A Possible Mechanism for Decrease in Serum Thyroxine Level by Polychlorinated Biphenyls in Wistar and Gunn Rats

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We have previously demonstrated that in mice, the decrease in serum thyroxine (T₄) level by polychlorinated biphenyls (PCBs) occurs without an increase in the UDP-glucuronosyltransferase (T₄-UDP-GT) for T₄ glucuronidation, although the PCB-induced decrease in rats is generally thought to occur through induction of T₄-UDP-GT, UGT1A1, and UGT1A6. In the present study, to further clarify the relationship between the decrease in serum T₄ level and the increase in UGT1A activity by PCB in rats, we examined the relationship using Wistar rats and Gunn rats, a mutant strain of Wistar rats deficient in UGT1A isoforms. The serum total T₄ level was markedly decreased not only in the Wistar rats but also in the Gunn rats 4 days after treatment with a PCB, Kanechlor-500 (KC500, 100 mg/kg) or 2,2',4,4',5-pentachlorobiphenyl (PentaCB, 112 mg/kg), and there was no significant difference in magnitude of the decrease between the two rat strains. At the same time, the level and activity of T₄-UDP-GT were significantly increased by treatment with either KC500 or PentaCB in Wistar rats but not in Gunn rats. In addition, no significant change in the level of serum total triiodothyronine (T₃) and thyroid-stimulating hormone by treatment with Kanechlor-500 (KC500, 100 mg/kg) or 2,2',4,4',5-pentachlorobiphenyl (PentaCB, 112 mg/kg), and there was no significant difference in magnitude of the decrease between the two rat strains. At the same time, the level and activity of T₄-UDP-GT were significantly increased by treatment with either KC500 or PentaCB in Wistar rats but not in Gunn rats. Furthermore, significant decrease in the activity of hepatic type-I deiodinase, which mediates the deiodization of T₄ to T₃, by treatment with KC500 or PentaCB was observed in both Wistar and Gunn rats. From the serum of KC500- or PentaCB-treated Wistar and Gunn rats, mono- and di-hydroxylated PCB metabolites, which would bind to T₄ binding protein (transthyretin), were detected. In conclusion, the present results suggest that the decrease in serum total T₄ level by either KC500 or PentaCB in Gunn rats was not dependent on the increase in hepatic T₄-UDP-GT activity. The findings further suggest that the PCB-mediated decrease in serum T₄ level might occur, at least in part, through formation of the hydroxylated PCB metabolites. Furthermore, even in Wistar rats, the PCB-mediated decrease in serum T₄ level might occur not only through the increase in hepatic T₄-UDP-GT but also via formation of hydroxylated PCB metabolites.

Key Words: polychlorinated biphenyls; Kanechlor-500; thyroid hormones; UDP-glucuronosyltransferases; Wistar rats; Gunn rats

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 (Craft et al., 2002; Li et al., 2001; Ness et al., 1993; Van Birgelen et al., 1995). Among the 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 [trans-thyretin (TTR)] are considered (Barter and Klaassen, 1992, 1994; Brouwer et al., 1998). In particular, the decrease in the level of serum thyroxine (T₄) 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 (T₄-UDP-GT) responsible for glucuronidation of T₄ (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). This hypothesis is supported by previous reports that a number of T₄-UDP-GT inducers, such as phenobarbital, 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, show ability to decrease serum thyroid hormone (Barter and Klaassen, 1994; De Sandro et al., 1992; Saito et al., 1991). However, the magnitude of decrease in the level of serum total T₄ is not necessarily correlated with that of increase in T₄-UDP-GT activity (Craft et al., 2002; De Sandro et al., 1992; Hood et al., 2003). Recently, we have found that treatment with Kanechlor-500 (KC500) resulted in a significant decrease in the serum T₄ level in both rats and mice, although a significant increase in T₄-UDP-GT activity occurred only in rats but not in mice (Kato et al., 2003).

In the present study, therefore, we examined a relationship between the decrease in serum total T₄ level and the increase in
the hepatic T₄-UDP-GT (UGT1A1 and UGT1A6) by PCB using Wistar and UGT1A-deficient Wistar rats (Gunn rats). In this way, we demonstrated that the PCB-mediated decrease in serum total T₄ level in rats was not necessarily dependent on the increase in hepatic T₄-glucuronidation.

