IN VIVO METABOLISM OF 2,2',4',5,5'-PENTACHLOROBIPHENYL AND ITS INDUCTIVE ACTIVITY OF DRUG-METABOLIZING ENZYMES : SPECIES DIFFERENCE BETWEEN RATS AND MICE

Yoshihisa Kato[1], Yasuhiko Shinmura[1], Koichi Haraguchi[2], Kimie Imai[3], Kiyomitsu Nemoto[1], Tachio Aimoto[3], Yoshito Masuda[2], Masakuni Degawa[1] and Ryohei Kimura[1]

[1]*School of Pharmaceutical Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422-8526, Japan*
[2]*Daiichi College of Pharmaceutical Sciences, 22-1, Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan*
[3]*Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan*

Introduction

A number of the methylsulfonyl ($MeSO_2$) metabolites of polychlorinated biphenyls (PCBs) have been found in several species of animals in Canada and Sweden[1-4] and in healthy humans[5-8].

We previously reported that nine 3-$MeSO_2$ metabolites of PCBs were the potent inducers of hepatic microsomal drug-metabolizing enzymes in rats[9-11]. Additionally we showed that the seven 3-$MeSO_2$ and two 4-$MeSO_2$ metabolites of tetra-, penta- and hexachlorobiphenyls (tetra-, penta- and hexaCBs) reduced the level of serum thyroxine[12-14], suggesting that the metabolites may act as endocrine-disrupters. Recently we indicated that increase in the hepatic T_4 glucuronidation through the seven 3-$MeSO_2$ and one 4-$MeSO_2$ metabolites-mediated induction of both UGT1A1 and UGT1A6 caused the reduction of serum T_4 levels[15]. These previous findings[9-15] indicated that the metabolites, mainly $MeSO_2$ metabolites, contributed to the biological effects such as endocrine-disruption and induction of drug-metabolizing enzymes by PCB congeners in several mammalian species.

Several mammalian species show different responses to PCB-derived toxicity such as endocrine-disruption, drug-metabolizing enzyme induction and so on[16]. The difference might be attributed to the species differences in the metabolism pattern of PCB congeners and/or the induction of drug-metabolizing enzymes by the chemicals.

In this study, we examined the species differences in the *in vivo* metabolism of 2,2',4',5,5'-pentaCB, which is one of major $MeSO_2$ metabolites accumulated in blubber of seals in the Baltic[1], and in the induction of drug-metabolizing enzymes by the compound between rats and mice.

Materials and Methods

Chemicals. 2,2',4',5,5'-pentaCB was synthesized by using the Cadogan coupling reactions[17]. 3- and 4-MeS, 3- and 4-MeSO, and 3- and 4-$MeSO_2$-2,2',4',5,5'-pentaCBs were prepared by the method as described before[18]. The purity of these compounds was >99% when analyzed by gas chromatography (GC). All other chemicals used in the present experiments were commercially obtained.

Animal treatments. Male Wistar rats, weighing 180-200 g, and male ddy mice, weighing 27-35 g, were housed three or four per cage in the laboratory with free access to commercial chow and tap water, and maintained on a 12-hr dark/light cycle (8:00 A.M.-8:00 P.M. light) in a room with controlled temperature (24.5 ± 1□) and humidity (55 ± 5%). Rats and mice received an intraperitoneal injection of 2,2',4',5,5'-pentaCB (342 μmol/kg) dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. All animals were starved for 18 hr prior to killing by decapitation at designated time after dosing.

Preparation of hepatic microsomes and soluble fractions, and enzyme assays. Microsomes and soluble fractions (105,000*g* supernatant) were prepared according to the procedure described previously[9]. The protein content was determined by the method of Lowry *et al.*[19] with bovine serum albumin as a standard. Total cytochrome P450 content was estimated according to the method of Omura and Sato[20]. The activity of alkoxyresorufin *O*-dealkylase in microsomes was determined by the method of Burke *et al.*[21]. The microsomal activities of UDP-glucuronosyltransferase (UDP-GT) toward 4-nitrophenol and chloramphenicol were determined as described by Isselbacher *et al.*[22] and Ishii *et al.*[23], respectively. The glutathione *S*-transferase (GST) activities were measured using four different substrates, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), ethacrynic acid (EA) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP) by the method of Habig *et al.*[24]

Determination of 2,2',4',5,5'-pentaCB and its sulfur containing metabolites in the liver and feces. The concentrations of 2,2',4',5,5'-pentaCB and its sulfur containing metabolites present in the liver and feces were determined with GC as described priviously[2].

Results and Discussion

The elimination rate of 2,2',4',5,5'-pentaCB and the concentrations of 3- and 4-$MeSO_2$ metabolites in the liver for 8 days after the administration of 2,2',4',5,5'-pentaCB were different between rats and mice (Fig. 1). The fecal excretion of 3-MeS metabolite during 8 days accounted for 0.3 and 0.6% of the dose of 2,2',4',5,5'-pentaCB in rats and mice, respectively. On the other hand, the fecal excretion of 4-MeS metabolite accounted for 2 and 1.3% of the dosage in rats and mice, respectively. The level of cumulative excretion of 3- and 4-$MeSO_2$ metabolites during 8 days was 5-15 times higher in mice than in rats.

