SPECIES DIFFERENCE AMONG MICE, HAMSTERS, RATS AND GUINEA PIGS IN 2,2',4',5,5'-PENTACHLOROBIPHENYL AND 2,2',3',4',5,6-HEXACHLOROBIPHENYL-INDUCED ALTERATIONS OF SERUM THYROID HORMONE LEVEL

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Introduction

Several mammalian species show different responses to PCB-derived toxicity such as endocrine-disruption, drug-metabolizing enzyme induction and so on[1]. 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.

We previously reported that there are marked differences between rats and mice in the hepatic concentration of the methylsulfonyl ($MeSO_2$) metabolites of 2,2',4',5,5'-pentachlorobiphenyl (PentaCB) and 2,2',3',4',5,6-hexachlorobiphenyl (HexaCB), and in the induction pattern of phase I microsomal cytochrome P450 enzymes, UDP-glucuronosyltransferase (UDP-GT) and glutathione *S*-transferase by PentaCB and HexaCB[2, 3]. In general, PCBs such as PentaCB, HexaCB, and Aroclor 1254 have been reported to decrease serum thyroid hormone level in rats and/or mice[4-8], and the decrease is thought to occur through the induction of thyroxine (T_4)-UDP-GTs[8-10]. We, however, have suggested that the reduction of serum T_4 levels is not necessarily correlate with increase in hepatic T_4 glucuronidation activity since PentaCB and HexaCB influence hardly the activity of 4-nitrophenol-UDP-GT (UGT1A6) responsible for the glucuronidation of T_4 in rats and mice[11].

In the present study, to clarify the relationship between decrease in serum thyroid hormone level and UDP-GT activity, we examined the species differences among mice, hamsters, rats and guinea pigs in the altered levels of drug-metabolizing enzymes, in the *in vivo* metabolism of PentaCB and HexaCB, and in the alteration of serum thyroid hormone level by PentaCB and HexaCB.

Materials and Methods

Chemicals. PentaCB and HexaCB were synthesized by using the Cadogan coupling reactions [11]. The purity of these compounds was >99% when analyzed by gas chromatography. All other chemicals used in the present experiments were commercially obtained.

Animal treatments. Male ddy mice weighing 27-35 g, male Syrian hamsters weighing 95-120 g, male Wistar rats weighing 150-200 g, and male Hartley guinea pigs weighing 400-540 g, were housed three or four per cage 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%). The animals received an intraperitoneal injection of PentaCB (11 mg/kg) and HexaCB (19 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. All animals were killed by decapitation on day 4 after the dosing, and the tissues were removed and weighed. Blood was collected from animals between 10:30 and 11:30 a.m. After clotting at room temperature, serum was separated by centrifugation and stored at -50 prior to determination of the levels of total T_4, total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) by radioimmunoassay using Amerlex-MT4, Amerlex-MT3 (Ortho-Clinical Diagnostics Co.; Amersham, UK) and Biotrak rTSH [^{125}I] assay system (Amersham Life Science Ltd.; Little Chalfont, UK), respectively.

Preparation of hepatic microsomes and the microsomal enzyme assays. Hepatic microsomes were prepared according to the procedure described previously[12]. The protein content was determined by the method of Lowry *et al.*[13] with bovine serum albumin as a standard. The activities of 7-ethoxyresorufin, 7-pentoxyresorufin and 7-benzyloxyresorufin *O*-dealkylases in hepatic microsomes were determined by the method of Burke *et al.*[14] The microsomal activities of UDP-GT toward chloramphenicol and T_4 were determined as described by Ishii *et al.*[15] and Barter and Klaassen[16], respectively.

Western blot analysis. Polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and a specific antibody against UGT2B1 isoform were used in Western blot studies. Western analyses for the UGTs were performed with microsomal preparations as described by Luquita *et al.*[17]

Determination of PCBs in the liver. The concentration of PCBs in the liver was determined with GC/MS as described by Mimura *et al.*[18] The quantification of hydroxyl (OH) and $MeSO_2$ metabolites was performed on GC/ECD (GC-14A, Shimadzu) by comparison with internal standards of 2,2',3,4',5,5',6-heptachloro-4-[^{13}C]-biphenylol for OH metabolites, and 4-methyl-3-$MeSO_2$-2,2',3',4',5'-pentachlorobiphenyl for $MeSO_2$ metabolites.

