# A POSSIBLE MECHANISM FOR THE DECREASE IN SERUM THYROXINE LEVEL BY POLYCHLORINATED BIPHENYLS IN WISTAR AND GUNN RATS

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## Introduction

Most polychlorinated biphenyl (PCB) congeners are known to decrease the levels of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats [1,2]. As possible mechanisms for the PCB-mediated decrease in level of serum thyroid hormone, enhancement of thyroid hormone metabolism by PCBs and displacement of the hormone from serum transport proteins (transthyretin (TTR)) are considered [3-5]. Especially, the decrease in the level of serum thyroxine ($T_4$) by 3,3',4,4',5-pentachlorobiphenyl, Aroclor 1254, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats is thought to occur mainly through the induction of the UDP-glucuronosyltransferase ($T_4$-UDP-GT) responsible for glucuronidation of $T_4$ [2,4]. However, the magnitude of decrease in level of serum total $T_4$ is not necessarily correlated with that of increase in $T_4$-UDP-GT activity [1,6]. Recently, we suggested that the decrease in serum total $T_4$ level by a single administration of either Kanechlor-500 (KC500) or 2,2',4,5,5'-pentachlorobiphenyl in UGT1A-deficient Wistar rats (Gunn rats) was not dependent on the increase in hepatic $T_4$-UDP-GT activity, and further suggested that even in Wistar rats, the PCB-mediated decrease in serum $T_4$ level might occur not only through the increase in hepatic $T_4$-UDP-GT [7]. In the environment, humans and animals are exposed to PCB of very low level extent over a long period of time.

In the present study, therefore, to clarify possible mechanisms for the PCB-mediated decrease in level of serum thyroid hormone, we examined a relationship between the decrease in serum total $T_4$ level and the increase in the hepatic $T_4$-UDP-GT (UGT1A1 and UGT1A6) by the consecutive treatment of Wistar and Gunn rats with PCB and demonstrated that the PCB-mediated decrease in serum total $T_4$ level in rats was not necessarily dependent on the increase in hepatic $T_4$-glucuronidation, but the decrease occurs through the increased transport of $T_4$ to the liver.

## Materials and Methods

**Animal treatments.** Male Wistar rats (160-200 g) and Gunn rats, (180-210 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male Wistar and Gunn rats were housed in three or four per cage with free access to commercial chow and tap water, and were maintained on a 12-h dark/light cycle (8:00 a.m.-8:00 p.m. light) in an air-controlled room (temperature: 24.5 ± 1℃, humidity: 55 ± 5%), and were handled with humane care under the guidelines of the University of Shizuoka (Shizuoka, Japan). The rats were treated with ip injection of KC500 (10 mg/kg) dissolved in Panacete 810 (5 ml/kg) at 24 h-intervals for 10 days. Control animals were treated with vehicle alone (5 ml/kg).

**A) *In vivo* study.** Rats were killed by decapitation on day 4 after the final dosing, and the liver was removed, and hepatic microsomes were prepared according to the method of Kato *et al.*[8] and stored at –85℃ until used. Blood was collected from each animal between 10:30 and 11:30 a.m. After clotting at room

temperature, serum was separated by centrifugation and stored at –50℃ until used.

**Analysis of serum hormones.** The levels of total $T_4$, free $T_4$, and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using the T4-RIABEAD (DAINABOT Co., Ltd, Tokyo, Japan), free $T_4$ (Diagnostic Products Corporation; Los Angels, CA), and Biotrak rTSH [$^{125}$I] assay systems (Amersham Life Science Ltd.; Little Chalfont, UK), respectivery.

**Hepatic microsomal UDP-GT assay.** The amount of protein was determined by the method of Lowry *et al*.[9] with bovine serum albumin as a standard. The activity of microsomal UDP-GT toward $T_4$ was determined by the method of Barter and Klaassen [3]. The UDP-GT activity was measured after activation of the UDP-GTs by 0.05% Brij 58.

