METABOLISM OF 2,3,3',4,4'-PENTACHLOROBIPHENYL IN HAMSTERS

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Introduction

2,3,3',4,4'-Pentachlorobiphenyl(PenCB)(CB105) is one of the components of PCB preparation, Kanechlor 400, which caused Yusho in Japan in 1968. Kuroki and Masuda[1] reported that both CB105 and 2,3',4,4',5-PenCB(CB118) disappeared from the serum and adipose tissues of Yusho patients or their concentrations were much lower than that of healthy volunteers. The fact suggested that in Yusho patients, liver cytochrome P450 (P450) was induced by PCB congeners and the induced P450 accelerated the metabolism of CB105 and CB118.

Generally, PCB congeners could be categorized into three types, phenobarbital (PB)-type, 3-methylcholanthrene (MC)-type and mixed-type with respect to the inducing ability of liver microsomal P450-mediated reactions[2,3]. PB-type PCB includes di-ortho-PCBs, whereas MC-type PCB consists of coplanar-PCBs. Since CB105 as well as CB118 is mono-ortho-PCB, it belongs to mix-type PCB, which means that CB105 could be metabolized by both PB- and MC-inducible isoforms of P450. However, there are few reports on the metabolism of CB105 in animals. Klasson-Wehler *et al.*[4] demonstrated five hydroxy-metabolites in the feces of mink and mouse administered CB105 orally. They were identified as 4-hydroxy-2,3,5,3',4'-PenCB, 5-hydroxy-CB105, 4'-hydroxy-2,3,5,3',4'-PenCB, 5'-hydroxy-CB105 and 2'-hydroxy-CB105. Recently, Haraguchi *et al.*[5] found nine metabolites in the feces of rats injected CB105 intraperitoneally. They included four hydroxy-metabolites except 2'-hydroxy-metabolites, and four methylthio-metabolites namely 5-, 6-, 5'- and 6'-methylthio-CB105.

The hamster is a unique animal with respect to the low sensitivity for TCDD toxicity[6] and the isoform of P450 metabolizing some tetrachlorobiphenyls[7,8]. Therefore, the *in vivo* metabolism of CB105 in hamsters and the effect of 450 inducers, PB and MC on its metabolism were studied and compared to rats.

Materials and Methods

CB105 was synthesized by the method of Cadogan[9] using 3,4-dichloroaniline and 1,2,3-trichlorobenzene as starting materials. 3-methoxy-2,4,5,3',4'-PenCB, 4- methoxy-2,3,5,3',4'-PenCB, 4'-methoxy-2,3,4,3',5'-PenCB, 6-methoxy-CB105 and 5-methoxy-CB105 were also synthesized by the method of Cadogan[9]. Finally, they were purified by alumina column and HPLC. All other chemicals used were of the highest quality commercially available.

P450 inducers, PB and MC, at a dose of 80 mg/kg/day and 20 mg/kg/day for 3 days, respectively, were intraperitoneally injected to male Golden Syrian hamsters (body weight about 90 g) and male Wistar rats (5 weeks old). CB105 was also intraperitoneally injected at a dose of 3 mg/body the next day after the last injection of PB and MC. Animals were killed 5 days after an injection of CB105. The feces was collected during the experiment and serum was collected at the sacrifice.

Dry powdered feces were extracted with chloroform for 14 h in a Soxhlet apparatus. Serum (0.5 ml) was acidified with 0.5 ml of 0.5M sulfuric acid and extracted with chloroform-methanol (2:1, v/v) and *n*-hexane. The extracts were methylated with diazomethane and applied to GC/MS and GC/ECD equipped with DB-1 (30 m x 0.25 mm, 0.25 μm thickness) or MSP50 (50 m x 0.25 mm, 0.25 μm thickness) fused capillary columns. CB105 and its metabolites were determined using a gas chromatograph HP5890 Series II equipped with ECD under the conditions as follows: DB-1 capillary column (for M-2 and M-3) and MPS50 capillary column (for M-1 and M-4); carrier gas, N_2 (1ml/min); column temp., 200-240° C; injection port temp., 250° C; detector temp., 250° C.

