In vivo metabolism of 2,2',3,4',5,5',6-heptachlorobiphenyl (CB187) in guinea pigs

Chiho Ohta[1], Koichi Haraguchi[2], Yoshihisa Kato[3], Nobuyuki Koga[1]

[1]Nakamura Gakuen University
[2]Daiichi College of Pharmaceutical Sciences
[3]University of Shizuoka

Introduction

2,2',3,4',5,5',6-Heptachlorobiphenyl (heptaCB) (CB187), one of the minor components in commercial PCB preparations, have been detected in the liver, adipose tissue and mother's milk of mammals at high concentration[1,2] as well as other 2,4,5-trichloro-substituted PCBs such as 2,2',3,4,4',5'-hexachlorobiphenyl (hexaCB) (CB138) and 2,2',4,4',5,5'- hexaCB (CB153). On the other hand, 4-hydroxy (OH)-CB187 have been reported to be present in human blood at the highest concentrations among 4-OH-metabolites of various PCB congeners. [1-3] Malmberg *et al*.[4] have reported that the half life of 4-OH-CB187 was 15.4 days in rat blood after an iv injection of the metabolite and was about 4 times longer than that of 4-OH-2,3,3',4',5-pentaCB (CB107), a major metabolite of 2,3',4,4',5-pentaCB (CB118). Our preceding studies showed that the guinea pig possesses high activity to hydrolyze some persistent PCBs such as CB138 and CB153.[5,6]

Very recently, we have demonstrated 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 guinea pig liver microsomes and that the formation of such metabolites is principally catalyzed by a phenobarbital (PB)-inducible guinea pig cytochrome P450 (P450), CYP2B18.[7] However, there is little report on the *in vivo* metabolism of CB187 in animals. Therefore, we examined the distribution of three OH-metabolites to the blood and feces of guinea pigs administered ip with CB187, and the effect of P450 inducers, PB and 3-methylcholanthrene (MC) was also investigated.

Materials and Methods

CB187 and its metabolites (4'-OH-CB151, 4'-OH-CB178 and 4-methoxy-CB187) were synthesized as described previously.[7] Twelve male Hartley guinea pigs (body weight about 280 g) were divided into untreated, PB- and MC-pretreated groups. Guinea pigs were administered ip with PB and MC at a dose of 80 and 20 mg/kg/day for two days, respectively, and two days after the last injection of PB and MC, CB187 was injected ip at a single dose of 80 μmol/kg. Guinea pigs were sacrificed 4 days after administration of CB187 and livers and blood were isolated. The feces were also pooled for 4 days. Dry powdered feces were extracted with acetone-*n*-hexane (2:1, v/v) for 24 h in a Soxhlet apparatus. 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.

For quantification of CB187 and its metabolites, the extracts were methylated with dimethyl sulfate (for feces) or diazomethane (for serum) and applied to GC/ECD using a gas chromatograph HP5890 Series II equipped with ECD. 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., 230ºC; detector temp., 250ºC. GC/MS was performed using Agilent 5973 inert MSD under the following conditions: HP-5 fused capillary column (60 m x 0.25 mm, 0.25 mm 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

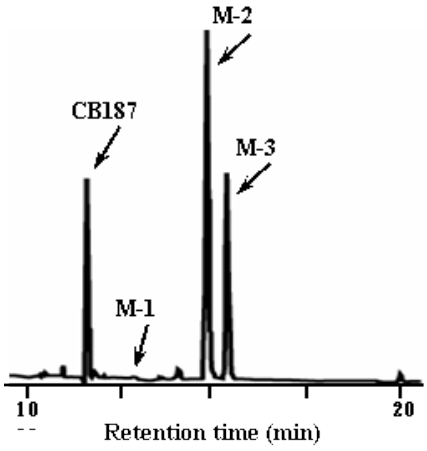

Figure 1: Gas chromatogram of CB187 metabolites in guinea pig serum.

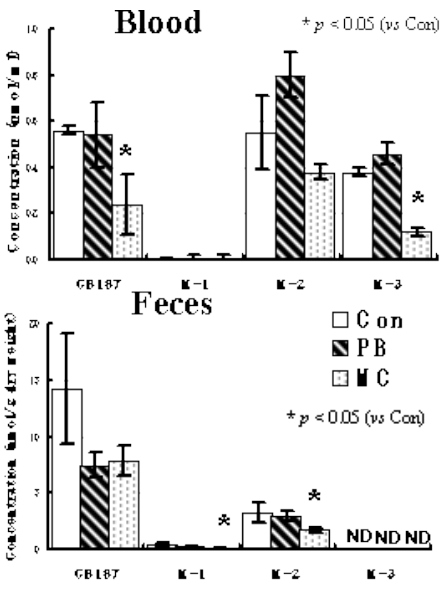

Figure 2: Effect of P450 inducers on the amount of CB187 metabolites in the blood and feces of guinea pigs.

