3,3′,4,4′-Tetrachlorobiphenyl-Mediated Decrease of Serum Thyroxine Level in C57BL/6 and DBA/2 Mice Occurs Mainly through Enhanced Accumulation of Thyroxine in the Liver

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Received October 6, 2013; accepted December 9, 2013; advance publication released online December 17, 2013.

A single intraperitoneal injection (50 mg/kg) of 3,3′,4,4′-tetrachlorobiphenyl (CB77), a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-type polychlorinated biphenyl, led to significant decreases in the levels of serum total thyroxine (T4) and free T4 without increase in the level of serum thyroid-stimulating hormone at 7 d later in both TCDD-sensitive C57BL/6 and TCDD-resistant DBA/2 mice. When [125I]T4 was injected into the mice 7d after treatment with CB77, the levels of biliary [125I]T4 and [125I]T4-glucuronide increased 90 to 120 min post injection in C57BL/6 mice, but not in DBA/2 mice, compared with levels in the corresponding control mice. In contrast, in both strains of mice, the CB77-pretreatment led to similar changes in the levels of the [125I]T4 bound to the serum transthyretin, albumin, and thyroxine-binding globulin. Consequently, treatment with CB77 promoted the clearance of [125I]T4 from the serum and further raised the steady-state volumes of distribution of [125I]T4, the concentration ratio (Kp value) of the liver to the serum, and the distribution of [125I]T4 in the liver in both strains of mice. The present findings indicate that in mice, the CB77-mediated decrease in the serum T4 level occurs through enhanced accumulation of hepatic T4 rather than through increased activity of hepatic thyroxine-uridine 5′-diphosphate-glucuronosyltransferase(s).

Key words 3,3′,4,4′-tetrachlorobiphenyl; glucuronosyltransferase; thyroxine; mouse

Studies on polychlorinated biphenyls (PCBs)-mediated toxicities1) have been performed over the last 40 years. Fukuoka Yusho (oil disease) and Taiwan Yu-Cheng patients are reported to show various symptoms, such as acneform eruptions, hypersecretion of meibomian glands, hyperpigmentation of the face, eyelids and gingival, enlargement and disorders of the liver, and disorder of thyroid hormone.2–4) Exposure of rats and mice to PCB congeners, including 3,3′,4,4′-tetrachlorobiphenyl (CB77), 2,3′,4,4′-pentachlorobiphenyl (CB118), 3,3′,4,4′,5-pentachlorobiphenyl (CB126), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB153), is known to decrease the level of thyroid hormone in serum and to increase the activities of drug-metabolizing enzymes in the liver.5–8) As possible mechanisms for the PCB-mediated decrease in serum thyroid hormone, the increase in excretion amounts of biliary thyroxine (T4)-glucuronide by the induction of hepatic thyroxine-uridine 5′-diphosphate (UDP)-glucuronosyltransferases (T4-UGTs), especially UGT1As, responsible for thyroid hormone metabolism,5,9) and the promotion of the release of T4 from a complex of serum T4-transthyretin (TTR) by a PCB congener and its hydroxylated metabolite(s) have been proposed.10) In addition, hydroxylated PCB metabolites are reported to exhibit a high affinity for binding to serum TTR.10,11) The decrease in serum T4 caused by exposure to Aroclor 1254, a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and TCDD-like PCBs in rats is thought to result from the induction of a hepatic T4-UGT by the aryl hydrocarbon receptor (AhR).12,13) However, we recently proposed that the decrease in serum T4 levels caused by exposure to Kanechlor-500 (KC500), a commercial PCB mixture, occurs through increased accumulation of T4 in several tissues, particularly the liver, rather than through an increase in activity of hepatic T4-UGT.14,15) More recently, we also demonstrated that decreases in the level of serum T4 caused by exposure to TCDD- and/or phenobarbital-like PCBs, such as CB126,16) CB153,17) and CB118,18) occur primarily through the enhanced accumulation of T4 in the liver.