**MATERIALS AND METHODS**

**Chemicals.** 2,2',4,5,5'-Pentachlorobiphenyl (PentaCB) was synthesized by using the Cadogan coupling reactions (Cadogan, 1962), Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). The [¹²⁵I]-reverse T₃ and [¹²⁵I]T₄, radiolabeled at the 5' position of the outer ring, was obtained from PerkinElmer Life Sciences, Inc. (Boston, MA). All other chemicals were obtained commercially in appropriate grades of purity.

**Animal treatments.** Male Wistar rats (160–200 g) and UGT1A-deficient Wistar rats (Gunn rats, 190–260 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male Wistar and Gunn rats were housed 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-conditioned room (temperature: 24.5 ± 1°C, humidity: 55 ± 5%), and were handled with humane care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Treatments of rats with KC500 (100 mg/kg) and PentaCB (112 mg/kg) were performed according to the method of Kato et al. (2001, 2003). Briefly, the rats received a single ip injection of KC500 (100 mg/kg) or PentaCB (112 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

**Analysis of serum hormones.** All rats were killed by decapitation on day 4 after the dosing, and the liver was removed. 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°C until used. The levels of total T₄, total triiodothyronine (T₃), free T₄, and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using the T-4 and T-3 RIABEAD (DAINABOT Co., Ltd, Tokyo, Japan), free T₄ (Diagnostic Products Corporation; Los Angeles, CA), and Biotrak rTSH [¹²⁵I] assay system (Amersham Life Science Ltd.; Little Chalfont, UK), respectively.

**Hepatic microsomal UDP-GT and deiodinase assays.** Hepatic microsomes were prepared according to the method of Kato et al. (1995). The amount of protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard. The activities of microsomal UDP-GT toward T₄ and chloramphenicol were determined by the methods of Barter and Klaassen (1992) and Ishii et al. (1994), respectively. All UDP-GT activities were measured after activation of the UDP-GTs by 0.05% Brij 58. The activity of hepatic microsomal type I outer-ring deiodinase was determined by the method of Hood and Klaassen (2000).

**Western blot analysis.** Polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and a specific antibody against UGT1A1, UGT1A6, or UGT2B1 were used (Kushiro et al., 1995, 1997). Western analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). The detection of protein was performed using a chemical luminescence (ECL detection kit, Amersham Pharmacia Biotech), and the band intensity was quantified densitometrically with LAS-1000 (FUJIFILM, Japan).

**Determination of hydroxylated PCB metabolites in the serum.** The extraction and sample clean-up procedures for serum PCB metabolites were performed by the method of Haraguchi et al. (1998). The identification of hydroxylated PCB metabolites was carried out on a GC/MS system (GC-17A, QP-5000, Shimadzu, Japan) with a DB-5 capillary column (60 m × 0.25 mm, i.d.). The temperature program was as follows: 100°C, 2 min, 100–250°C at 20°C/min, 250–280°C at 2°C/min (Mimura et al., 1999). Quantification of the hydroxylated PCB metabolites was performed on GC/EC/ED (GC-14A, Shimadzu, Japan) by comparison with an internal standard of 2,2',3,4,5,5',6-heptachloro-4-[¹³C]biphenylol. The major hydroxylated PCB metabolites (>5 ng/g liver) were analyzed.

**Statistics.** The data obtained were statistically analyzed according to Dunnett’s test after the analysis of variance (ANOVA).

**RESULTS**

**Serum Hormone Levels**

Serum constitutive levels of total T₄, free T₄, total T₃, and TSH were more than 1.5-fold higher in Gunn rats than in Wistar rats. The effects of KC500 and PentaCB on the concentration of serum thyroid hormones were next examined in Wistar and Gunn rats (Fig. 1). In both Wistar and Gunn rats, levels of serum total T₄ and free T₄ were significantly decreased by treatment with either KC500 or PentaCB, and the magnitude of the decrease was almost the same in the both rats. In contrast, no significant change in the level of serum total T₃ and TSH was observed in either Wistar or Gunn rats, with the exception of the slight decrease of serum total T₃ in PentaCB-treated Gunn rats.