Results and Discussion

Serum total T_4 level was significantly decreased by PentaCB treatment in mice, hamsters, and rats but not in guinea pigs (Fig. 1). HexaCB treatment resulted in a significant decrease of level of serum total T_4 (Fig. 1) and a significant increase in the activity of UDP-GT (UGT1A1 and UGT1A6) toward T_4 in only mice (Fig. 2). The content of UGT1A isoforms was increased by PentaCB and HexaCB in rats. Serum total T_3 level was significantly decreased by PentaCB treatment in only mice. No significant change in the level of serum TSH by either PCB treatment was observed in the all species of animals used. PentaCB administration resulted in significant increases in hepatic microsomal enzyme activities: benzyloxyresorufin *O*-dealkylase activity, 6.9-fold; pentoxyresorufin *O*-dealkylase activity (CYP2B1/2), 4.1-fold; ethoxyresorufin *O*-dealkylase activity (CYP1A1/2), 1.9-fold in rats, and significant decreases in the enzyme activities: benzyloxyresorufin *O*-dealkylase activity, 54%; pentoxyresorufin *O*-dealkylase activity, 39%; ethoxyresorufin *O*-dealkylase activity, 31% in guinea pigs, respectively. HexaCB administration resulted in significant increases in the enzyme activities: benzyloxyresorufin *O*-dealkylase activity, 2.1- and 3.9-fold in mice and rats, respectively; pentoxyresorufin *O*-dealkylase activity, 1.6-fold in mice; ethoxyresorufin *O*-dealkylase activity, 1.4-fold in rats, and

in a significant decrease in the enzyme activity: benzyloxyresorufin *O*-dealkylase activity, 51% in guinea pigs. PentaCB and HexaCB treatments resulted in significant increases in the activity of UDP-GT (UGT2B1) toward chloramphenicol in rats and the content of the UGT2B1 isoform in rats and hamsters. The differences in total hepatic concentration of OH and $MeSO_2$ metabolites and in the activities of benzyloxyresorufin, pentoxyresorufin and ethoxyresorufin *O*-dealkylases among PentaCB- and HexaCB-treated mice, hamsters, rats and guinea pigs were not correlated with that in the decrease in serum total T_4 level.

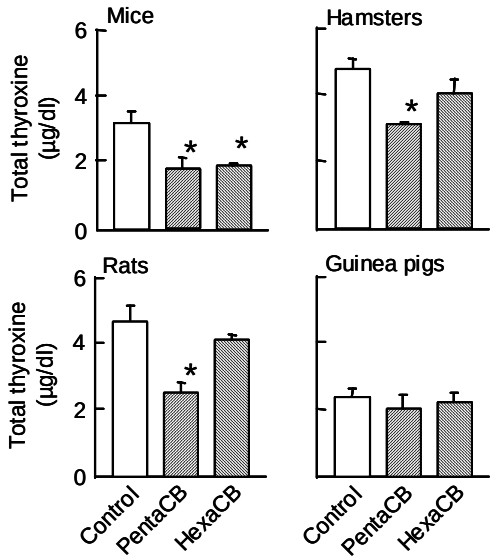

Fig. 1. Effects of PentaCB and HexaCB on serum total thyroxine concentration in mice, hamsters, rats and guinea pigs. Animals were given PentaCB (11 mg /kg) and HexaCB (19 mg/kg) i.p. and killed at 4 days after the administration. Each column represents the mean } S.E. (vertical bars) for five to six animals. *P< 0.05, significantly different from each control.

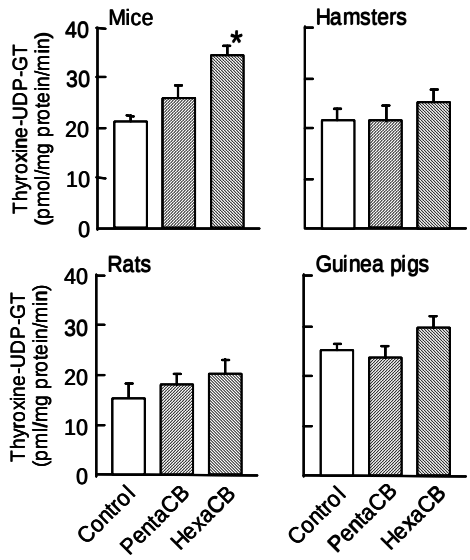

Fig. 2. Effects of PentaCB and HexaCB on UDP-glucuronyltransferase activity toward thyroxine of liver microsomes in mice, hamsters, rats and guinea pigs. The experimental conditions were the same as described in the note to Fig. 1. Each column represents the mean } S.E. (vertical bars) for five to six animals. *P<0.05, significantly different from each control.

In conclusion, there are marked differences in the decrease of serum total T_4 and T_3 levels and in the induction of UGT1A1, UGT1A6 and UGT2B1 by PentaCB and HexaCB among mice, hamsters, rats and guinea pigs. The difference among mice, hamsters, rats and guinea pigs in the metabolism of PentaCB and HexaCB was not necessarily correlated with that in the decrease in serum total T_4 level. The present findings suggest that the reduction of serum total T_4 level by HexaCB in mice would occur at least in part by an increase in T_4 glucuronidation through the induction of hepatic T_4-UDP-GT, especially UGT1A1 and UGT1A6[19], while the decrease of serum total T_4 level by PentaCB in mice, hamsters and rats may occur through alternative mechanisms.

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