

4'-MeO-2,2',3,3',5,5',6-heptaCB (CB178) were obtained by the diazo-coupling reaction with 2,3,5,6-tetrachloroaniline (Aldrich, St. Louis, MO) and 2,3,6-trichlorophenol (Lancaster Synthesis, Morecambe, UK) (Hutzinger et al. 1971) and subsequent methylation with dimethylsulfate. Finally, they were purified by silica gel 60 (0.040–0.063 mm, Merck) column chromatography, TLC (silica gel 60F₂₅₄, Merck) and reverse-phase HPLC. The HPLC conditions were as follows; column, ODS (20 × 250 mm, 5 μm, YMC); mobile phase, acetonitrile; flow rate, 4 ml min⁻¹; detection wavelength, 250 nm. PB (Na salt) and MC were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Nicotine adenine dinucleotide phosphate (NADP⁺) and glucose-6-phosphate (G-6-P) were purchased from Oriental Yeast Co., Ltd (Tokyo). G-6-P dehydrogenase and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of the highest quality commercially available.

4-MeO-CB187 (M-3). ¹H-NMR (500 MHz, chloroform-*d*) δ 3.990 (3H, s, 4-CH₃O), 7.287 (1H, s, 3'-H), 7.646 (1H, s, 6'-H); MS(EI) *m/z* (relative intensity) 422(100) [M⁺], 424(227) [M⁺ + 2], 426(214) [M⁺ + 4], 428(118) [M⁺ + 6], 396(27) [M⁺ + 8], 407(15) [M⁺-CH₃], 379(46) [M⁺-COCH₃], 309(54) [M⁺-COCH₃Cl₂].

3'-MeO-CB187. ¹H-NMR (500 MHz, chloroform-*d*) δ 3.967 (3H, s, 3'-CH₃O), 7.106 (1H, s, 6'-H), 7.700 (1H, s, 4-H); MS(EI) *m/z* (relative intensity) 422(100) [M⁺], 424(223) [M⁺ + 2], 426(214) [M⁺ + 4], 428(113) [M⁺ + 6], 430(36) [M⁺ + 8], 407(13) [M⁺-CH₃], 379(40) [M⁺-COCH₃], 372(12) [M⁺-CH₃Cl₂], 309(56) [M⁺-COCH₃Cl₂].

4'-MeO-CB178 (M-2). ¹H-NMR (500 MHz, chloroform-*d*) δ 4.004 (3H, s, 4'-CH₃O), 7.155 (1H, s, 6'-H), 7.697 (1H, s, 4-H); MS(EI) *m/z* (relative intensity) 422(100) [M⁺], 424(218) [M⁺ + 2], 426(206) [M⁺ + 4], 428(106) [M⁺ + 6], 430(33) [M⁺ + 8], 407(28) [M⁺-CH₃], 379(41) [M⁺-COCH₃], 309(74) [M⁺-COCH₃Cl₂].

2'-MeO-2,3,5,6,3',5'-hexaCB (CB165). MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(189) [M⁺ + 2], 392(153) [M⁺ + 4], 394(66) [M⁺ + 6], 396(15) [M⁺ + 8], 338(154) [M⁺-CH₃Cl], 318(22) [M⁺-Cl₂], 275(40) [M⁺-COCH₃Cl₂].

3'-MeO-2,3,5,6,2',5'-hexaCB (CB151). MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(194) [M⁺ + 2], 392(155) [M⁺ + 4], 394(67) [M⁺ + 6], 396(9) [M⁺ + 8], 345(24) [M⁺-COCH₃], 318(9) [M⁺-Cl₂], 275(46) [M⁺-COCH₃Cl₂].

4'-MeO-CB151 (M-1). ¹H-NMR (500 MHz, chloroform-*d*) δ 5.778 (1H, s, 4'-OH), 7.149 (1H, s, 3'-H), 7.218 (1H, s, 6'-H), 7.675 (1H, s, 4-H); MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(190) [M⁺ + 2], 392(152) [M⁺ + 4], 394(66) [M⁺ + 6], 396(16) [M⁺ + 8], 373(5) [M⁺-CH₃], 345(34) [M⁺-COCH₃], 318(7) [M⁺-Cl₂], 275(43) [M⁺-COCH₃Cl₂].

5'-MeO-2,3,5,6,2',4'-hexaCB (CB147). MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(183) [M⁺ + 2], 392(147) [M⁺ + 4], 394(63) [M⁺ + 6], 396(15) [M⁺ + 8], 373(20) [M⁺-CH₃], 345(27) [M⁺-COCH₃], 318(7) [M⁺-Cl₂], 275(50) [M⁺-COCH₃Cl₂].

6'-MeO-CB151. MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(192) [M⁺ + 2], 392(154) [M⁺ + 4], 394(66) [M⁺ + 6], 396(16) [M⁺ + 8], 338(86) [M⁺-CH₃Cl], 318(43) [M⁺-Cl₂], 275(36) [M⁺-COCH₃Cl₂].