**Hepatic UDP-GT and Type-I Deiodinase Activities**

It has been reported that T₄ glucuronidation is primarily mediated by the UGT1A enzymes UGT1A1 and UGT1A6 in the rat liver (Visser, 1996). Therefore, we examined the effects of KC500 and PentaCB on the hepatic T₄-UDP-GT activity in Wistar and Gunn rats. In addition, we examined whether Gunn rats show the response for the PCB-mediated induction of another UGT isoform, UGT2B1. Constitutive activity of the T₄-UDP-GT was more than 2.1-fold higher in Wistar rats than in Gunn rats. The activity of T₄-UDP-GT (UGT1A1 and UGT1A6) was significantly increased by either KC500 or PentaCB in Wistar rats but not in Gunn rats (Fig. 2). In contrast, treatment with each PCB resulted in a significant increase in the activity of UDP-GT (UGT2B1) toward chloramphenicol in both rats, although the increased level was more than 2.5-fold higher in Wistar rats than in Gunn rats.

Hepatic type-I T₄-deiodinase activity in Wistar rats was significantly decreased by KC500 but not by PentaCB, although in Gunn rats, it was significantly decreased by either PentaCB or KC500 (Fig. 3).

**Immunoblot Analysis for UGT1As**

The intensities of immunoreactive bands for hepatic UGT1A isoforms, such as UGT1A1 and UGT1A6, were increased by either KC500 or PentaCB in Wistar rats but not in Gunn rats (Figs. 4 and 5). In addition, no constitutive expression of the UGT1A isoforms was confirmed in Gunn rats. In contrast, the level of UGT2B1 was increased by either KC500 or PentaCB in both Wistar and Gunn rats, and the increased level was higher in Wistar rats than in Gunn rats (Figs. 4 and 5).
Hydroxylated PCB Metabolites in Serum

KC500 or PentaCB was administered to Wistar and Gunn rats, and 4 days after administration, hydroxylated PCB metabolites in each serum were analyzed (Table 1). In KC500-treated Wistar and Gunn rats, three mono-hydroxylated metabolites (3-OH-2,2',4,4',5-pentachlorobiphenyl, 4-OH-2,3,3',4',5-pentachlorobiphenyl, and 3'-OH-2,2',3,4,4',5'-hexachlorobiphenyl) and three dihydroxylated metabolites (3,4-(OH)2-2,3',4',5-tetrachlorobiphenyl, 3',4'-(OH)2-PentaCB, and
3,4-(OH)₂-2,3',4',5,6-pentachlorobiphenyl) were detected. 4-OH-2,3,3',4',5-pentachlorobiphenyl was a main hydroxylated metabolite, and the amounts in Wistar and Gunn rats were 89% and 56%, respectively, of the total hydroxylated metabolites detected. The sum of dihydroxylated metabolites, 3,4-(OH)₂-2,3',4',5-tetrachlorobiphenyl, 3',4'-((OH)₂-PentaCB, and 3,4-(OH)₂-2,3',4',5,6-pentachlorobiphenyl was 37% of the total hydroxylated PCB metabolites detected in KC500-treated Gunn rats, whereas in KC500-treated Wistar rats, these dihydroxylated metabolites were hardly detected (Table 1). In addition, total amounts of the hydroxylated metabolites in Wistar and Gunn rats were almost the same.

In PentaCB-treated Wistar and Gunn rats, three monohydroxylated metabolites (3-OH-PentaCB, 3'-OH-PentaCB, and 4'-OH-PentaCB) and one dihydroxylated metabolite, 3',4'-((OH)₂-PentaCB, were detected in the serum (Table 1). The relative levels of 3',4'-((OH)₂-PentaCB to the total hydroxylated metabolites of PentaCB detected in Wistar and Gunn rats were almost the same (about 83% of total hydroxylated metabolites in the corresponding rats), although the absolute level of the dihydroxylated metabolite was 2.6-fold higher in Gunn rats than in Wistar rats. In addition, serum concentrations of PentaCB, which were determined according to the method for hydroxylated PCB metabolites, were 91.6 and 127.8 ng/ml in PentaCB-treated Wistar and Gunn rats, respectively. Namely, the serum concentrations of total OH-PentaCBs in PentaCB-treated Wistar

FIG. 4. Representative immunoblot patterns for hepatic microsomal UGT isoforms in KC500-treated or PentaCB-treated Wistar and Gunn rats.

