THE DECREASE IN LEVEL OF SERUM THYROXINE BY 2,2',4,5,5'-PENTACHLOROBIPHENYL IN RATS AND MICE : NO CORRELATION WITH FORMATION OF METHYL SULFONYL METABOLITES

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ABBREVIATIONS: MeSO₂, methylsulfonyl; PCB, polychlorinated biphenyl; PentaCB, 2,2',4,5,5'-pentachlorobiphenyl; T₄, thyroxine.
ABSTRACT

A relationship between formation of methylsulfonyl (MeSO₂) metabolites of 2,2',4,5,5'-pentachlorobiphenyl (PentaCB) and decrease in serum thyroxine (T₄) level was examined in the rats and mice after a single i.p. injection of PentaCB (342 µmol/kg body weight). In either rats or mice, levels of the 3- and 4-MeSO₂ metabolites of PentaCB in the liver and feces increased in a time-dependent fashion up to day 8 after PentaCB-treatment. However, there was a remarked difference between rats and mice in the amount of the metabolites formed, and cumulative amount of the either MeSO₂ metabolite for 8 days after PentaCB treatment in the liver was 4-15 times higher in mice than in rats. On the other hand, 40-60% decrease in level of serum total T₄ occurred in both rats and mice at day 1 after PentaCB treatment, and the decrease retained up to day 8 after PentaCB treatment. Thus, there was a marked difference between rats and mice in the formation of MeSO₂ metabolites from PentaCB, but not significant difference between rats and mice in PentaCB-induced decrease in level of serum total T₄, indicating that PentaCB-induced decrease in level of serum total T₄ is not necessarily dependent on the MeSO₂ metabolites formed.
Introduction

Polychlorinated biphenyls (PCBs) are environmental pollutants that accumulate in the food chain due to their high lipophilicity and low biotransformation rate (Safe, 1993). Their bioaccumulation has been detected in the environment (Olafsson et al., 1987; Kannan et al., 1989) and in human serum, adipose tissue, and milk (Safe et al., 1985; Dewailly et al., 1991; Fängström et al., 2002).

A number of methylsulfonyl (MeSO$_2$) metabolites of PCBs have been found in several species of animals in Canada, Sweden, and East Greenland (Haraguchi et al., 1992; Bergman et al., 1994; Letcher et al., 1995; Chu et al., 2003; Sandala et al., 2004) and in both healthy humans and Yusho patients in Japan (Haraguchi et al., 1986, 1989). Additionally, MeSO$_2$-PCBs were identified in human milk, blood, liver and adipose tissue (Norén et al., 1996; Weistrand et al., 1997; Weistrand and Norén, 1997; Guvenius et al., 2002). The main MeSO$_2$-PCBs found are 3- and 4-MeSO$_2$ derivatives of non-planar PCBs (Haraguchi et al., 1992; Bergman et al., 1994; Letcher et al., 1995; Norén et al., 1996; Guvenius et al., 2002; Sandala et al., 2004).

The 3-MeSO$_2$-PCBs derived from non-planar PCBs, including 2,2',4,5,5'-pentachlorobiphenyl (PentaCB) 2,3',4',5-tetrachlorobiphenyl, and 2,2',3',4',5-pentachlorobiphenyl, show much greater activities for inducing hepatic drug-metabolizing enzymes than the corresponding parent PCBs (Kato et al., 1995, 1999b), while the 4-MeSO$_2$-PCBs have no such capacity (Kato et al., 1995). More recently, we have found that some of MeSO$_2$ metabolites, including 3-MeSO$_2$- and 4-MeSO$_2$-PentaCBs (Fig. 1), were able to reduce level of serum thyroxine (T$_4$) and/or to increase level of serum thyroid stimulating hormone (TSH) in rats (Kato et al., 1998,
Furthermore, the MeSO₂ metabolite-induced decrease in serum T₄ levels was suggested to occur, at least in part, through increase in hepatic UDP-glucuronosyltransferases (UDP-GTs), UGT1A1 and UGT1A6, responsible for glucuronidation of T₄ (Kato et al., 2000b). Thus, MeSO₂ metabolites of non-planar PCBs seem to play important roles in PCB-induced toxicities.

There is a species-difference in the response to PCB-induced toxicities, including induction of drug-metabolizing enzymes, endocrine-disruption, carcinogenicity, and impairment of immune system (Safe, 1994). As one of reasons for the species-difference, difference in a PCB metabolism would be considered.

