC-MS. The metabolite M-1 was quantified by a calibration curve of authentic CB182 for the peak area in GC. The conditions of GC-ECD (HP5890 Series II) were as follows: column, DB-1 capillary column (30 m x 0.25 mm, 0.25 μm thickness) and SP-2330 capillary column (30 m x 0.25 mm, 0.20 μm thickness); carrier gas, N_2 (1 ml/min); column temp., 230ºC; injection port temp., 250ºC; detector temp., 250ºC. The conditions of GC-MS (Shimadzu QP2010) were: column, DB-1 capillary column (30 m x 0.25 mm i.d., 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 and Discussion

When a 30 m DB-1 column was used, CB182 and CB187 was detected at the same retention time in GC. However, a 30 m SP-2330 column could separate CB182 and CB187 at a retention time of 12.25 min and 13.17 min, respectively (data not shown). Next, CB182 was incubated for 60 min with liver microsomes of rats, guinea pigs and human. As a result, a metabolite named M-1 was observed at a retention time of 20.15 min on SP-2330 column (Fig. 1) and of 19.73 min on DB-1 column in GC-ECD. In rats, untreated microsomes produced only trace amount of M-1. PB-treated microsomes metabolized CB182 to M-1 at an extremely higher rate of 1,367 pmol/hr/mg protein. This activity for CB182 was about 10 times higher than that for CB187 (7). However, MC-treated microsomes did not produce M-1 at all. In guinea pigs, all microsomes produced M-1 at rates of around 20 pmol/hr/mg protein. On the other hand, pooled human liver microsomes showed lower activity to produce M-1 than guinea pig microsomes.

To get some information about chemical structure of the metabolite, large scale incubation (100 ml) using liver microsomes of PB-treated rats was conducted at 37ºC for 60 min. CB182 and M-1 extracted with the organic solvents were methylated by diazomethane and applied to GC-MS. The mass spectral data and retention time of the methylated M-1 were shown in Table 1. The methylated M-1 had the molecular weight of 422 and the potent fragment ion of $[M^{+}-43]$ (m/z 379), indicating that it was the metabolite with methoxy-group at meta-position. Moreover, its mass fragmentation and retention time in GC-MS was completely agreed with synthetic 3'-methoxy-CB182, assuming that M-1 was 3'-OH-CB182. The postulated metabolic pathway is shown in Fig. 2.

Our previous study demonstrated that CB187 was metabolized in guinea pigs more rapidly than in rats (7,8). In this study, CB182 was hydroxylated at 3'-position very easily by liver microsomes of PB-treated rats. In contrast to rats, the formation of 3'-OH-CB182 was little affected by pretreatment of PB in guinea pigs. Although the reason why the metabolic profile of CB182 was different from that of CB187 is unclear at present, it would be considered that CB182 is a good substrate for rat CYP2B1, but not for guinea pig CYP2B18 and human CYP2B4. Also, it appears that rat CYP2B1 prefers 2,4,6-trichloro-substituted PCBs rather than 2,4,5-trichloro-substituted ones.

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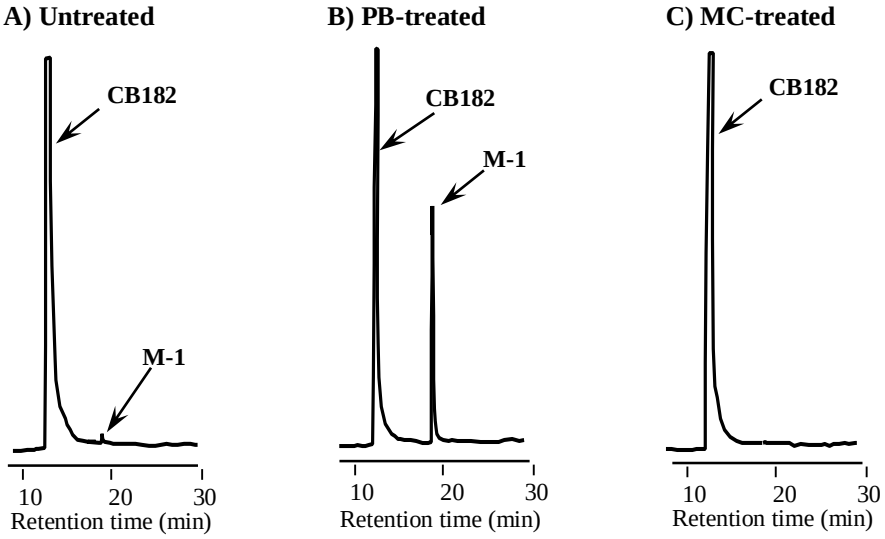

Fig. 1 GC-ECD chromatograms of the methylated derivative of a CB182 metabolite formed by liver microsomes of untreated (A), PB-treated (B) and MC-treated (C) rats

Table 1 Metabolism of CB182 by liver microsomes of rats, guinea pigs and humans and effects of cytochrome P450 inducers on CB182 metabolism

	M-1 formed (pmol/hr/mg protein)		
Animal	Untreated	PB-treated	MC-treated
Rat	B.D.	1368.8 ± 162.4	N.D.
Guinea pig	18.7 ± 12.6	26.7 ± 11.7	17.7 ± 11.1
Human*	14.3 ± 2.0	-	-

N.D., not detected. B.D., below detection limit.
Each value represents the mean ± S.D. of four animals.
*The value represents the mean ± S.D. of triplicate determination.

Table 2 Mass spectral data and retention times of the methylated derivative of a CB182 metabolite and its synthetic compound

Compound	Molecular weight	Mass spectral data				Retention time (min)
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	$[M^+-50]$	
M-1	422	100	47	27	14	15.54
3'-CH_3O-CB182	422	100	45	29	18	15.54

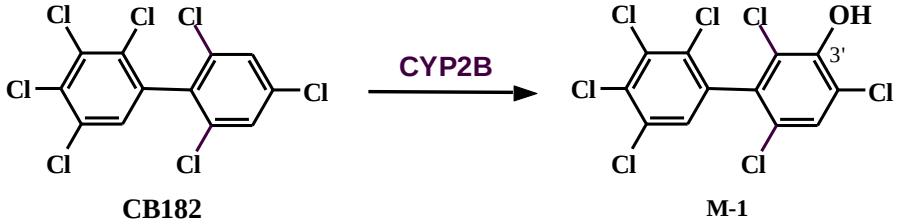

Fig. 2 Postulated metabolic pathway of CB182 in the liver