FIG. 5. Effects of KC500 and PentaCB on the level of hepatic microsomal UGT isoforms in Wistar and Gunn rats. After the immunoblot as shown in Figure 4, the isolated bands responsible for UGT isoforms were densitometrically quantified as described in Materials and Methods. The data are represented as the mean ± SE (vertical bars) for five to ten animals. *p < 0.01, significantly different from each control. ND: not detectable.
Serum Concentrations of Hydroxylated PCB Metabolites After the Administration of KC500 or PentaCB to Wistar and Gunn Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolite</th>
<th>OH-PCB concentration (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wistar</td>
</tr>
<tr>
<td>KC500</td>
<td>3-OH-2',4',4',5',6-pentachlorobiphenyl</td>
<td>98.1 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>4-OH-2,3',5',6-pentachlorobiphenyl</td>
<td>1206.3 ± 131.6</td>
</tr>
<tr>
<td></td>
<td>3'-OH-2',3,4',5',5'-hexachlorobiphenyl</td>
<td>45.3 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>3,4-(OH)2-2',3',4',5'-tetrachlorobiphenyl</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>3',4'-(OH)2-PentaCB</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>3,4-(OH)2-2',3',4',5,6-pentachlorobiphenyl</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>Sum of OH-PCBs</td>
<td>1349.8 ± 145.9</td>
</tr>
<tr>
<td>PentaCB</td>
<td>3-OH-PentaCB</td>
<td>28.6 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>3'-OH-PentaCB</td>
<td>10.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>4'-OH-PentaCB</td>
<td>12.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>3',4'-(OH)2-PentaCB</td>
<td>229.3 ± 20.3</td>
</tr>
<tr>
<td></td>
<td>Sum of OH-PentaCB</td>
<td>281.5 ± 23.1</td>
</tr>
</tbody>
</table>

Note.—The experimental conditions were the same as described in Figure 1. Results are expressed as mean ± SE for 3–6 animals.

and Gunn rats were 3.1 time higher and 5.7 times higher, respectively, than those of PentaCB in the corresponding rats.

DISCUSSION

In the present study, we found that treatment with either KC500 or PentaCB resulted in a drastic decrease in serum total T₄ and free T₄ levels in both Wistar and Gunn rats, although a significant increase in the activity of T₄-UDP-GT occurred only in Wistar rats. The present findings demonstrate that in Gunn rats, the PCB-mediated decrease in level of serum T₄ does not occur through induction hepatic T₄ glucuronidation enzymes. Although decreases in serum T₄ level by Aroclor 1254 in Gunn rats has been reported (Collins and Capen, 1980), the biochemical mechanism for the PCB-mediated decrease in serum T₄ has remained unclear. In addition, the decrease in serum T₄ level without any increase in T₄-UDP-GT activity has been reported in clofibrate-treated Gunn rats (Visser et al., 1993).

In general, PCBs, including 3,3',4,4',5-pentachlorobiphenyl, 2,2',4,4',5,5'-hexachlorobiphenyl, and Aroclor 1254 have been thought to decrease the level of serum T₄ through increase in the activity of hepatic T₄-UDP-GT (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). However, it has been reported that the difference between rats and mice in magnitude of decrease in level of serum total T₄ by 2,2',4,4',5,5'-hexachlorobiphenyl is not well correlated with that of increase in activity of T₄-UDP-GT (Craft et al., 2002). Furthermore, we have found that KC500 resulted in a significant decrease in the serum T₄ level in both rats and mice, although a significant increase in T₄-UDP-GT activity occurred only in rats but not in mice (Kato et al., 2003). In addition, in the serum level of total T₄ by PentaCB or 2,2',3,3',4,6'-hexachlorobiphenyl occurred in both rats and mice, although a significant change in activity of UDP-GT, specially UGT1A6, was hardly observed in the both species (Kato et al., 2001). These previous reports strongly support the finding that the decrease in serum total T₄ level by PCB does not occur only through an increase in hepatic T₄-UDP-GT activity.