**Western blot analysis.** Polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, and UGT2B1 were used [10]. Western analyses for microsomal UGT isoforms were performed by the method of Luquita *et al*.[11]

**B) *Ex vivo* study.** At 4 day after last treatment with KC500, animals were anesthetized with 50 mg/ml sodium pentobarbital combined 1:1 with 1 mg/ml potassium iodide at 2 mg/ml. The femoral artery was cannulated and primed with heparinized saline. Fifteen minutes later, animals were given [$^{125}$I]$T_4$ i.v. at 15 μCi/ml in 10 mM NaOH saline including 1 % normal each animal serum.

**Clearance of [$^{125}$I]$T_4$ from serum.** Five minutes following i.v. administration of [$^{125}$I]$T_4$ and five more times at the indicated time, a portion of blood was sampled from the artery, and serum was collected and stored at -50℃ for assay. Two aliquots were taken from serum samples for γ-counting.

**Analysis of [$^{125}$I]$T_4$ binding to serum proteins.** The levels of serum [$^{125}$I]$T_4$-albumin, [$^{125}$I]$T_4$-thyroxine binding protein, and [$^{125}$I]$T_4$-TTR complexes were determined by the method of Kato *et al.*[12]

**Tissue distribution of [$^{125}$I]$T_4$.** At 60 min after the administration of [$^{125}$I]$T_4$, blood was sampled from abdominal aorta, and tissues were removed and weighted. Radioactivities in serum and tissues were determined by γ-counting, and concentration ratios of the tissue to serum were determined.

**Results and Discussion**

The serum total $T_4$ and free $T_4$ levels were markedly decreased not only in the Wistar rats but also in the Gunn rats 4 days after final treatment with KC500 (10 mg/kg, i.p., once daily for 10 days), and there was no significant difference in magnitude of the decrease between Wistar and Gunn rats (Fig. 1). At the same time, the level and activity of $T_4$-UDP-GT (UGT1A1 and UGT1A6) were significantly increased by treatment with KC500 in Wistar rats but not in Gunn rats (Fig. 2). In contrast, the level of UGT2B1 was increased by KC500 in both Wistar and Gunn rats. Hepatic microsomal enzyme activities (benzyloxyresorufin *O*-dealkylase activity, 48- and 35-fold; pentoxyresorufin *O*-dealkylase activity (CYP2B1/2), 17- and 10-fold; ethoxyresorufin *O*-dealkylase activity (CYP1A1/2), 64- and 10-fold in Wistar and Gunn rats, respectively) were significantly increased by KC500 treatment. In addition, no significant change in the level of serum TSH by the KC500 treatment was observed in either Wistar or Gunn rats.

Furthermore, significant increases in the disappearance of [$^{125}$I]$T_4$ from the serum and in the distribution volume of [$^{125}$I]$T_4$ by KC500 treatment were observed in both Wistar and Gunn rats. A concentration ratio of the liver to serum was approximately one in either Wistar or Gunn rats, and KC500-treatment increased the ratio by 4 times. The concentration of [$^{125}$I]$T_4$ appeared to be the highest in the liver in both Wistar and Gunn rats. The hepatic levels of [$^{125}$I]$T_4$ in both rats were further increased by KC500 treatment. More than 40% of [$^{125}$I]$T_4$ dosed was transported to the liver of both rats (Fig. 3). In contrast, a significant increase in liver weight was observed in KC500-treated Wistar rats but not in the Gunn rats. In addition, significant decrease in the binding of [$^{125}$I]$T_4$ to serum TTR and significant increase in the binding to serum albumin by KC500 treatment were observed in either Wistar or Gunn rats.

In conclusion, the present findings demonstrate that the PCB-mediated decrease in serum total $T_4$ level in Gunn rats occurs without an increase in hepatic $T_4$-UDP-GT activity; they further suggest that in both strain rats, the PCB-mediated decrease occurs through the increased transportation of $T_4$ to the liver. Furthermore, the decrease in the binding of $T_4$ to serum TTR and hepatic hyperplasia might be attributed to the increase in the level of $T_4$ in the liver. In Wistar rats, however, the PCB-mediated induction of $T_4$-UDP-GT might, at least in part, contribute to the decrease. Further studies are necessary for understanding the susceptibility toward a PCB-mediated decrease in serum $T_4$ level in animals including humans.