In the present study, we examined a species-difference in the in vivo metabolism of PentaCB between rats and mice and a relationship between formation of MeSO₂ metabolites and decrease in level of serum total T₄. The results demonstrate that there is a remarked species-difference in formation of MeSO₂ metabolites between rats and mice, whereas no species-difference in PentaCB-induced decrease in level of serum total T₄ was observed, indicating that there is not necessarily correlation between formation of MeSO₂ metabolites and decrease in serum total T₄.
Materials and Methods

Chemicals. PentaCB was synthesized by using the Cadogan coupling reactions (Cadogan, 1962). 3-MeSO₂⁻ and 4-MeSO₂⁻-PentaCBs were prepared by the method as described previously (Haraguchi et al., 1987). The purity of these compounds was >99% when analyzed by gas chromatography. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). All other chemicals were obtained commercially.

Animal treatments. Male Wistar rats, weighing 180-200 g, and male ddy mice, weighing 27-35 g, were housed in three or four per cage with free access to commercial chow and tap water, and 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 °C, humidity: 55 ± 5%). All animals were handled with human care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Treatments of rats and mice with PentaCB were performed according to the method of Kato et al. (1995, 1999b, 2004). Briefly, the rats and mice received a single i.p. injection of PentaCB (342 µmol/5 ml/kg) dissolved in Panacete 810. In addition, control animals were treated with a vehicle alone (5 ml/kg). All animals were killed by decapitation on designated time after the dosing, and the liver was removed and kept at -50°C until examined. Liver microsomal fractions were prepared according to the method of Kato et al. (1995) and stored at −85°C until used.

Level of serum thyroid hormone T₄. 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. Level of serum total T₄, was measured by radioimmunoassays using an Amerlex-MT4 assay system (Amersham Life Science Ltd.; Little Chalfont, UK).

**Hepatic microsomal T₄-UDP-GT activity.** Amount of microsomal protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Activity of microsomal T₄-UDP-GT was determined by the method of Barter and Klaassen (1992).

**Determination of PentaCB and its MeSO₂ metabolites.** Amounts of PentaCB and its MeSO₂ metabolites in the liver and feces were determined with gas chromatography as described previously (Bergman et al., 1992). In the present study, the total area under the liver concentration versus time curve was calculated by the trapezoidal rule and shown as an AUC.

**Statistics.** The data obtained were statistically analyzed according to Student’s t-test.
Results

MeSO₂ metabolites of PentaCB. We have previously reported that when rats and mice were treated with PentaCB (34 µmol/kg), sum of hepatic 3- and 4-MeSO₂ metabolites detected in mice was two-fold higher than that in the rats (Haraguchi et al., 2005). To further clarify a difference between rats and mice in the level of the MeSO₂ metabolites from PentaCB, we examined levels of the MeSO₂ metabolites in both the liver and feces from the PentaCB (342 µmol/kg)-treated rats and mice. Amounts of PentaCB and its MeSO₂ metabolites, 3- and 4-MeSO₂-PentaCBs, after the PentaCB treatment in either the liver or the feces were clearly different between rats and mice. Level of PentaCB in the liver of either rats or mice reached the maximum day 2 after the treatment and then decreased. The maximum levels in rats and mice were about 10 nmol/g tissue and about 35 nmol/g tissue, respectively. Levels of 3- and 4-MeSO₂-PentaCBs in the mouse liver increased in a time-dependent fashion up to day 4 after the PentaCB treatment, and the maximum levels of 3- and 4-MeSO₂-PentaCBs were about 4 nmol/g tissue and about 7 nmol/g tissue, respectively (Fig. 2). On the other hand, in the rat liver, the 3- and 4-MeSO₂ metabolites were hardly produced, and levels of the metabolites were less than 1 nmol/g tissue at the any time examined. AUC values in 3- and 4-MeSO₂-PentaCBs in the liver were 17.50 and 31.54 nmol·day/g tissue, respectively, in mice and 2.92 and 4.83 nmol·day/g tissue, respectively, in rats. Thus, the AUC values obtained were much higher in mice than in rats.