As possible mechanisms for the PCB-mediated decrease in serum T₄, changes in type-I deiodinase activity and serum TSH level might also be considered. However, no increase (significant decrease) in hepatic activity of microsomal type-I deiodinase, which mediates the deiodization of T₄ and T₃, was observed in either Wistar or Gunn rats. Similar results have been reported in previous study using Aroclor 1254-treated Sprague-Dawley rats (Hood and Klassen, 2000). Accordingly, a PCB-mediated decrease in serum T₄ level is thought to occur through a type-I deiodinase-independent pathway. Furthermore, the level of serum TSH in both Wistar and Gunn rats was not significantly changed by either KC500 or PentaCB, indicating that TSH is not related to the PCB-mediated decrease in the serum T₄ level. In addition, it had been reported that the serum TSH level was little affected by PCB (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995; Kato et al., 2003).

As another possible mechanism, binding of hydroxylated PCBs to TTR, a major T₄-transporting protein, might be considered, (1) because hydroxylated PCB metabolites show the binding affinity for TTR (Brouwer et al., 1998; Lans et al., 1993) and (2) because the binding affinity of 4-OH-2,3,3',4',5'-pentachlorobiphenyl, which was detected as a main hydroxylated metabolite in KC500-treated rats in the present experiments, is 3.3-fold higher than that of the natural ligand T₄ (Meerts et al., 2002). The present findings and previous reports suggest that the decrease in the level of serum T₄ in either
PCB-mediated decrease in the serum T4 level remains unclear. (Kester and Hansen, 2003) and (2) the increase in estrogen sulfotransferase activity in part, to a decrease in the level of serum T4 in either KC500- or PentaCB-treated rats, the dihydroxylated metabolite was hardly detected. In addition, in PentaCB-treated Wistar and Gunn rats, the amount of \(3',4',4''\)-(OH)\(_2\)-PentaCB was more than 80% of the total dihydroxylated PCB metabolites detected in the serum. Furthermore, PentaCB, which shows a weaker affinity for TTR than natural T\(_4\) (Chauhan et al., 2000), was also detected in the serum at a low level, as compared with the total hydroxylated metabolites. Accordingly, the binding of dihydroxylated PCB metabolites and PentaCB to TTR might also be attributed, in part, to a decrease in the level of serum T\(_4\) in either KC500- or PentaCB-treated rats. However, an increase in the serum free T\(_4\) level did not occur in any rats treated with either KC500 or PentaCB, although Pedraza and colleagues (1996) have shown that the synthetic flavinoid EM-21388, which displaces T\(_4\) from TTR, increases the serum free T\(_4\) level. Considering the dihydroxylated metabolites of the PCBs examined, the decrease in serum total T\(_4\) level by KC500 or PentaCB seems to occur, at least in part, through a TTR-associated pathway, although the reason that the serum level of free T\(_4\) was decreased remains unclear. Furthermore, two other factors might be considered as possible mechanisms for the PCB-mediated decrease in the level of serum T\(_4\): (1) the change in the performance of the hypothalamo-pituitary-thyroid-axis (Khan et al., 2002; Khan and Hansen, 2003) and (2) the increase in estrogen sulfotransferase, which efficiently catalyzes the sulfation of iodothyronines (Kester et al., 1999). However, the exact mechanisms for the PCB-mediated decrease in the serum T\(_4\) level remains unclear.

In conclusion, the present findings demonstrate that the decrease in serum total T\(_4\) level by PCB in Gunn rats occurs without an increase in hepatic T\(_4\)-UDP-GT activity; they further suggest that in rats, especially Gunn rats, the PCB-mediated decrease might occur, at least in part, through formation of the hydroxylated PCB metabolites. In Wistar rats, however, the PCB-mediated induction of T\(_4\)-UDP-GT might also 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.

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