# 2,2',4,5,5'-PENTACHLOROBIPHENYL-MEDIATED INHIBITION OF A SERUM $T_4$-TRANSTHYRETIN COMPLEX FORMATION IS ONE OF CAUSES FOR THE PCB-INDUCED CHANGES IN THE SERUM AND HEPATIC $T_4$ LEVELS IN MICE

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## Introduction

As a possible mechanism for polychlorinated biphenyl (PCB)-mediated decrease in serum thyroid hormone ($T_4$), increases in hepatic $T_4$-UDP-glucuronosyltransferases ($T_4$-UGTs), especially UGT1A1 and UGT1A6[1], responsible for thyroid hormone metabolism have been proposed[2,3,4]. However, we have demonstrated that decreases in level of serum total $T_4$ in rats and mice by PCBs, including a commercial PCB Kanechlor-500 (KC500), 2,2',4,5,5'-pentachlorobiphenyl (PentaCB), and 3,3',4,4',5-pentachlorobiphenyl (CB126), occur through increased accumulation of $T_4$ in several tissues, especially the liver, rather than an increase in hepatic $T_4$-UGT activity[5,6,7].

Furthermore, we have recently found that KC500-mediated decreases in the serum $T_4$ level in hamsters and guinea pigs occur mainly through increase in the accumulation (transportation from serum to tissues) of $T_4$ in the liver[8]. As a possible mechanism for the PCB-mediated accumulation in the liver, serum transthyretin (TTR)-related pathway is considered, because TTR plays an important role in homeostasis of serum total $T_4$ level[9] and because PCB and its hydroxylated metabolites show inhibitory effects on $T_4$-TTR complex formation[5,10,11,12].

In the present work, therefore, association of serum TTR to PentaCB-mediated changes in the levels of serum total $T_4$ and hepatic $T_4$ was examined using [TTR(+/+)] C57BL/6 (TTR-WT) and its TTR-deficient [TTR(-/-)] (TTR-null) mice. The results indicated that PentaCB-mediated decrease in serum $T_4$ level in WT mice was mainly dependent on the enhanced accumulation of $T_4$ in the liver along with the chemical-mediated inhibition of serum $T_4$-TTR complex formation.

## Materials and methods

**Chemicals.** PentaCB was synthesized by using the Cadogan coupling reactions[13]. The purity of the compound was >99% when analyzed by gas chromatography. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan).

**Animal Treatments.** Male C57BL/6 mice [TTR(+/+), TTR-WT] (19-30 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). TTR-deficient TTR(-/-) (TTR-null) mice (19-26 g) were generated by using a homologous recombination method as described previously[9]. Male TTR-heterozygous TTR(+/-) mice were backcrossed to female TTR-WT mice for eight generations. The genotype of each pup was determined based on the presence of the mutant TTR allele by polymerase chain reaction with genomic DNA taken from the tail. Male TTR-WT and TTR-null 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). Treatment of mice with PentaCB was performed according to the method of Kato *et al.*[5]. Briefly, mice received a single intraperitoneal injection of PentaCB (112 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 mg/kg).

**A) *In Vivo* Study.** Mice were killed by decapitation 4 days after the administration of PentaCB (112 mg/kg) or a vehicle alone. The liver was removed and weighed. Hepatic microsomes were prepared according to the method of Kato *et al.*[14] 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.

**Western blot analysis.** The amount of hepatic microsomal protein was determined by the method of Lowry *et al*.[15] with bovine serum albumin as a standard. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita *et al*.[16] using specific antibody against rat UGT1A1[17,18]. Mouse Ugt1a1, which corresponds to rat UGT1A1, was measured by use of an ECL detection kit (GE Healthcare UK, Ltd), and the level of protein was determined densitometrically with LAS-1000 (Fuji Photo Film. Co., Ltd., Tokyo, Japan).

**B) *Ex Vivo* Study.** At 4 days after treatment with PentaCB (112 mg/kg), 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 (polyethylene tube SP8, Natsume Inc., Tokyo, Japan) 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.

**Clearance of [$^{125}$I]$T_4$ from serum.** Clearance of [$^{125}$I]$T_4$ from serum was measured according to the method of Oppenheimer *et al*.[19]. In brief, after the administration of [$^{125}$I]$T_4$, a portion (80 μl) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at -50°C until use. An aliquot (15 μl) of serum was used for determination of the level of [$^{125}$I]$T_4$ by a gamma-counter (Cobra II Auto-Gamma 5002; Perkin Elmer Life and Analytical Sciences), and the assay was performed in duplicate.

**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]$T_4$-TTR complexes were determined according to the method of Davis *et al*.[20].

**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*.[19]. In brief, at 5 min after administration of [$^{125}$I]$T_4$ to PentaCB-pretreated mice, blood was sampled from abdominal aorta. Then, the cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adreanal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, caecum, brown fat, skeletal muscle, bone marrow, skin, spinal cord and fat were removed and weighed. Radioactivities in the serum and tissues were determined by a gamma-counter (Cobra II Auto-Gamma 5002; Perkin Elmer Life and Analytical Sciences), and then, the amounts of [$^{125}$I]$T_4$ in the tissues were represented as ratios to the amount in serum.