CB126 and CB77 are representative inducers of TCDD-type.19) Toyama et al.20) suggested that the CB126-induced decrease in T4 level in blood in C57BL/6J mice occurred in an AhR-dependent manner, while the CB77-induced decrease occurred in an AhR-independent manner.

In the present study, we verified the validity of our proposed theory of the mechanism by which PCB exposure causes the level of serum T4 to decrease. We selected CB77, a TCDD-like PCB that was detected in the blood and adipose tissue of the patients with Yusho.21) We herein examined whether or not there are the differences between TCDD-sensitive C57BL/6 mice and TCDD-insensitive DBA/2 mice in the CB77-induced alteration of the levels of serum thyroid hormone and hepatic T4, and discussed the results.

The authors declare no conflict of interest.


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MATERIALS AND METHODS

Chemicals  Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co., Ltd. (Tokyo, Japan). The $^{[125]}$I$^{+}$, which was radiolabelled at the $\Sigma$-position of the outer ring, was obtained from PerkinElmer, Inc. Life and Analytical Sciences (Waltham, MA, U.S.A.). CB77 was purchased from Cambridge Isotope Laboratories, Inc. (MA, U.S.A.). All other chemicals used herein were obtained commercially in appropriate grades of purity.

Animal Treatments  Male C57BL/6 and DBA/2 mice (weighing 16–31 g and 17–29 g, respectively) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Male C57BL/6 and DBA/2 mice were housed with 3 or 4 mice per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (light, 8:00 a.m. to 8:00 p.m.) in an air-controlled room (temperature, 24.5°C±1°C; humidity, 55%±5%), and handled in accordance with the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received an intraperitoneal injection of CB77 (50 mg/kg) dissolved in Panacete 810 (5 mL/kg). The control animals were treated with vehicle alone (5 mL/kg).

In Vivo Studies. Preparation of Serum  Mice were killed by decapitation 7 d after the administration of CB77. The thyroid and liver of each mouse were removed and weighed. Blood was collected from each animal between 10:30 a.m. and 11:30 a.m. The blood samples were held at room temperature to promote clotting, after which the serum was separated by centrifugation and stored at −50°C until use.

Analysis of Serum Hormones  The levels of total T$_4$, free T$_4$, and thyroid-stimulating hormone (TSH) in serum were measured in accordance with the method previously described by Kato et al.\textsuperscript{16}

Ex Vivo Studies  Seven days after the treatment with CB77, the mice were treated according to the method previously described by Kato et al.\textsuperscript{16}

Clearance of $^{[125]}$I$^{+}$ from Serum  Clearance of $^{[125]}$I$^{+}$ from serum was measured in accordance with the method previously described by Kato et al.\textsuperscript{16}

Assays for the Levels of Total $^{[125]}$I$^{+}$ and $^{[25]}$I$^{+}$ Glucuronide in Bile  After the $^{[25]}$I$^{+}$ was administered, bile was collected at 30-min intervals for 2 h and kept on ice. The volume of the bile was determined gravimetrically. The amounts of total $^{[25]}$I$^{+}$ and $^{[25]}$I$^{+}$ glucuronide in the bile were determined by using the method previously described by Kato et al.\textsuperscript{16}

Analysis of the Amount of $^{[125]}$I$^{+}$ Bound to Serum Proteins  The amounts of $^{[125]}$I$^{+}$-thyroxine binding globulin (TBG), $^{[125]}$I$^{+}$-albumin, and $^{[125]}$I$^{+}$-TTR complexes in the serum were determined in accordance with the method previously described by Kato et al.\textsuperscript{16}

Tissue Distribution of $^{[125]}$I$^{+}$  The tissue distribution of $^{[125]}$I$^{+}$ was assessed in accordance with the method previously described by Kato et al.\textsuperscript{16}

Statistical Analysis  The data were statistically analyzed with the method previously described by Kato et al.\textsuperscript{16}

RESULTS AND DISCUSSION

The serum total T$_4$ and free T$_4$ levels in both C57BL/6 and DBA/2 mice were markedly decreased by treatment with CB77 (Fig. 1), while no significant change in the level of serum TSH was observed in either strain of mice. Several reports have indicated that the level of TSH in the serum of rats and mice is not significantly changed by exposure to PCBs.\textsuperscript{14–17,22–24}

