IN VITRO METABOLISM OF 2,2',4,4',5-PENTACHLOROBIPHENYL (CB99) BY RAT AND GUINEA PIG LIVER MICROSOMES

Ohta C[1]*, Haraguchi K[2], Kato Y[3], Endo T[4], Kimura O[4], Koga N[1]

[1] Faculty of Nutritional Sciences, Nakamura Gakuen University, Fukuoka, Japan; [2] Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan; [3] Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Kagawa, Japan; [4] Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan

Introduction

2,4,5-trichloro-substituted PCB congeners such as CB153, CB138 and CB180 are well-known as persistent organic compounds present at high level in human blood and liver[1-3]. Recently, Todaka *et al.* have analyzed all PCB congeners in the blood of Yusho patients 37 years after the outbreak of Yusho poisoning and demonstrated that 2,4,5-trichloro- or 2,3,4,5-tetrachloro-substituted PCB congeners such as CB153, CB180, CB138, CB182/187, CB170, CB156 and CB146 were detected in Yusho patients at 1.6 to 3.9 times higher concentration than those in normal controls[4]. According to a series of our studies on PCB metabolism using animal liver microsomes, the 2,4,5-trichloro- or 2,3,4,5-tetrachloro-substituted PCB congeners are metabolized at very slow rates and a species difference between rats and guinea pigs is observed in the metabolic pattern of each PCB congener[5-12]. In addition, they have also reported that blood concentration of another 2,4,5-trichloro-substituted PCB congener, 2,2',4,4',5-pentachlorobiphenyl (pentaCB) (CB99), in Yusho patients was 1.4-fold that of normal controls[4]. However, there is no report on CB99 metabolism by animal liver microsomes. Therefore, we examined the *in vitro* metabolism of CB99 by rat and guinea pig liver microsomes and the effects of cytochrome P450 inducers, phenobarbital (PB) and 3-methylcholanthrene (MC), on the metabolism.

Materials and methods

CB99 and its three metabolites were synthesized by the method of Cadogan[13]. The chemical purities of these compounds were more than 91% as determined by GC. Liver microsomes from male Wistar rats (body weight about 200 g) and Hartley guinea pigs (body weight about 280 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. CB99 (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 liver microsomes in a total volume of 1 ml. After incubation, unchanged CB99 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 organic layer was pooled and evaporated to dryness. The residue was methylated with diazomethane and applied to GC-ECD and GC-MS. CB99 metabolites were quantified by a calibration curve of authentic CB99 for GC peak area. The conditions of GC-ECD (HP5890 Series II) were: column, DB-1 capillary column (30 m x 0.25 mm id, 0.25 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 as follows: 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

The effects of cytochrome P450 inducers, PB and MC, on CB99 metabolism were examined using rat and guinea pig liver microsomes. GC-ECD chromatograms of the methylated derivatives of CB99 metabolites formed by liver microsomes of PB-treated rats and guinea pigs were shown in Fig. 1. In PB-treated rats, two metabolites (M-1 and M-2) were detected at retention times of 16.7 min and 18.8 min, and were produced at rates of 164 and 61 pmol/hr/mg protein, respectively. On the other hand, PB-treated guinea pigs produced M-1 at a rate of 10 pmol/hr/mg protein. However, untreated and MC-treated liver microsomes of both animals did not metabolize CB99 at all. These results suggest that PB-inducible CYP2B enzymes, namely rat CYP2B1 and

guinea pig CYP2B18[7], are responsible for CB99 metabolism and the activity of rat CYP2B enzymes for CB99 is much higher than that of guinea pig CYP2B enzymes.

