A POSSIBLE MECHANISM FOR THE DECREASE IN SERUM THYROXINE LEVEL BY 4-OH-2,2',3,4',5,5',6-HEPTACHLOROBIPHENYL IN MICE

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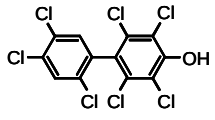
Introduction

A large number of hydroxylated polychlorinated biphenyls (OH-PCBs) have been found in the blood of humans, birds, seals and polar bears [1-3]. The concentration of the OH-PCBs may exceed 10% of total amount of polychlorinated biphenyl (PCB) in human serum [1,2]. Recently, high concentration of OH-PCBs was detected in the serum from pregnant Faroese women and Eastern Slovakia human [4,5]. The major OH-PCBs identified in the serum were 4-OH-2,3,3',4',5-pentachlorobiphenyl (4-OH-CB107), 3-OH-2,2',4,4',5,5'-hexachlorobiphenyl (3-OH-CB153), 4-OH-2,2',3,4',5,5'-hexachlorobiphenyl (4-OH-CB146), 3'-OH-2,2',3,4,4',5'-hexachlorobiphenyl (3'-OH-CB138) and 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl (4-OH-CB187) (Fig. 1). These hydroxylated PCB metabolites, especially 4-OH-CB187, show very high binding affinity for serum transport protein (transthyretin (TTR)) [6,7], and the binding affinity of 4-OH-CB187 is 5.3-fold-higher than that of endogenous ligand thyroxine (T_4) [8]. Thus, 4-OH-CB187 appears to change the metabolic fate and action of serum thyroid hormone.

Most PCB congeners are known to decrease the levels of serum thyroid hormone in rats [9,10]. As possible mechanisms for the PCB-mediated decrease, induction of hepatic glucuronosyltransferases (UGTs), especially UGT1As, responsible for thyroid hormone metabolism and competitive inhibition on the formation of thyroid hormone-TTR complex are considered [6,11]. However, we have recently demonstrated that a consecutive treatment 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) 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-glucuronosyltransferase (T_4-UDP-GT) activity [12].

In the present study, we selected 4-OH-CB187 as a major hydroxylated PCB detected from serum of the wildlife including humans, and its effect on level of serum thyroid hormone was examined in mice. The present results revealed that 4-OH-CB187 showed a definite ability to decrease serum T_4 level and strongly suggested that its decrease occurred mainly through increase in accumulation of T_4 in the liver rather than the increase in hepatic T_4-glucuronidation activity.



4-OH-CB187

Fig. 1. Chemical structure of 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl

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). TTR-null (TTR-/-) mice (15-24 g) were generated by using a homologous recombination method as described previously [13]. Male TTR+/- mice were backcrossed to C57BL/6 TTR+/+ female mice for 8 generations. The genotype of each pup was determined on the basis of the presence of the mutant TTR allele by PCR with genomic DNA taken from the tail. Male C57BL/6, DBA/2, TTR-heterozygous (TTR +/-) and TTR-/- 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 4-OH-CB187 (1.0 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 4 days after the administration of 4-OH-CB187. The liver was removed, and hepatic microsomal fractions were prepared according to the method of Kato *et al* [14]. 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 T_4-Metabolizing Enzyme assay. Amount of hepatic microsomal protein was determined by the method of Lowry *et al* [15]. with bovine serum albumin as a standard. Microsomal T_4-UDP-GT activity was determined by the method of Barter and Klaassen [16].

Western blot analysis. Polyclonal anti-peptide antibodies [17] 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* [18].

***Ex Vivo* Study.** At 4 days after treatment with 4-OH-CB187, 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.* [19]. 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 glucuronide. Amount of biliary [^{125}I]T_4 glucuronide was determined with HPLC as described of Vansell and Klaassen [20] .

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* [21].

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* [19]. In brief, at 5 min after administration of [^{125}I]T_4 to 4-OH-CB187-pretreated mice, blood was sampled from abdominal aorta. Then, tissues were removed and weighted. 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 [22].

Results and Discussion

Serum total T_4 and free T_4 levels were markedly decreased in both dioxin-sensitive C57BL/6 mice and dioxin-resistant DBA/2 mice 4 days after the treatment with 4-OH-CB187 (1.0 mg/kg, i.p.) (Fig. 2). At the same time, the level and activity of T_4-UDP-GT (UGT1a and UGT1a1) in the liver were not significantly changed in either C57BL/6 or DBA/2 mice. In addition, no significant changes in the amount of biliary [^{125}I]T_4-glucuronide and in the level of serum TSH after 4-OH-CB187-pretreatment were observed in the either strain of mice.

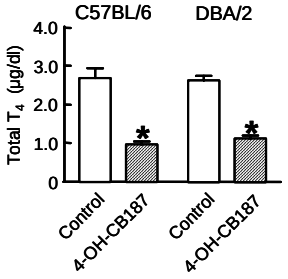


Fig. 2. Effect of 4-OH-CB187 on the level of serum total T_4 in mice

Animals were killed 4 days after the administration of 4-OH-CB187 (1.0 mg/kg). Each column represents the mean ± S.E. (vertical bars) for four to eight animals. *P<0.01, significantly different from each control.

Furthermore, significant increases in clearance of [^{125}I]T_4 from the serum and in tissue distribution volume of [^{125}I]T_4 by 4-OH-CB187-pretreatment were observed in both C57BL/6 and DBA/2 mice. A concentration ratio of the liver to serum was 0.5-0.6 in the control (4-OH-CB187-untreated) C57BL/6 and DBA/2 mice, and pretreatment of the mice with 4-OH-CB187 resulted in increase in the ratio by 1.5-1.6 times. Tissue distribution of [^{125}I]T_4 was the highest in the liver in control mice. Hepatic level of [^{125}I]T_4 was increased by 4-OH-CB187-pretreatment in both C57BL/6 and DBA/2 mice. Forty % of [^{125}I]T_4 dosed was transported to the liver, although no significant change in liver weight by 4-OH-CB187 treatment was observed in the either strain of mice. Furthermore, significant decrease in the level of serum [^{125}I]T_4-TTR complex and significant increase in the binding level of T_4 to serum albumin and TBG were observed in the 4-OH-CB187-pretreated mice. In addition, 4-OH-CB187-mediated decrease in serum total T_4 and free T_4 levels were observed in C57BL/6 TTR+/+ and TTR+/- mice but not in TTR-/- mice.

In conclusion, the present findings demonstrate that 4-OH-CB187 possesses the ability to reduce serum thyroid hormone levels in mice and further indicate that the 4-OH-CB187-mediated decrease in serum T_4 level in both C57BL/6 and DBA/2 mice occurs without an increase in hepatic T_4-UDP-GT activity. These results strongly suggest that the 4-OH-CB187-mediated decrease in serum T_4 level occurs through increase in accumulation (transportation from serum to tissues) of T_4 in tissues, especially the liver, but not through induction of hepatic T_4-UDP-GT. In addition, the present data obtained by use of TTR-heterozygous (TTR +/-) and TTR-/- mice suggest that the 4-OH-CB187-mediated increase in accumulation of T_4 in the liver might occur through a reduction of T_4-TTR complex formation in serum.

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