**Statistics**. The data obtained were statistically analyzed according to the Student's *t* test or Dunnett's test after analysis of variance. In addition, the clearance of [$^{125}$I]$T_4$ from serum 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. The pharmacokinetic parameters of [$^{125}$I]$T_4$ were estimated with noncompartmental methods as described previously[21].

## Results and discussion

The constitutive levels of serum total $T_4$ and free $T_4$, including $T_4$-TTR complex level, were much higher in TTR-WT mice than in TTR-null mice (Table 1). Treatment of TTR-WT mice with PentaCB (112 mg/kg) resulted in significant decrease in the total $T_4$ and free $T_4$ levels along with decrease in serum $T_4$-TTR complex, and the levels of serum total $T_4$ and free $T_4$ in PentaCB-treated TTR-WT mice were almost the same as those in control TTR-null mice (Table 1). In addition, only slight decrease in serum total $T_4$, but not free $T_4$ by PentaCB treatment was observed in TTR-null mice (Table 1). Furthermore, no significant PentaCB-mediated changes in the levels of serum total $T_3$ and TSH were observed in either strain of mice. The amounts of Ugt1a1 protein in the both strains of mice were significantly increased by PentaCB treatment.

Furthermore, clearance of [$^{125}$I]$T_4$ from the serum after [$^{125}$I]$T_4$-administration was promoted by the PentaCB-pretreatment in either strain of mice, especially TTR-WT mice. Significant increase in the mean total body clearance of [$^{125}$I]$T_4$ by the PentaCB (112 mg/kg)-pretreatment was observed in either TTR-WT or TTR-null mice. In addition, significant increases in the steady-state volume of distribution in the PentaCB-pretreated TTR-WT and TTR-null mice were increased. Furthermore, in TTR-WT mice, PentaCB-pretreatment resulted in significant decrease and increase in the serum levels of [$^{125}$I]$T_4$-TTR complex and the [$^{125}$I]$T_4$-albumin complex,

respectively. On the other hand, in PentaCB-pretreated TTR-null mice, significant increase and decrease in the levels of serum [$^{125}$I]$T_4$-albumin and TBG complexes, respectively. Consequently, accumulation level of [$^{125}$I]$T_4$ in the liver, but not in extrahepatic tissues, and the concentration ratio of the liver to serum were enhanced in the PentaCB-pretreated TTR-WT and TTR-null mice (Table 2). In addition, no significant PentaCB-mediated increases in the liver weight were observed in either TTR-WT or TTR-null mice.

Table 1. Effects of PentaCB on the levels of serum total $T_4$ and free $T_4$ in TTR-WT and TTR-null mice

| | TTR-WT | | TTR-null | |
|---|---|---|---|---|
| Treatment | Control | PentaCB | Control | PentaCB |
| Total $T_4$ (μg/dl serum) | 1.79 ± 0.09 | 0.86 ± 0.04* | 0.60 ± 0.09† | 0.48 ± 0.09 |
| Free $T_4$ (ng/dl serum) | 0.38 ± 0.22 | 0.16 ± 0.03* | 0.13 ± 0.02† | 0.15 ± 0.02 |

Animals were killed 4 days after administration of PentaCB (112 mg/kg), and levels of serum thyroid hormones were measured, as described in *Materials and Methods*. The values shown are expressed as the mean ± S.E. for five to six mice. *Significant differences from the strain-matched control: $P<0.05$. †Significant differences from the corresponding control TTR-WT mice: $P<0.001$.

Table 2. Tissue distribution of [$^{125}$I]$T_4$ after the administration of [$^{125}$I]$T_4$ to PentaCB-pretreated TTR-WT and TTR-null mice

| | Tissue distribution (% dose) | | | |
|---|---|---|---|---|
| | TTR-WT | | TTR-null | |
| Tissue | Control | PentaCB | Control | PentaCB |
| Thyroid gland | 0.03 ± 0.01 | 0.02 ± 0.001 | 0.02 ± 0.002 | 0.02± 0.001 |
| Liver | 28.65 ± 2.11 | 52.21 ± 1.13* | 39.74 ± 1.35† | 54.94 ± 3.14* |
| Kidney | 4.71 ± 0.37 | 4.12 ± 0.12 | 5.06 ± 0.26 | 4.44± 0.38 |

Four days after treatment with PentaCB (112 mg/kg), [$^{125}$I]$T_4$ was administered to the mice, and after 5 min of the [$^{125}$I]$T_4$ administration, the radioactivity in each tissue was measured, as described in *Materials and Methods*. The values shown are expressed as the mean ± S.E. for four to six animals. *Significant differences from the strain-matched control: $P<0.05$. †Significant differences from the corresponding control TTR-WT mice: $P<0.001$.

The present findings demonstrate that PentaCB-mediated decrease in serum $T_4$ level occurs mainly through increase in accumulation level of $T_4$ in the liver and further indicate that the lack of TTR and PentaCB-mediated inhibition of serum $T_4$-TTR complex formation promote a decrease of serum total $T_4$. In addition, the clearance profile of serum [$^{125}$I]$T_4$ in the PentaCB-pretreated TTR-WT mice was almost the same as that in PentaCB-untreated (control) TTR-null mice, indicating that serum TTR plays an important role in homeostasis of serum $T_4$.

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