To elucidate the chemical structures of two metabolites, we performed the large scale incubation (100 ml) for 60 min with liver microsomes of PB-treated rats, methylated two metabolites with diazomethane and applied to GC-MS. As shown in Table 1, the GC-MS data of the methylated metabolites were compared to those of synthetic authentic compounds. The mass fragmentation and retention times of methylated derivatives of M-1 and M-2 in GC-MS agreed well with 3'-methoxy (MeO)-CB99 and 5'-MeO-CB99, suggesting that M-1 and M-2 are 3'-hydroxy (OH)-CB99 and 5'-OH-CB99, respectively.

We previously observed species differences in the metabolism of 2,4,5-trichloro-substituted PCB congeners such as CB187, CB138, CB153, CB180, CB149, CB146 and CB101 by animal liver microsomes. Guinea pig liver microsomes metabolized CB187[7], CB138[6], CB153[5], CB180[12] and CB146[10] to their OH-metabolites more rapidly than rat liver microsomes. In contrast, rat liver microsomes metabolized CB149[11] and CB101[9] much more rapidly than guinea pig microsomes. In this study, CB99 was metabolized in rats at higher rate than in guinea pigs as well as CB149 and CB101. From these results, it is suggested that rat CYP2B enzymes catalyze the *meta*-hydroxylation of 2,4- or 2,5-dichlorobenzene and 2,3,6-trichlorobenzene moiety more preferentially than the 3-hydroxylation of 2,4,5-trichlorobenzene moiety.

Acknowledgements
This work was partially supported by a Health and Labour Scientific Research Grants (H25-food-designated-014, Koga N) from Ministry of Health, Labour and Welfare of Japan, and a Grant-in-Aid for Scientific Research (B) (No. 24310049, Haraguchi K) and a Grant-in-Aid for Scientific Research (C) (No. 23510083, Kato Y) from Japan Society for the Promotion of Science.

References

1. Fängström B, Athanasiadou M, Grandjean P, Weihe P, Bergman Å. (2002); *Environ Health Perspect.* 110: 895-9
2. Sandala GM, Sonne-Hansen C, Dietz R, Muir DC, Valters K, Bennett ER, Born EW, Letcher RJ. (2004); *Sci Total Environ*. 331: 125-41
3. Park J-S, Linderholm L, Charles MJ, Athanasiadou M, Petril J, Kocan A, Drobna B, Trnovec T, Bergman Å, Hertz-Picciotto I. (2007); *Environ Health Perspect.* 115: 20-7
4. Todaka T, Hori T, Yasutake D, Yoshitomi H, Hirakawa H, Onozuka D, Kajiwara J, Iida T, Yoshimura T, Furue M. (2009); *Fukuoka Igaku Zasshi* 100: 156-65
5. Ariyoshi N, Oguri K, Koga N, Yoshimura H, Funae Y. (1995); *Biochem Biophys Commun Res*. 212: 455-60
6. Koga N, Kanamaru T, Oishi N, Kato Y, Kimura R, Haraguchi K, Masuda Y. (2001); *Fukuoka Igaku Zasshi* 92: 167-76
7. Ohta C, Haraguchi K, Kato Y, Koga N. (2005); *Xenobiotica* 35: 319-30
8. Ohta C, Haraguchi K, Kato Y, Ozaki M, Koga N. (2006); *Organohalogen Compd.* 68: 1733-6
9. Koga N, Ohta C, Haraguchi K, Matsuoka M, Kato Y, Endo T. (2007); *Organohalogen Compd.* 69: 1757-60
10. Ohta C, Haraguchi K, Kato Y, Endo T, Matsuoka M, Koga N. (2008); *Organohalogen Compd.* 70: 1185-8
11. Ohta C, Haraguchi K, Kato Y, Endo T, Koga N. (2009); *Organohalogen Compd.* 71: 298-301
12. Ohta C, Haraguchi K, Kato Y, Endo T, Matsubara F, Koga N. (2010); *Organohalogen Compd.* 72: 1796-9
13. Cadogan JIG. (1962); *J Chem Soc*. 4257-8

