SPECIES DIFFERENCES IN THE IN VITRO METABOLISM OF 2,2',3',4,4',5-HEXACHLOROBIPHENYL

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Introduction

PCBs demonstrate extremely high lipophilicity and biological stability, and as a result these substances are not easily eliminated from the body once they are ingested. However, the extent of their biotransformation varies greatly in the congeners and isomers and from species to species. The initial event of biotransformation of PCBs is hydroxylation catalyzed by liver microsomal cytochrome P450. The metabolism of PCBs depends upon the number of chlorine on the biphenyl ring and the 5 or more chlorine-substituted PCBs can be metabolized much more slowly than the lower chlorinated biphenyls.

Of all animal species, dogs and guinea pigs have been known to show much higher activity to metabolize the 5 or more chlorine-substituted PCBs such as 2,2',3',4,4',5-hexachlorobiphenyl (HCB) (CB138) and 2,2',4,4',5,5'-HCB (CB153)[1-3], which were detected in blood and adipose tissues of mammals and human mother's milk at high concentration. In addition, Ariyoshi *et al.*[2,3] found three major metabolites, namely 3-hydroxy (OH)-CB153, 2-OH-2',3,4,4',5,5'-HCB and 2-OH-2',4,4',5,5'-pentachlorobiphenyl (PenCB) in the feces of guinea pigs administered with CB153. On the other hand, there is no report about metabolism *in vivo* or *in vitro* of CB138 in animals. Therefore, we examined CB138 metabolism by liver microsomes of rats, hamsters and guinea pigs and found that there is a big species difference in CB138 metabolism.

Materials and Methods

CB138 was synthesized from 2,4,5-trichloroaniline and 1,3,4-trichlorobenzene as starting materials according to the method of Cadogan[4]. 2-Methoxy (MeO)-3,4,5,2',3',4'-HCB (S-4) was synthesized from 2,3,4-trichloroaniline and 2,3,4-trichlorophenol by the method reported previously[2]. Similarly, 3-MeO-CB138 (S-3a) and 4-MeO-2,3,5,2',3',4'-HCB (S-3b) were obtained by the method of Cadogan[4] from 2,3,6-trichloroanisole and 2,3,4-trichloroaniline.

Nine male Wistar rats (body wt. about 170 g), nine male Golden Syrian hamsters (body wt. about

90 g) and nine male Hartley guinea pigs (body wt. about 280 g) were used and divided to three groups, untreated, PB- and 3-methylcholanthrene (MC)-pretreated groups. PB and MC were dissolved in saline and corn oil, and injected intraperitoneally at a dose of 80 and 20 mg/kg/day for 3 days, respectively. Animals were killed the next day after the last injection of each P450 inducer and their livers were removed. Liver microsomes were prepared by a conventional centrifugation method.

CB138 was incubated for 2 hr at 37℃ with NADPH-generating system, $MgCl_2$ and animal liver microsomes in 100 mM HEPES buffer (pH 7.4) under aerobic conditions. After extraction with organic solvents such as chloroform-methanol (2:1) and *n*-hexane and methylation by diazomethane, CB138 and its metabolites were analyzed using a gas chromatograph HP5890 Series II equipped with an electron capture detector (ECD) under the conditions as follows: column, DB-1 capillary column (30 m x 0.25 mm i.d., 0.33 μm thickness); carrier gas, N_2 (1 ml/min); column temp., 230℃; injection port temp., 250℃; detector temp., 250℃.

Results

Species differences in the *in vitro* metabolism of CB138 was examined using liver microsomes from rats, hamsters and guinea pigs. The order of ability to metabolize CB138 was guinea pigs ≫ rats = hamsters. As shown in Fig. 1, guinea pig liver microsomes formed four metabolites named as M-1, M-2, M-3 and M-4 with retention times of 17.08 min, 17.28 min, 21.68 min and 22.09 min, respectively, in GC/ECD and all metabolites were increased to about 4-5 fold of untreated microsomes by PB-pretreatment. On the other hand, rats and hamsters showed much less activity to metabolize CB138 than guinea pigs. Only PB-treated microsomes produced very small amounts of M-3 in rats and M-1, M-2 and M-3 in hamsters, whereas untreated and MC-treated microsomes did not.