In feces, level of PentaCB was higher in rats than in mice, whereas levels of 3- and 4-MeSO₂ metabolites of PentaCB were much higher in mice than in rats (Fig. 3). In addition, the 3-MeSO₂ metabolite was hardly detected in the rat feces.
Serum thyroid hormone level. Effects of PentaCB on the levels of serum thyroid hormone T₄ in rats and mice were next examined. Levels of serum total T₄ in both rats and mice were significantly decreased by treatment with PentaCB (Fig. 4). In either rats or mice, level of serum total T₄ decreased to 40-60% of the corresponding control level at day 1 after PentaCB treatment, and the decrease was maintained up to day 8 in each species of animal. In addition, no significant change in level of serum TSH after treatment with PentaCB was found in either rats or mice (data not shown).

Hepatic T₄-UDP-GT activity. We examined effects of PentaCB on hepatic T₄-UDP-GT activity in rats and mice. Hepatic activities of T₄-UDP-GT day 4 after treatment with PentaCB in rats and mice increased to 2-fold and 1.6-fold over the corresponding control levels, respectively (Table 1).
**Discussion**

We have previously reported that there are species differences between rats and mice in the level of PentaCB and its metabolites, including methylsulfonylated and hydroxylated PentaCBs, and in their tissue distributions (Haraguchi et al., 2005). In the present study, clear difference in the formation of MeSO$_2$ metabolites between the PentaCB-treated rats and mice was confirmed. A possible mechanism for the formation of the sulfur-containing metabolites from PentaCB is showed in Fig. 5 (Hansen, 1999). In brief, PentaCB is first oxidized to arene oxide intermediate in the liver, and the resultant arene oxide is converted to glutathion-conjugated form by hepatic glutathione S-transferase. The glutathion conjugate is introduced to a mercapturic acid pathway and thereafter, excreted as a cysteine conjugate into the gastrointestinal tract via the bile. C-S bond of the cysteine conjugate is cleaved to thiol form by intestinal microflora C-S lyase. The resultant thiol compound is methylated to methylsulfide form. Thereafter, the methylsulfide is oxidized to methylsulfoxide and further to methylsulfone (MeSO$_2$ metabolites) in the liver. In addition, Koga et al. (2002) have suggested that S-oxidation of the methylsulfide is catalyzed by cytochrome P450 enzymes, especially CYP2B subfamily enzymes in rats, hamsters, and guinea pigs. Considering a possible process of formation of MeSO$_2$ metabolites, difference between rats and mice in the amount of hepatic MeSO$_2$ metabolites formed from PentaCB would be attributed to the difference in the activity of CYP2B subfamily enzymes, glutathione S-transferase, and/or intestinal microflora C-S lyase.

We have previously reported that 3-MeSO$_2$ metabolite of PentaCB showed higher activity than a parent compound PentaCB for inducing hepatic drug-metabolizing
enzymes (Kato et al., 1995, 1999b) and further demonstrated that some MeSO₂ metabolites, including 3-MeSO₂- and 4-MeSO₂-PentaCBs, could reduce serum total T₄ level in rats (Kato et al., 1998, 1999a, 2000a). Accordingly, it has been expected that MeSO₂-PentaCB metabolites contribute to decrease in the level of serum T₄ in PentaCB-treated animals. However, despite levels of MeSO₂-PentaCB metabolites in both the liver and feces were much higher in mice than in rats, magnitudes of decrease in the level of serum total T₄ in rats and mice were almost the same. The present findings indicate that PentaCB-induced decrease in serum T₄ level is not dependent on only the MeSO₂ metabolites formed.

Decrease in the level of serum T₄ by PCB has been thought to occur through increase in hepatic T₄-UDP-GT activity (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). However, it has been reported that 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). Likewise, we have reported that treatment with Kanechlor-500 resulted in a significant decrease in serum T₄ level in both rats and mice, although a significant increase in the activity of T₄-UDP-GT enzymes, including UGT1A1 and UGT1A6, by the PCB occurred only in rats but not in mice (Kato et al., 2003) and further demonstrated that treatment with either Kanechlor-500 or PentaCB resulted in a drastic decrease in serum total T₄ level even in UGT1A1/1A6-deficient (Gunn) rats (Kato et al., 2004). These previous reports strongly propose that the decrease in serum total T₄ level by PCBs including PentaCB does not occur only through increase in hepatic T₄-UDP-GT activity, although significant increase in T₄-UDP-GT activity by PentaCB was observed in both rats and mice (Table 1).
In the present study, level of serum TSH in either rats or mice was not significantly changed by PentaCB, indicating that TSH is not related to the PentaCB-induced decrease in serum T₄ level. In addition, it had been reported that serum TSH level was little affected by PCBs (Liu et al., 1995; Hood et al., 1999; Hallgren et al., 2001; Kato et al., 2003).