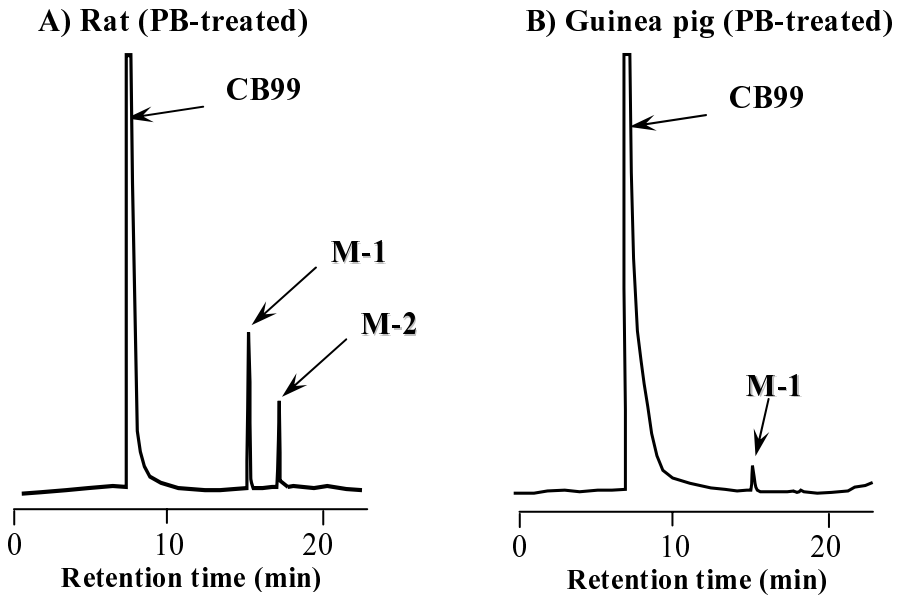

Fig. 2 GC-ECD chromatograms of the methylated derivatives of CB99 metabolites formed by liver microsomes of PB-treated rats (A) and PB-treated guinea pigs (B)