The CB77-treatment resulted in significant increase in the activity of hepatic 7-ethoxresorufin O-deethylase (Cyp1a1/2), which is induced in an AhR-dependent manner by a TCDD-type chemical,\textsuperscript{25} in C57BL/6 mice, but not in DBA/2 mice (data not shown). On the other hand, no significant increases in the levels of the protein and activity of hepatic T$_4$-UGTs, such as Ugt1a and Ugt1a1, by CB77-treatment were observed in either strain of mice (data not shown). Accordingly, CB77-mediated decreases in the levels of serum T$_4$ in both the strains of mice were not dependent on hepatic T$_4$-UGT activity, although as a possible mechanism for the TCDD-like PCB-induced decrease in serum thyroid hormones in rats, hepatic T$_4$-UGT-associated pathway is considered.\textsuperscript{13} The levels of biliary $^{[125]}$I$^{+}$ and $^{[25]}$I$^{+}$-glucuronide after an intravenous (i.v.) injection of $^{[125]}$I$^{+}$ were slightly increased in CB77-pre-treated C57BL/6 mice, but not in DBA/2 mice, compared with those in the corresponding control mice (Fig. 2). These findings suggest that there is a strain-difference between C57BL/6 and DBA/2 mice in the activity/amount of the transporter(s) responsible for their excretion to the bile duct. Incidentally, it has been reported that T$_4$ homeostasis is regulated, at least in

![Fig. 1. Effects of CB77 on the Levels of Serum Total T$_4$, Free T$_4$, and TSH.](image-url)
part, by several transporters that excrete $T_4$ and $T_4$-glucuronide into the bile duct.\(^{26-28}\)

We have previously demonstrated that the PCB-mediated decrease in serum total thyroid hormones occurred through an increased accumulation of $T_4$ in the liver rather than through an increase in formation of $[^{125}\text{I}]T_4$-glucuronide using Wistar and Gunn ($T_4$-UGT-deficient) rats.\(^{15}\) Therefore, we examined the distribution analyses of $[^{125}\text{I}]T_4$ in serum and several tissues 5 min after the $[^{125}\text{I}]T_4$ administration to CB77-pretreated mice. After administrations of $[^{125}\text{I}]T_4$ to the CB77-pretreated C57BL/6 and DBA/2 mice, serum $[^{125}\text{I}]T_4$ concentrations were measured at the indicated times (Fig. 3). In both C57BL/6 and DBA/2 mice, pretreatment with CB77 resulted in the clearance of $[^{125}\text{I}]T_4$ from serum, and their serum $[^{125}\text{I}]T_4$ levels were decreased to approximately 30% to 40% of the corresponding control level within 5 min. These decreases remained up to 120 min later. The steady state volumes of distribution of the $[^{125}\text{I}]T_4$ estimated from the data obtained in the CB77-pretreated C57BL/6 and DBA/2 mice (Fig. 3) were 1.5 and 1.9 times, respectively, compared with those in the corresponding control mice (Table 1). Furthermore, after treatment with CB77, the tissue-to-serum concentration ratio ($K_p$ value) of $[^{125}\text{I}]T_4$ was markedly increased for several tissues in both strains of mice, especially the liver and kidney (Fig. 4). In addition, no significant increase in the $K_p$ value of the thyroid was observed in either strain. In both C57BL/6 and DBA/2 mice, pretreatment with CB77 resulted in significant increases in the levels of total $[^{125}\text{I}]T_4$ in several tissues, especially the liver, and in the accumulated level in the liver accounting for approximately 40% of the total dose of $[^{125}\text{I}]T_4$ (Fig. 5). The accumulation levels of $[^{125}\text{I}]T_4$ in the liver (per g liver) of C57BL/6 and DBA/2 mice treated with CB77 were also increased (Table 2). In addition, the CB77-treatment resulted in