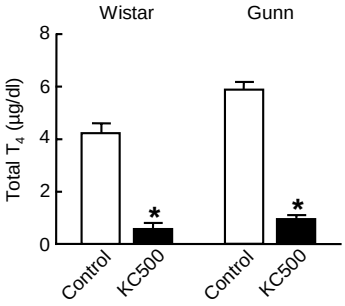


Fig. 1. Effect of KC500 on the level of serum total thyroxine in Wistar and Gunn rats

Animals were killed 4 days after the final administration of KC500 (10 mg/kg, i.p., once daily for 10 days). Each column represents the mean ± S.E. (vertical bars) for five to six animals. *$P$<0.01, significantly different from each control.

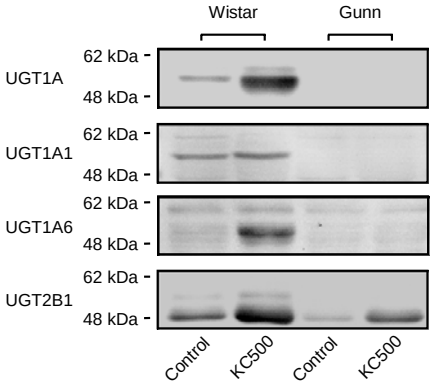


Fig. 2. Representive Western blot patterns for hepatic microsomal UGT isoforms in KC500-treated Wistar and Gunn rats

Animals were killed 4 days after the final administration of KC500 (10 mg/kg, i.p., once daily for 10 days).

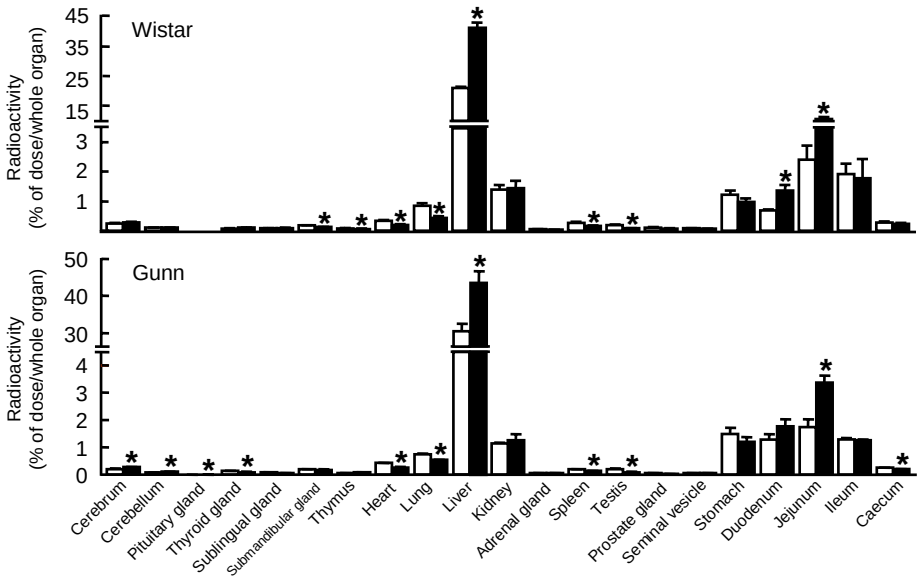


Fig. 3. Tissue distribution of total radioactivity after the administration of [$^{125}$I]$T_4$ to the KC500-treated Wistar and Gunn rats

KC500 (10 mg/kg) was given i.p. to animals at 24 hr-intervals for 10 days. The radioactivity of each tissue was measured at 60 min after the i.v. administration of [$^{125}$I]$T_4$. Each column represents the mean ± S.E. (vertical bars) for three to six animals. *$P$<0.05, significantly different from each control. ☐, control; ■, KC500.

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