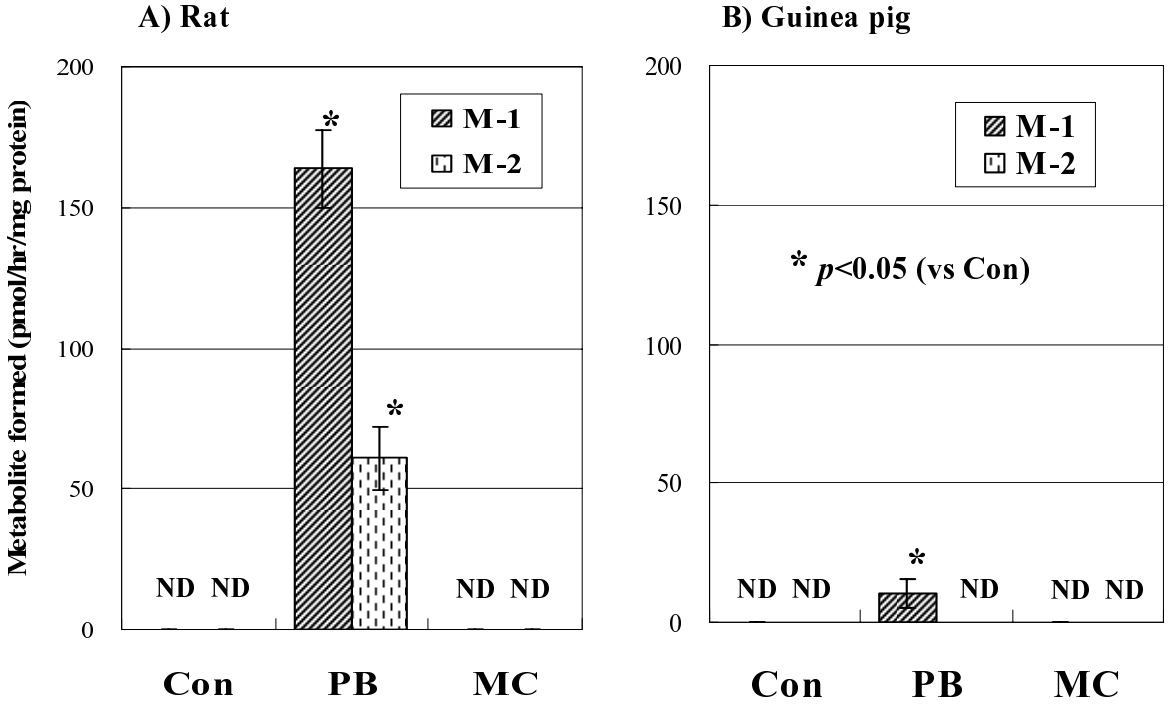

Fig. 3 Effects of cytochrome P450 inducers on CB99 metabolism by liver microsomes of rats (A) and guinea pigs (B). Each bar represents mean ± S.D. of four animals.

Table 1 Mass spectral data and retention times of the methylated derivatives of two CB99 metabolites and their synthetic compounds

Compound	Molecular weight	Mass spectral data (Relative abundance, %)				Retention time (min) in GC-MS
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	$[M^+-50]$	
M-1 (methylated)	354	100	11	38	8	12.34
M-2 (methylated)	354	100	32	39	4	12.91
2'-MeO-CB120	354	100	3	-	169	11.80
3'-MeO-CB99	354	100	19	39	12	12.34
3'-MeO-CB102	354	100	18	38	3	12.36
5'-MeO-CB99	354	100	16	32	7	12.91
4'-MeO-CB120	354	100	76	34	-	13.21
3-MeO-CB99	354	100	8	32	11	13.61

-, not detected. CB102, 2,2',4,5,6'-pentaCB; CB120, 2,3',4,5,5'-pentaCB.

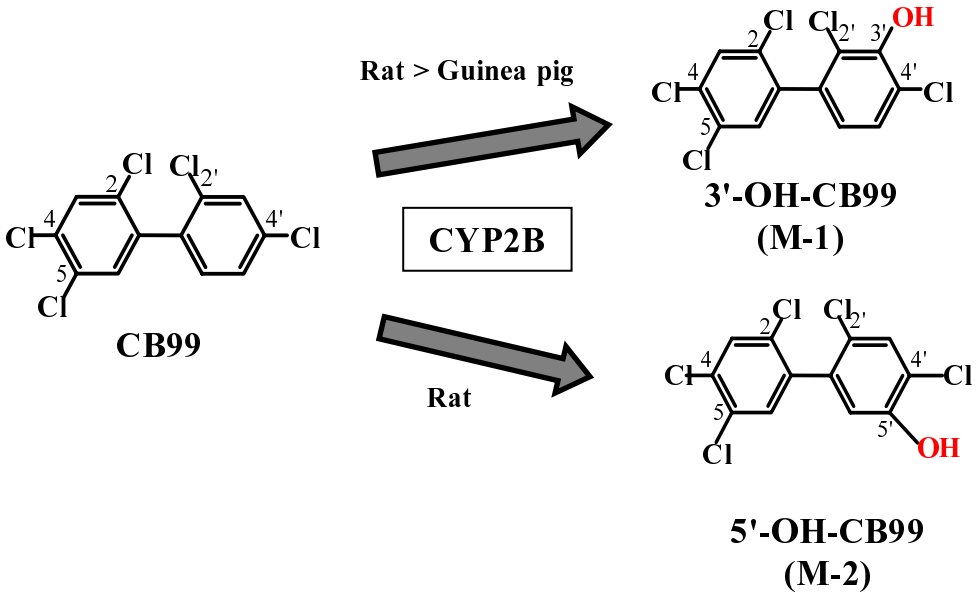

Fig. 3 Postulated metabolic pathways of CB99 in rat and guinea pig liver