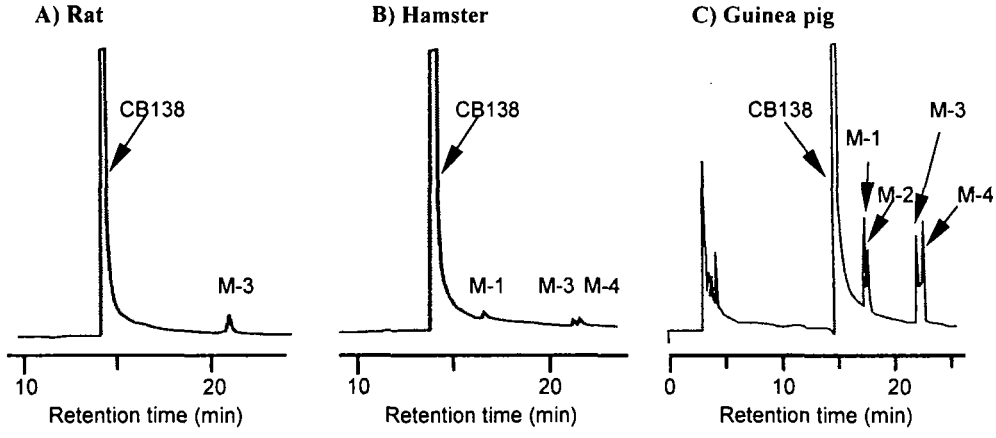

Fig. 1. Gas chromatograms of the methylated derivatives of CB138 metabolites formed by liver microsomes of PB-treated rats (A), hamsters (B) and guinea pigs (C).

When mass spectra of the methylated derivatives of M-1, M-2, M-3 and M-4 were measured in

GC/MS, the former two possess the molecular ion of 354 and the latter two had the molecular ion of 388 (Table 1). In addition, the mass fragmentation pattern indicated that both M-2 and M-3 having a fragment ion [M^+-43] are substituted with a methoxy group at 3(3')- or 5(5')-position and also M-1 and M-4 have a methoxyl group at 2(2')- or 6(6')-position because the high intensity of a fragment ion [M^+-50] was observed. From these results, they were assumed to be 2-OH-2',3',4,4',5-PenCB (M-1), 5-OH-2,2',3',4,4'-PenCB (M-2), 3-OH-CB138 (M-3) and 2-OH-2',3,3',4,4',5-HCB (M-4), respectively.

To determine the chemical structures of M-3 and M-4, 3-MeO-CB138 (S-3a) and 2-MeO-2',3,3',4,4',5-HCB (S-4) were synthesized and compared their chromatographic behaviors in GC/MS and GC/ECD with those of the methylated derivatives of M-3 and M-4. As a result, the data of S-3a and S-4 were in almost complete agreement with those of M-3 and M-4, respectively (Table 1).

Table 1 Mass spectral data and retention times of methylated derivatives of four CB138 metabolites and three synthetic compounds in GC/MS and GC/ECD

Compound	Molecular weight	Mass spectral data					Retention time (min)
		[M^+]	[M^+-15]	[M^+-43]	[M^+-50]	[M^+-70]	
CB138	358	100	-	-	-	61	14.57
M-1	354	100	-	-	88	-	17.08
M-2	354	100	-	22	41	-	17.28
M-3	388	100	-	32	13	-	21.68
M-4	388	100	-	-	101	-	22.09
S-3a	388	100	8	40	15	-	21.68
S-3b	388	100	54	46	-	-	21.86
S-4	388	100	-	-	155	17	22.09

Discussion

The metabolizing activity for CB138 was the highest in guinea pigs, and was stimulated by PB-pretreatment in every animal species examined. The postulated pathways of CB138 in guinea pig liver are shown in Fig. 2. In guinea pigs, four major metabolites were produced not only in PB-treated microsomes but also in untreated microsomes. This fact suggests that CB138 metabolism in guinea pig liver is mainly catalyzed by a constitutive and PB-inducible P450, namely CYP2B18[5-7]. Interestingly, CB138 possesses strong PB-type inducing ability of drug metabolizing enzymes in rat liver similarly to CB153[8].