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**Table 1.** Distribution Volume of $[^{125}\text{I}]T_4$ after the Administration of $[^{125}\text{I}]T_4$ to the CB77-Pretreated Mice

<table>
<thead>
<tr>
<th>Distribution volume (mL)</th>
<th>C57BL/6</th>
<th>DBA/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.29±0.24</td>
<td>5.05±0.37*</td>
</tr>
<tr>
<td>CB77</td>
<td>5.05±0.37*</td>
<td>2.42±0.13</td>
</tr>
</tbody>
</table>

The data shown were calculated from the data shown in Fig. 6. The values shown are expressed as the mean±S.E. of four or five mice. *p<0.05, significantly different from each control.
a significant increase in liver weight in C57BL/6 mice, but not DBA/2 mice, while it showed no significant effects on thyroid weight in either strain of mice (Table 3).

As a possible mechanism for CB77-mediated enhancement of T₄ accumulation in the liver, a TTR-associated pathway might be considered, because PCB and its hydroxylated metabolites act as competitors for the T₄ binding to TTR. Namely, a decrease in T₄-TTR complex formation results in

![Graph showing effects of CB77 Pretreatment on the Tissue-to-Serum Concentration Ratios (Kp Values) of [125I]T4 in Various Tissues](image1)

Fig. 4. Effects of CB77 Pretreatment on the Tissue-to-Serum Concentration Ratios (Kp Values) of [125I]T₄ in Various Tissues

A portion of [125I]T₄ (15 µCi/mL) was intravenously administered to the mice pretreated with CB77 or vehicle alone (control), and at 5 min after the [125I]T₄ administration, the radioactivity in each tissue was measured. Each column represents the mean±S.E. (vertical bars) of three or four mice. *p<0.05, significantly different from each control. □ control; □ CB77.

![Graph showing tissue distribution of [125I]T₄ after administration of [125I]T₄ to CB77-Pretreated Mice](image2)

Fig. 5. Tissue Distribution of [125I]T₄ after Administration of [125I]T₄ to CB77-Pretreated Mice

CB77 (50 mg/kg) was given to the mice, and at 168 h after the treatment, [125I]T₄ was intravenously injected into the mice. At 5 min after the [125I]T₄ treatment, the radioactivity in each tissue was measured as described in Materials and Methods. Each column represents the mean±S.E. (vertical bars) of three or four mice. *p<0.05, significantly different from each control. □ control; □ CB77.
increases in serum free T₄ and a subsequent uptake of T₄ into the liver. Therefore, we examined the effects of CB77 treatment on the binding of [¹²⁵I]T₄ to serum proteins such as TTR, albumin, and TBG in C57BL/6 and DBA/2 mice (Fig. 6). In the CB77-pretreated C57BL/6 and DBA/2 mice, the serum [¹²⁵I]T₄-TTR complex levels were significantly decreased, while the binding of [¹²⁵I]T₄ to serum albumin and TBG was increased at the indicated times after [¹²⁵I]T₄ was administered. These results strongly suggest that a TTR-associated pathway partially contributes to the CB77-mediated increase in the accumulation of [¹²⁵I]T₄ in the liver.

In conclusion, we have demonstrated that in mice, the CB77-mediated decrease in serum T₄ level occurred mainly through increased accumulation of T₄ in the liver and partially through increased excretion of biliary [¹²⁵I]T₄ metabolite(s) and/or development of liver hypertrophy. In the present study, we verified our hypothesis that the decrease in serum T₄ level caused by exposure to PCBs occurs mainly through increased accumulation of T₄ in the liver, although an exact mechanism for the PCB-mediated increase in the liver-selective T₄ accumulation remains unclear. Since the several transporters, including apical and basolateral T₄-transporters, are reported to exist in the liver cells, further studies on PCB-mediated changes in the expression and activity of hepatic T₄ transporter(s) are necessary to understand the liver-selective accumulation of T₄.

Acknowledgment  This work was supported in part by a Grant-in-Aid for Scientific Research (C) [No. 23510083, Y.K.] from the Japan Society for the Promotion of Science.

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