2,2',4,4',5,5'-HEXACHLOROBIPHENYL-MEDIATED DECREASE IN SERUM THYROXINE LEVEL IN MICE

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Introduction

We have demonstrated that a commercial polychlorinated biphenyl (PCB) mixture, the Kanechlor-500-mediated decrease occurs through increased accumulation of thyroxine (T_4) in several tissues, especially the liver, rather than an increase in hepatic T_4-UDP-glucuronosyltransferase (UGT) activity[1], and further indicated that decrease in serum T_4 level by 3,3',4,4',5-pentachlorobiphenyl (CB126) occurs not only through the induction of hepatic T_4-UGT but also through the enhanced accumulation of hepatic T_4 along with development of liver hypertrophy[2]. More recently, we have found that phenobarbital (PB)-mediated decreases in the serum T_4 level in mice, hamsters, and rats occur mainly through an increase in the accumulation level of T_4 in the liver[3]. Furthermore, since the magnitude of induction of T_4-UGT is not well correlated with the magnitude of decrease in the serum total T_4 level in the rodent exposed to 2,2',4,4',5,5'-hexachlorobiphenyl (CB153) (Fig. 1), a PB-type inducer of drug-metabolizing enzymes[4], an exact mechanism for the chemical-mediated decrease in serum T_4 level remains unclear.

In the present work, therefore, to further clarify an exact mechanism for the decrease in the serum total T_4 level in the mice treated with CB153, a PB-type inducer of drug-metabolizing enzymes, we examined whether or not there is a strain difference in CB153-mediated decrease of serum total T_4 level between C57BL/6 and DBA/2 mice, which are responsive and non-responsive mice, respectively, to the chemical-mediated induction of T_4-UGTs. The results indicated that there is no strain difference in the pattern of the CB153-mediated decrease in serum total T_4 level. Namely, the CB153-mediated decreases in the serum total T_4 level in both strains of mice were clarified to be primarily dependent on the enhanced accumulation of T_4 in the liver and slightly on the increase in excretion amounts of biliary T_4 and T_4-glucuronide[5].

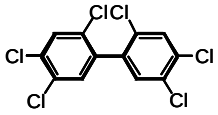

Fig. 1. Chemical structure of 2,2',4,4',5,5'-hexachlorobiphenyl

Materials and methods

Animal Treatments. Male C57BL/6 mice (16-31 g) and the DBA/2 mice (17-29 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male C57BL/6 and DBA/2 mice were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C, humidity, 55 ± 5%), and handled with animal care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received a single i.p. injection of CB153 (12.5,

25, 50, 100 or 200 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

***In Vivo* Study.** Mice were killed by decapitation 3 days after the administration of CB153. The thyroid gland and liver were removed and weighed. Hepatic microsomes were prepared according to the method of Kato *et al*.[6] and stored at –85°C until use. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at –50°C until use.

Analysis of serum hormones. Levels of total T_4, free T_4, total triiodothyronine (T_3), and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using Total T4 and Free T4 kit (Diagnostic Products Corporation; Los Angels, CA), T-3 RIABEAD (Dainabot Co., Ltd, Tokyo, Japan), and the rTSH [^{125}I] Biotrak assay system (GE Healthcare UK, Ltd., Little Chalfont, Buckinghamshine, UK), respectively.

Hepatic microsomal enzyme assay. The amount of hepatic microsomal protein was determined by the method of Lowry *et al*.[7] with bovine serum albumin as a standard. Microsomal *O*-dealkylase activities of 7-benzyloxy-, 7-ethoxy-, and 7-pentoxyresorufins were determined by the method of Burke *et al*.[8]. The activity of microsomal UGT toward T_4 (T_4-UGT activity) was determined by the method of Barter and Klaassen[9].

Western blot analysis. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita *et al*.[10] using polyclonal anti-peptide antibodies against the common region of rat UGT1A isoforms and specific antibody against rat UGT1A1 (Ikushiro *et al*. 1995, 1997)[11, 12].

***Ex Vivo* Study.** At 3 days after treatment with CB153, the mice were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated and primed with heparinized saline (33 units/ml), and then animal's body was warmed to 37°C. Fifteen minutes later, the mice received a single i.v. injection of 1.5μCi [^{125}I]T_4 (0.1 ml) dissolved in saline containing 10 mM NaOH and 1 % normal mouse serum.

Biliary excretion of [^{125}I]T_4 and [^{125}I]T_4 glucuronide. After the administration of [^{125}I]T_4, bile was collected on ice for 2 hr at 30 min-intervals. Bile volume was determined gravimetrically. The amounts of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide in bile were determined by the method of Vansell and Klaassen[13].

Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine binding globulin (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-transthyretin (TTR) complexes were determined according to the method of Davis *et al*.[14].

Tissue distribution of [^{125}I]T_4. Tissue distribution of [^{125}I]T_4 was performed according to the modified method of Oppenheimer *et al*.[15]. In brief, at 5 min after administration of [^{125}I]T_4 to CB153-pretreated mice, cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adreanal gland, spleen, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, and caecum were removed and weighed. Radioactivities in the tissues were determined by a gamma-counter.

Statistics. The data obtained were statistically analyzed according to Student's *t* test or Dunnett's test after analysis of variance. In addition, amount of biliary [^{125}I]T_4 glucuronide and the binding level of [^{125}I]T_4 bound to serum proteins were statistically analyzed according to Newman-Keuls' test after analysis of variance.

Results and discussion:

Serum total T_4 and free T_4 levels 3 days after the CB153 treatment were markedly decreased in both C57BL/6 and DBA/2 mice (Table 1). The magnitude of the decrease in the levels of serum total T_4 and free T_4 in C57BL/6 mice was greater than that in DBA/2 mice, although absolute levels of the hormone after CB153 treatment were almost the same in both strains of mice. Serum total T_3 level 3 days after the treatment with CB153 was significantly decreased in C57BL/6 mice, but not in DBA/2 mice. On the other hand, no significant increase in the level of serum TSH by the CB153 treatment was observed in either strain of mice.

Treatments of C57BL/6 and DBA/2 mice with CB153 resulted in significant increases in hepatic microsomal enzyme activities: 1.6 and 1.5-fold for ethoxyresorufin *O*-dealkylase (Cyp1a1/2) activity, respectively; 7.4 and 8.3-fold for benzyloxyresorufin *O*-dealkylase (Cyp2b1/2 and Cyp3a1/2) activity, respectively; 3.7 and 3.0-fold for pentoxyresorufin *O*-dealkylase (Cyp2b1/2) activity, respectively.

No significant increase in the activity of hepatic T_4-UGT was observed in either strain of mice. The amounts of hepatic Ugt1a and Ugt1a1 enzyme proteins were not significantly changed by CB153 treatment in either strain of mice. In addition, the amounts of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide after i.v. injection of [^{125}I]T_4 were slightly increased by CB153-pretreatment in C57BL/6 mice, but not in DBA/2 mice. No CB153-mediated change in the binding level of [^{125}I]T_4 to each serum protein after [^{125}I]T_4 administration was observed in either

strain of mice.

Table 1. Effects of CB153 on the levels of serum total T_4 and free T_4

	C57BL/6		DBA/2	
Treatment	Control	CB153	Control	CB153
Total T_4 (μg/dl serum)	2.74 ± 0.23	0.92 ± 0.12*	2.36 ± 0.12	1.34 ± 0.15*
Free T_4 (ng/dl serum)	0.58 ± 0.08	0.16 ± 0.02*	0.41 ± 0.03	0.24± 0.02*

Animals were killed 3 days after the administration of CB153 (100 mg/kg). Each column represents the mean ± S.E. (vertical bar) for three to four animals. *P<0.05, significantly different from each control.

In the control C57BL/6 and DBA/2 mice, the accumulation of [^{125}I]T_4 was the highest in the liver among all the tissues examined. In the both strains of mice, pretreatment with CB153 resulted in increase in the level of hepatic total [^{125}I]T_4, and the more than 44% and 34% of the [^{125}I]T_4 dosed were accumulated in the liver of C57BL/6 and DBA/2 mice, respectively. Furthermore, the accumulation levels per gram liver in the CB153-pretreated C57BL/6 and DBA/2 were significantly increased to 1.41- and 1.23- times, respectively, as compared with those in the corresponding control animals. In the both strains of mice, no significant increases in the accumulation levels of [^{125}I]T_4 by CB153-pretreatment were observed in any extrahepatic tissues examined. Furthermore, no significant changes in the weights of the liver and thyroid gland after CB153-treatment were observed in either strain of mice.

In conclusion, we confirmed in the present study that the CB153-mediated decrease in serum T_4 level in mice occurs mainly through the increase in accumulation of T_4 in the liver and slightly through an increase in the excretion of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide[5]. Further studies on the effects of PCBs, including CB153, on the function of hepatic T_4-transporters would be necessary for understanding of an exact mechanism for the PCB-induced decrease in the serum T_4 level.

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