2,2',4',5,5'-pentaCB administration resulted in the increase in hepatic benzyloxyresorufin *O*-dealkylase activity: 34- and 11-fold, pentoxyresorufin *O*-dealkylase activity (CYP2B1/2): 16- and 6-fold, and ethoxyresorufin *O*-dealkylase activity (CYP1A1/2): 1.7- and 1.3-fold in rats and mice, respectively, at 8 days after the administration (Fig. 2). The administration of 2,2',4',5,5'-pentaCB extensively increased the UDP-GT (UGT2B1) activity toward chloramphenicol in rats, and slightly increased that in mice. No increase in UDP-GT (UGT1A6) activity toward 4-nitrophenol by 2,2',4',5,5'-pentaCB was observed in both species. In addition, the species difference was observed in the alteration of GST activities using four substrates after the administration of 2,2',4',5,5'-pentaCB.

In conclusion, there are marked differences in the elimination and metabolism of 2,2',4',5,5'-pentaCB and in induction pattern of phase I microsomal drug-metabolizing enzymes, UDP-GT and GST by 2,2',4',5,5'-pentaCB between rats and mice. These differences might be correlated with those in the susceptibility of animals to PCBs-derived toxicity.

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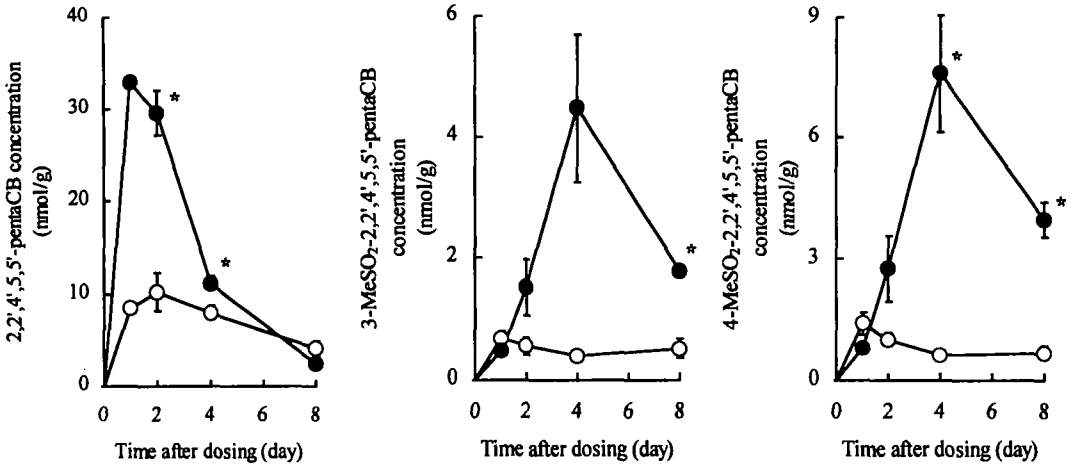

Fig. 1. Time courses of liver concentrations of 2,2',4',5,5'-pentaCB, and its 3- and 4-$MeSO_2$ metabolites after the administration of 2,2',4',5,5'-pentaCB to rats and mice. Animals were given 2,2',4',5,5'-pentaCB (342 μmol/kg) i.p. and killed at the indicated times after the administration. Each point represents single values and the mean ± S.E. (vertical bars) for two to three animals.
*$P<0.05$, significantly different from the corresponding value of rats.
(o) Rats, (•) mice.

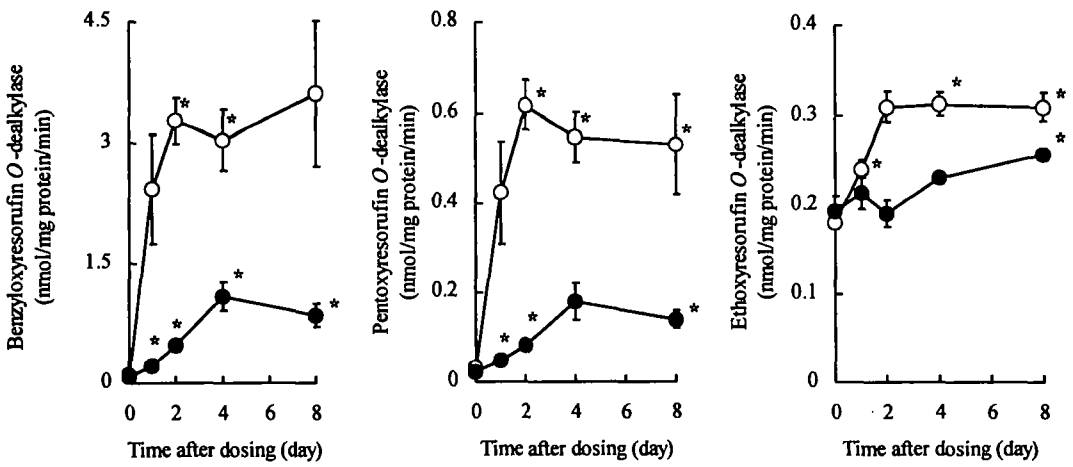

Fig. 2. Effects of 2,2',4',5,5'-pentaCB on the activities of drug-metabolizing enzymes of hepatic microsomes in rats and mice. The experimental conditions were the same as described in the note to Fig. 1. Each point represents the mean ± S.E. (vertical bars) for three to four animals.
*$P<0.05$, significantly different from control (0 day).
(o) Rats , (•) mice.

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