Results

In 5 days-feces of hamsters administered with CB105 intraperitoneally at a dose of 3 mg/body, three metabolites, named M-1, M-2 and M-3, with the retention times of 11.82 min, 15.82 min and 16.27 min, were detected on a DB-1 column (Fig. 1a). Of three metabolites, M-2 and M-3 were major metabolites in hamster feces similarly to rats. In hamster serum at 5 days after CB105 administration, a metabolite, named M-4, was detected at the same retention time to M-1. To elucidate whether M-1 and M-4 were identical or not, their mass spectra in GC/MS were compared. The methylated derivatives of both metabolites showed the same molecular ion at m/z 354 and the characteristic fragment ion of [M^+-15], suggesting the chemical structure of 4- or 4'-methoxy-PCB. When DB-1 column was exchanged to MPS50 column, 4- and 4'-methoxy-PenCB could be eluted at the retention times of 29.88 min and 30.12 min, respectively (data not shown). As a result, it has become clear that M-1 and M-4 were 4'-hydroxy-2,3,3',4,5'-PenCB and 4-hydroxy-2,3,3',4',5-PenCB, respectively. On the other hand, from the fact that the methylated derivatives of M-2 and M-3 had the molecular ion at m/z 354, the fragment ion of [M^+-43] and the

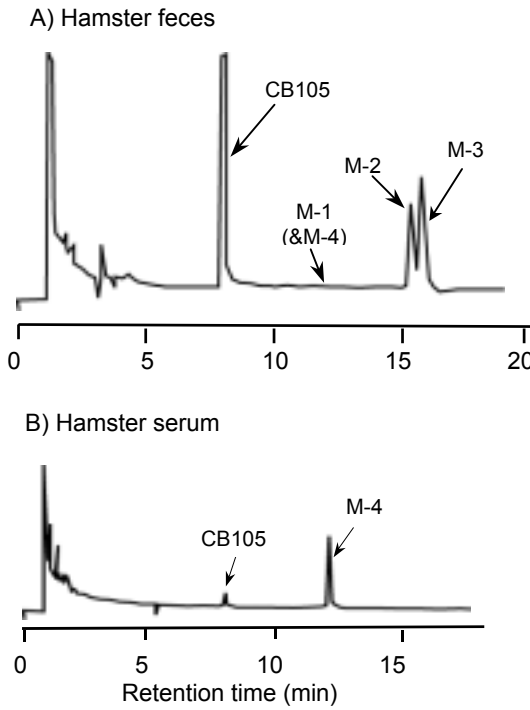

Fig. 1 Gas Chromatogram of metabolites in the feces (A) and the serum (B) of hamsters

same retention time to authentic samples in GC/MS, M-2 and M-3 were identified as 5'-hydroxy-CB105 and 5-hydroxy-CB105, respectively.

The effect of P450 inducers on the amount of the CB105 metabolites in hamster feces was shown in Fig. 2. The formation ratio of four metabolites was 0.2:1:39:84 (M-4: M-1: M-2: M-3) in untreated hamsters. The fecal excretion of M-2 and M-3 accelerated to about 1.7- and 1.5-fold of control by the pretreatment of PB and MC, respectively. The fecal excretion of M-4 and M-1 was rather increased by MC-pretreatment than by PB-pretreatment. In rats, the order of fecal excretion was M-2 > M-3 >> M-4 = M-1 and all of them were increased to about 2 to 3-fold by the pretreatment of PB and MC (data not shown).

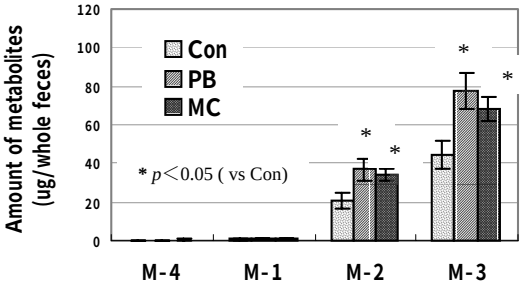

Fig. 2 Effect of P450 inducers on the amount of CB105 metabolites in hamster feces

Fig. 3 showed the effect of P450 inducers on the concentration of M-4 in the serum of hamsters and rats. The concentration of M-4 in hamster serum was 0.39 μg/ml and was increased significantly to 1.8-fold of untreated hamsters by PB treatment and 2.6-fold by MC treatment. In contrast, the concentration of M-4 in rat serum was 0.28 μg/ml and the treatment of rats with PB and MC did not show such an increase of serum M-4.