Two mechanisms have been considered so far for the hydroxylation of aromatic hydrocarbons including PCBs; 1) an arene oxide formation followed by rearrangement, and 2) an insertion of oxygen between carbon-hydrogen bond (direct hydroxylation). The formation of an arene oxide often results in an NIH-shift of either the hydrogen or chlorine atom. In this study, we observed the formation of 2-OH-2',3,3',4,4',5-HCB (M-4) and 2-OH-2',3',4,4',5-PenCB (M-1) as major metabolites, which indicates that CB138 metabolism in guinea pigs principally proceeds via 2,3-

epoxide as an intermediate. In contrast, only 3-OH-CB138 (M-3) was formed by liver microsomes of PB-treated rats. This result suggests that M-3 might be formed in a direct hydroxylation mechanism. In conclusion, it is apparent that the metabolic pattern of CB138 in animals is similar to that of CB153 reported by Ariyoshi et al.[2,3].

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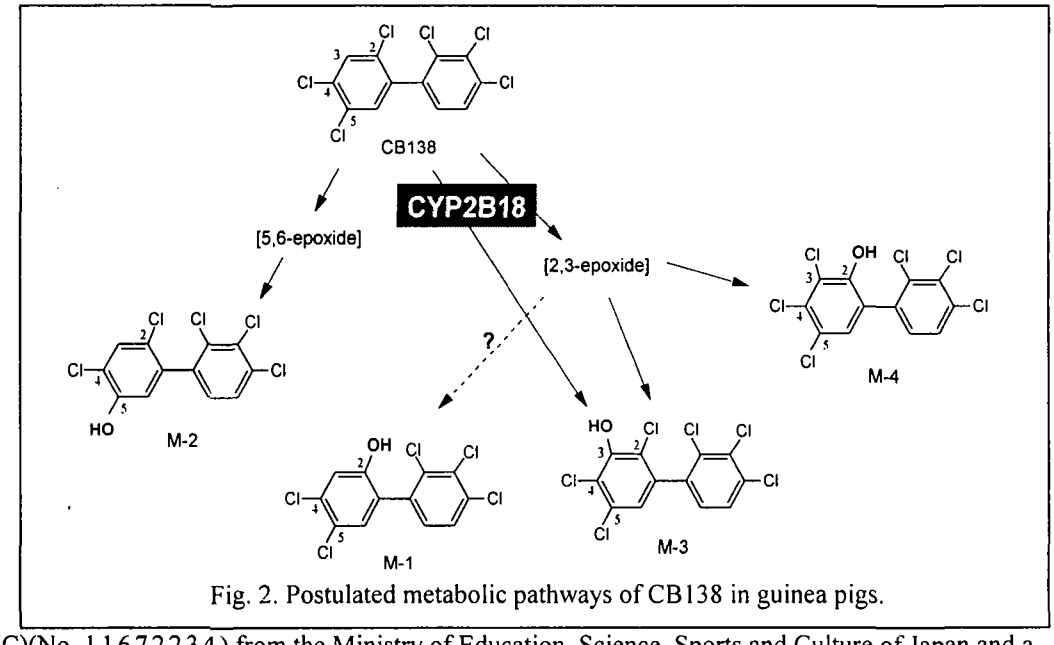

Fig. 2. Postulated metabolic pathways of CB138 in guinea pigs.

References

1. Schnellmann, R.G., Vickers, A.E.M. and Sipes, I.G. (1985) Biochem. Toxicol. 7, 247-282.
2. Ariyoshi, N., Koga, N., Oguri, K. and Yoshimura, H. (1992) Xenobiotica 22, 1275-1290.
3. Ariyoshi, N., Koga, N., Yoshimura, H. and Oguri, K. (1997) Xenobiotica 27, 973-983.
4. Cadogan, J.I.G. (1962) J. Chem. Soc., 4257-4258.
5. Ariyoshi, N., Oguri, K., Koga, N., Yoshimura, H. and Funae, Y. (1995) Biochem. Biophys. Res. Commun. 212, 455-460.
6. Koga, N., Kanamaru, T., Kikuichi, N., Oishi, N., Kato, S. and Yoshimura, H. (1998) Bull. Environ. Contamin. Toxicol. 60, 898-903.
7. Koga, N., Kikuichi, N., Kanamaru, T., Kuroki, H., Matsusue, K., Ishida, C., Ariyoshi, N., Oguri, K. and Yoshimura, H. (1998) Chemosphere 37, 1985-1904.
8. Parkinson, A., Safe, S.H., Robertson, L.W., Thomas, P.E., Ryan, D.E., Reik, L.M. and Levin, W. (1983) J. Biol. Chem. 258, 5967-5976.