Figure 1 shows a typical gas chromatogram of CB187 and its methylated metabolites found in guinea pig serum 4 days after CB187 administration. In the serum, M-2 and M-3 which corresponded to 4'-OH-CB178 and 4-OH-CB187, respectively, were detected as major metabolites in addition to unchanged CB187. A trace amount of M-1, 4'-OH-CB151 was also observed. On the other hand, in the 4-days feces, only M-2 was a major metabolite and M-1 was also detected as a minor metabolite. However, no M-3 was found (data not shown).

The effect of P450 inducers on the *in vivo* metabolism of CB187 in guinea pigs is shown in Figure 2. In the serum of untreated guinea pigs, the concentration of M-2 and M-3 was 0.55 and 0.38 nmol/ml of serum, respectively. PB-pretreatment increased the formation of M-2 and M-3 to 1.5-fold and 1.2-fold of control animals, whereas MC-pretreatment decreased both metabolites to 74% and 36% of control animals, respectively. In the 4 days-feces, the amount of M-1 and M-2 was determined to be 0.47 and 3.30 nmol/g dry weight of feces, respectively. Both metabolites were decreased to 57% and 89% of untreated guinea pigs by PB-pretreatment. MC-pretreatment also resulted in a significant decrease of both metabolites to 18% and 53% of untreated guinea pigs. M-3 was not detected in all groups.

Discussion

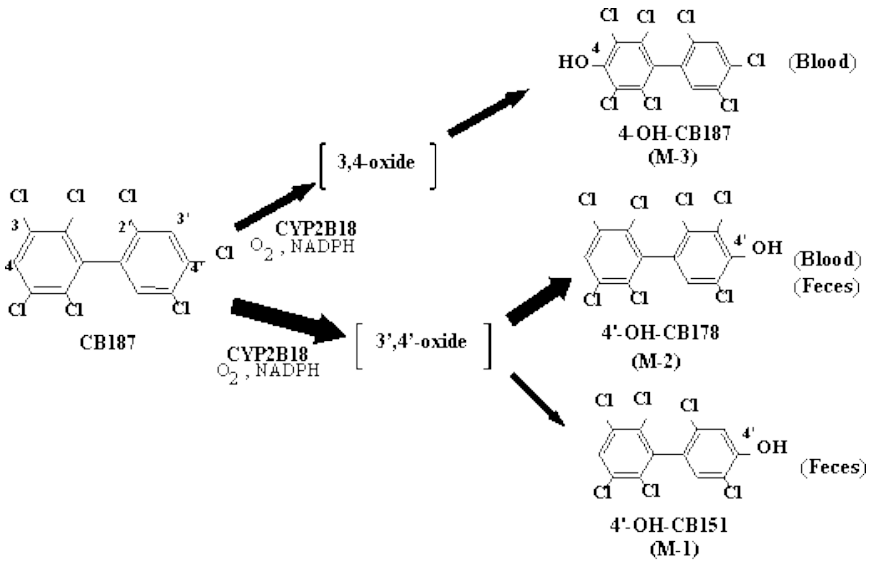

Figure 3: Postulated pathways of CB187 in guinea pigs.

In this study, we found two metabolites (M-1and M-2) in the feces and three metabolites (M-1, M-2 and M-3) in the serum of guinea pigs. From the mass spectral data and the retention times in GC/MS and GC/ECD, they were in agreement with the *in vitro* metabolites formed by guinea pig liver microsomes, namely, 4'-OH-CB151, 4'-OH-CB178 and 4-OH-CB187. The postulated metabolic pathways are shown in Figure 3. 4-OH-CB187 (M-3) was exclusively present in the blood but not in the feces during the experiment. 4'-OH-CB178 (M-2) was distributed both to the blood and the feces. Our recent study have demonstrated that the formation ratio of 4-OH-CB187 to 4'-OH-CB178 was about one twelfth in the *in vitro* study using liver microsomes of PB-treated guinea pigs.[7] However, in this study, the ratio was one half in the blood of PB-treated guinea pigs, indicating that 4-OH-CB187 formed in the liver was retained in guinea pig blood rather than 4'-OH-CB178. Since such a blood affinity of 4-OH-metabolites could be explained by the potency of affinity for transthyretin, a protein transporting thyroid hormone and vitamin A in the blood,[8] 4-OH-CB187 would probably have higher affinity for the transthyretin than 4'-

OH-CB178. 4'-OH-CB151 (M-1) was observed in the blood and feces to a lesser extent than that in the *in vitro* study.[6] The result might suggest the distribution of 4'-OH-CB151 into the other organs such as liver, kidney, lung and adipose tissues. Further study on the distribution of CB187 metabolites are needed to evaluate the toxicity of CB187.

Compared with the *in vitro* metabolism,[7] PB-pretreatment did not affect the formation of the metabolites so strongly in the *in vivo* metabolism. The fact suggests the possibility that CB187 possesses the PB-type inducing ability of liver microsomal enzymes such as CYP2B18 which accelerates the metabolism of CB187 in guinea pigs. In contrast, MC-pretreatment appears to suppress the *in vivo* metabolism of CB187 similarly to the *in vitro* metabolism.

Acknowledgments

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