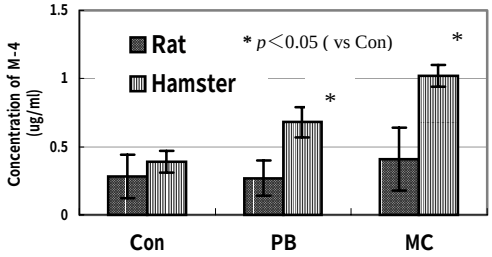

Fig. 3 Effect of P450 inducers on the concentration of M-4 in the serum of rats and hamsters

Discussion

In this study, we found four metabolites in the feces of hamsters administered CB105 intraperitoneally. They were in agreement with the previous reports by Klasson-Wehler *et al.*[4] and Haraguchi *et al.*[5] However, we did not detect 2'-hydroxy-metabolite and four methylthio-metabolites. The postulated metabolic pathways were shown in Fig. 4. Although M-2 and M-3 were major metabolites in both animals, species difference in the formation ratio of M-2 and M-3 was observed between hamsters and rats. The amount of M-3 excreted in rat feces was one third of M-2, whereas that in hamster feces was twice that of M-2. These results suggested that the hamster oxidized 2,3,4-trichloro-substituted benzene ring predominantly rather than 3',4'-dichloro-substituted benzene ring differently from the rat.

Similarly to Klasson-Wehler *et al.*[4] and Haraguchi *et al.*[5], we observed M-4 as well as unchanged CB105 in the serum of hamsters and rats. The concentration of M-4 in hamster serum was about 1.5- to 2.5-times as high as that in rat serum, indicating that M-4 formed in hamster liver was distributed and retained in blood to an extent more than that formed in rat liver. This may be because hamster transthyretin, a protein transporting thyroid hormone in blood, has higher affinity to M-4 than rat transthyretin[10].

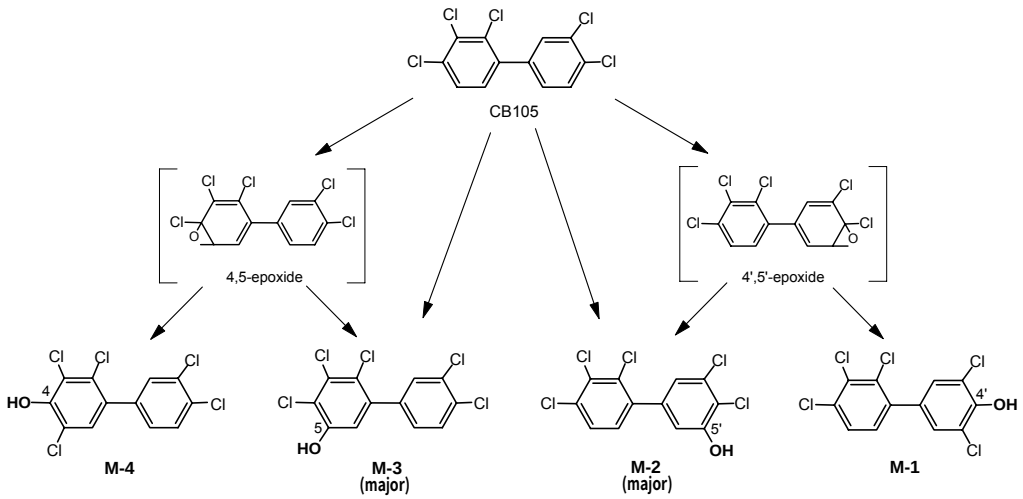

Fig. 4 Postulated pathways of 2,3,3',4,4'-PenCB in hamsters.

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