A POSSIBLE MECHANISM FOR THE DECREASE IN SERUM THYROXINE LEVEL BY TCDD-LIKE PCB, 3,3',4,4',5-PENTACHLOROBIPHENYL IN MICE

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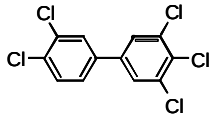
Introduction

Most polychlorinated biphenyl (PCB) congeners, such as 2,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl (CB126), and 2,2',4,4',5,5'- and 2,3,3',4,4',5-hexachlorobiphenyls, are known to decrease the levels of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats and mice [1,2]. As possible mechanisms for the PCB-mediated decrease in level of serum thyroid hormone, induction of hepatic UDP-glucuronosyltransferases (UDP-GTs), especially UGT1As, responsible for thyroid hormone metabolism and competitive inhibition on the formation of thyroid hormone-TTR complex are considered [1,3]. Especially, the decrease in the level of serum thyroxine (T_4) by Aroclor 1254, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and TCDD-like PCB, CB126, in rats is reported to occur mainly through the induction of the UDP-GT responsible for glucuronidation of T_4 (T_4-UDP-GT) through aryl hydrocarbon (Ah) receptor-mediated mechanism [2,4]. However, we have demonstrated that a single and consecutive treatments with Kanechlor-500 (KC500) resulted in significant decrease in level of serum total T_4 not only in Wistar but also in Gunn rats (UGT1A-deficient Wistar rats) [5,6] and further indicated that the KC500-induced decrease would occur through increase in accumulation of T_4 in several tissues, especially the liver, rather than increase in hepatic T_4-UDP-GT activity [6].

In the present study, therefore, to clarify possible mechanisms for the decrease in level of serum thyroid hormone by TCDD-like PCB, we examined a relationship between the decrease in serum total T_4 level and the increase in the hepatic T_4-UDP-GT by the treatment of the TCDD-sensitive C57BL/6 and the resistant DBA/2 strains of mice with CB126 (Fig. 1) and strongly suggested that CB126-mediated decrease in serum total T_4 level in mice occurred mainly through increase in accumulation of T_4 in the liver (transportation from serum to the liver) rather than the increase in hepatic T_4-glucuronidation activity.



CB126

Fig. 1. Chemical structure of 3,3',4,4',5-pentachlorobiphenyl

Materials and Methods

Animal treatments. Male C57BL/6 mice (18-31 g) and the DBA/2 mice (18-28 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male C57BL/6 and DBA/2 mice were housed in 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-8:00

PM light) in an air-controlled room (temperature, 24.5 ± 1℃, humidity, 55 ± 5%), and handled with human care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received intraperitoneal injection of CB126 (2.5 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 7 days after the administration of CB126. The liver was removed, and hepatic microsomal fractions were prepared according to the method of Kato *et al* [7]. and stored at –85℃ until used. 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℃ until used.

Analysis of serum hormones. Levels of total T_4, free T_4, and thyroid-stimulating hormone (TSH) were measured by the radioimmunoassays using Total T_4 and Free T_4 kits (Diagnostic Products Corporation; Los Angels, CA) and the rTSH [^{125}I] Biotrak assay system (GE Healthcare UK, Ltd., Little Chalfont, Buckinghamshire, UK), respectively.

Hepatic microsomal Enzyme assay. Amount of hepatic microsomal protein was determined by the method of Lowry *et al* [8]. with bovine serum albumin as a standard. The activities of 7-ethoxyresorufin, 7-pentoxyresorufin and 7-benzyloxyresorufin *O*-dealkylase in hepatic microsomes were determined by the method of Burke *et al* [9]. Microsomal T_4-UDP-GT activity was determined by the method of Barter and Klaassen [10].

Western blot analysis. Polyclonal anti-peptide antibodies [11] against the common region of rat UGT1A isoforms and specific antibodies against rat UGT1A1 and UGT1A6 were used. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita *et al* [12].

***Ex Vivo* Study.** At 7 days after treatment with CB126, the mice were anesthetized with a saline (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, and then animals were warmed to 37℃. Fifteen minutes later, the mice were given i.v. 1 ml of [^{125}I]T_4 (15 μCi/ml) dissolved in the saline containing 10 mM NaOH and 1 % normal mouse serum.

Clearance of [^{125}I]T_4 from serum. The study on the clearance of [^{125}I]T_4 from serum was performed according to the method of Oppenheimer *et al.* [13]. In brief, after the administration of [^{125}I]T_4, a portion (0.3 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at -50℃ until used. [^{125}I]T_4 level in each serum sample (15 μl) was determined by a gamma-counter.

Biliary excretion of [^{125}I]T_4 and [^{125}I]T_4 glucuronide. Amounts of biliary [^{125}I]T_4 and [^{125}I]T_4 glucuronide were determined with HPLC as described of Vansell and Klaassen [14].