In the PentaCB-treated rats and mice, 3-OH-, 3'-OH-, 4'-OH- and 3',4'-(OH)₂-PentaCBs were found in the both liver and serum (Haraguchi et al., 2005). Mono- and di-hydroxylated PCB derivatives (Meerts et al., 2002; Lans et al., 1993), including 4-OH-2,3,3',4',5-pentachlorobiphenyl, 4,4'-(OH)₂-3,3',5,5'-tetrachlorobiphenyl, and 4,4'-(OH)₂-2,3,3',5,5'-pentachlorobiphenyl, have been reported to bind to T₄-transporting serum protein transthyretin (TTR). Therefore, PentaCB-induced decrease in the level of serum total T₄ might occur, in part, through formation of the hydroxylated metabolites showing ability to bind to TTR.

In conclusion, we demonstrate herein that there is a remarked difference between rats and mice in the formation of MeSO₂ metabolites from PentaCB and further suggest that PentaCB-induced decrease in level of serum total T₄ is not necessarily dependent on the formation of the MeSO₂ metabolites. Additionally, the present findings demonstrate that PentaCB-induced decrease in serum total T₄ level in either rats or mice occurs without increase in serum TSH level. Although the PentaCB-induced decrease might occur, at least in part, through induction of T₄-UDP-GT and/or formation of the hydroxylated metabolites from PentaCB, exact mechanism for PentaCB-induced decrease in serum thyroid hormones remains unclear. Further studies on PentaCB-induced alterations of the level and function of T₄-transporters in the liver and extrahepatic tissues would be necessary for understanding
of the exact mechanism.
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Reduction of thyroid hormone levels by methylsulfonyl metabolites of tetra- and


Footnotes

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Legends for figures

FIG. 1. Chemical structures of PentaCB and its MeSO₂ derivatives.

FIG. 2. Levels of parent PCB and its MeSO₂ metabolites in the liver of PentaCB-treated rats and mice. Animals were killed at the indicated times after treatment of PentaCB (342 µmol/kg, i.p.). Each point represents the mean ± SE (vartical bars) for three to four animals. ○: Rats, ●: mice.

FIG. 3. Levels of parent PCB and its MeSO₂ metabolites in the feces of the PentaCB-treated rats and mice. Animals were given PentaCB (342 µmol/kg, ip, each). Each point represents the mean ± SE (vartical bars) for three to four animals. ○: Rats, ●: mice.

FIG. 4. Effects of PentaCB on levels of serum total T₄ in rats and mice. Levels of serum thyroid hormone T₄ after treatment of PentaCB (342 µmol/kg, i.p.) to animals were measured as described in “Materials and Methods”, and the data were represented as percentages of the corresponding controls (constitutive level). Constitutive levels: total T₄ (µg/dl), 1.53 ± 0.08 in rats and 1.17 ± 0.12 in mice. Each point represents the mean ± SE (vertical bars) for three to eight animals. *P<0.05, significantly different from the corresponding controls (0 hr). ○: Rats, ●: mice.

FIG. 5. A possible pathway for formation of MeSO₂ metabolites from PentaCB.
TABLE 1

*Effects of PentaCB on hepatic \( T_f \)-UDP-GT activity in rats and mice*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Activity (pmol/mg protein/min)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PentaCB</td>
</tr>
<tr>
<td>Rats</td>
<td>12.7 ± 1.2</td>
<td>25.7 ± 2.6*</td>
</tr>
<tr>
<td>Mice</td>
<td>21.2 ± 3.0</td>
<td>34.8 ± 4.8*</td>
</tr>
</tbody>
</table>

Animals were given PentaCB (342 \( \mu \)mol/kg, i.p.) and killed at day 4 after the treatment. Data represent the mean ± SE for 3 - 6 animals. *\( p<0.05 \), significantly different from the corresponding controls.
Fig. 1

PentaCB

3-MeSO_2-PentaCB

4-MeSO_2-PentaCB
Fig. 2
Fig. 3

- PentaCB (% of total dose)
- 3-MeSO₂-PentaCB (% of total dose)
- 4-MeSO₂-PentaCB (% of total dose)

Day after administration
Fig. 4

Total thyroxine (% of control)

Day after administration

0 2468

0

25

50

75

100

125

* * * * * * * *
Fig. 5