Analysis of [^{125}I]T_4 bound to serum proteins. Levels of [^{125}I]T_4-albumin, [^{125}I]T_4-thyroxine binding protein (TBG), and [^{125}I]T_4-TTR complexes in the serum were determined according to the method of Davis *et al* [15].

Tissue distribution of [^{125}I]T_4. The study on the tissue distribution of [^{125}I]T_4 was performed according to the modified method of Oppenheimer *et al* [13]. In brief, at 5 min after administration of [^{125}I]T_4 to CB126-pretreated mice, blood was sampled from abdominal aorta. Then, tissues were removed and weighed. Radioactivities in the serum and tissues were determined by a gamma-counter, and amounts of [^{125}I]T_4 in the tissues were shown as a ratio of the tissue to serum.

Statistics. The data obtained were statistically analyzed according to Student's *t* test or Dunnett's test after analysis of variance. In addition, data on the clearance of [^{125}I]T_4 from serum and the level of [^{125}I]T_4 bound to serum proteins were statistically analyzed according to the Newman-Keuls test after analysis of variance. The pharmacokinetic parameters of [^{125}I]T_4 were estimated with noncompartmental methods as described previously [16].

Results and Discussion

Serum total T_4 and free T_4 levels 7 days after the treatment with CB126 (2.5 mg/kg, i.p.) were markedly decreased in TCDD-sensitive C57BL/6 mice but not in TCDD-resistant DBA/2 mice (Fig. 2). At the same time, the level and activity of hepatic T_4-UDP-GT (UGT1a and UGT1a1) were significantly in C57BL/6 mice, whereas level of hepatic UGT2b1 was decreased. These CB126-meidated changes did not occur in DBA/2 mice. In addition, significant increases in the weight of liver and thyroid by CB126-treatment were observed in C57BL/6 mice but not in DBA/2 mice.

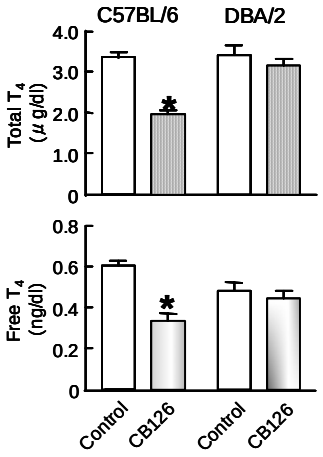


Fig. 2. Effect of CB126 on the levels of serum total thyroxine and free thyroxine in mice

Animals were killed 7 days after the administration of CB126 (2.5 mg/kg). Each column represents the mean ± S.E. (vertical bars) for four to seven animals. *P<0.05, significantly different from each control.

Treatment of C57BL/6 mice with CB126 resulted in remarkable increase in hepatic microsomal enzyme activities; ethoxyresorufin *O*-dealkylase activity (CYP1A1/2): 73-fold, pentoxyresorufin *O*-dealkylase activity (CYP2B1/2): 7-fold, and benzyloxyresorufin *O*-dealkylase activity: 3-fold. On the other hand, no such CB126-mediated increase was observed in DBA/2 mice.

The amounts of biliary [^{125}I]T_4 and [^{125}I]T_4-glucuronide after i.v. injection of [^{125}I]T_4 were greater in CB126-pretreated C57BL/6 mice than in the control (untreated) mice, while no effect of the CB126-pretreatment was observed in DBA/2 mice. In addition, no significant increase in the level of serum TSH after CB126-pretreatment was observed in the either strain of mice.

Furthermore, a significant increase in clearance of [^{125}I]T_4 from the serum by CB126-pretreatment was observed in C57BL/6 mice but not in DBA/2 mice. On the other hand, no significant changes in the tissue distribution volume (Vd) of [^{125}I]T_4 and in the concentration ratio (Kp value; 0.5-0.6) of the liver to serum by CB126-pretreatment was observed in either strain of mice. Tissue distribution of [^{125}I]T_4 was the highest in the liver in control mice, and hepatic level of [^{125}I]T_4 was increased by CB126-pretreatment in C57BL/6 mice but not in DBA/2 mice. In CB126-pretreated C57BL/6 mice, 49 % of [^{125}I]T_4 dosed was transported to the liver with development of hypertrophy and hyperplasia. In addition, no significant change in liver distribution of [^{125}I]T_4 (% of dose/g liver) by the treatment was observed in either strain of mice. Furthermore, slight increase in the level of serum [^{125}I]T_4-TTR complex and slight decrease in the binding level of [^{125}I]T_4 to serum albumin and TBG were observed in the CB126-pretreated C57BL/6 mice but not in the CB126-pretreated DBA/2 mice.

In conclusion, the present findings suggest that the CB126-mediated decrease in serum T_4 level occurs mainly through the increase in accumulation (transportation from serum to liver) of T_4 in the liver along with development of the hypertrophy and hyperplasia, although the enhancement of biliary excretion of T_4 through CB126-mediated induction of T_4-UDP-GT might be one of factors that mediate the decrease in serum T_4 level.

Acknowledgements

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