6-MeO-CB146. ¹H-NMR (500 MHz, chloroform-*d*) δ 3.605 (3H, s, 6-CH₃O), 7.327 (1H, s, 4-H), 7.609 (1H, s, 3'-H), 7.643 (1H, s, 6'-H); MS(EI) *m/z* (relative intensity) 388(100)

[M⁺], 390(193) [M⁺+2], 392(156) [M⁺+4], 394(86) [M⁺+6], 396(16) [M⁺+8], 338(134) [M⁺-CH₃Cl], 318(29) [M⁺-Cl₂], 275(39) [M⁺-COCH₃Cl₂].

5-MeO-2,3,6,2',4',5'-hexaCB (CB149). ¹H-NMR (500 MHz, chloroform-*d*) δ 3.951 (3H, s, 5-CH₃O), 7.127 (1H, s, 4-H), 7.284 (1H, s, 3'-H), 7.634 (1H, s, 6'-H); MS(EI) *m/z* (relative intensity) 388(100) [M⁺], 390(190) [M⁺+2], 392(154) [M⁺+4], 394(65) [M⁺+6], 396(16) [M⁺+8], 373(4) [M⁺-CH₃], 345(30) [M⁺-COCH₃], 338(16) [M⁺-CH₃Cl], 318(6) [M⁺-Cl₂], 275(42) [M⁺-COCH₃Cl₂].

Animal treatment

Nine male Wistar rats (body weight about 200 g), nine male Golden Syrian hamsters (body weight about 90 g) and nine male Hartley guinea pigs (body weight about 300 g) were used and divided into three groups, untreated, PB-treated and MC-treated groups. PB and MC were dissolved with saline and corn oil and injected i.p. to animals at a dose of 80 and 20 mg kg⁻¹ day⁻¹ for 3 days, respectively. Animals were killed 24 h after the last injection of each inducer, and liver microsomes were prepared as described elsewhere (Koga et al. 1990).

Quantification of metabolites

In the *in vitro* study, 40 μM CB187 was incubated at 37°C with 1 mg of protein from animal liver microsomes, NADPH-generating system (0.33 mM NADP⁺, 5 mM G-6-P, 1 unit of G-6-P dehydrogenase), 6 mM MgCl₂ and 100 mM HEPES buffer (pH 7.4) in a total volume of 1 ml. After incubation for 60 min, unchanged CB187 and its metabolites were extracted three times with chloroform-methanol (2:1, v/v) and *n*-hexane. The pooled organic layer was evaporated to dryness, methylated with diazomethane and applied to a gas chromatograph HP5890 Series II equipped with ECD under the following conditions: DB-1 capillary column (30 m × 0.25 mm, 0.25 μm thickness); carrier gas, N₂ (1 ml min⁻¹); column temperature, 230°C; injection port temperature, 250°C; detector temperature, 250°C. The CB187 metabolites were quantified by a standard calibration curve for GC peak area. Analysis of CB187 and its metabolites by GC/MS was performed using Agilent 5973 inert MSD under the following conditions: HP-5 fused capillary column (60 m × 0.25 mm, 0.25 μm thickness); carrier gas, He (1 ml min⁻¹); column temperature, 70°C (1.5 min)–20°C min⁻¹–230°C (0.5 min)–4°C min⁻¹–280°C (5 min); injection port temperature, 250°C; detector temperature, 230°C.

Inhibition study with antiserum against guinea pig CYP2B18

Rabbit antiserum against guinea pig CYP2B18 was prepared as reported previously (Koga et al. 1998a). Antiserum (100, 200 and 300 μl each) was preincubated with liver microsomes (1 mg of protein) of PB-treated guinea pigs at 4°C for 16 h in 100 mM HEPES buffer (pH 7.4). After the addition of 6 mM MgCl₂, 40 μM CB187 and NADPH-generating system (0.33 mM NADP⁺, 5 mM G-6-P, 1 unit of G-6-P dehydrogenase), the incubation was performed at 37°C for 60 min. Analysis of metabolites was done in a same manner as described above.

Other methods

Protein measurement was made by the method of Lowry et al. (1951) using BSA as a standard.

Results

Identification of CB187 metabolites

When CB187 was incubated for 60 min with guinea pig liver microsomes, three metabolites were observed at retention times of 17.57 min, 21.49 min and 22.51 min in GC/ECD and named tentatively M-1, M-2 and M-3, respectively, as shown in Figure 1. To obtain some information on the chemical structure of the metabolites, a large-scale incubation (100 ml) with liver microsomes of PB-treated guinea pigs was conducted at 37°C for 60 min, and the metabolites were extracted with organic solvents, methylated by diazomethane and applied to GC/MS. Table I shows the mass spectral data and retention times of three methylated metabolites. The methylated derivatives of M-2 and M-3 gave the same molecular weight of 422 and the fragment ion of M^+-15 (m/z 407) indicating monomethoxylated compounds at the 4- or 4'-positions. Moreover, their retention times in GC/MS were completely identical with synthetic 4'-MeO-CB178 and 4-MeO-CB187, indicating that M-2 and M-3 were 4'-OH-CB178 and 4-OH-CB187, respectively. On the other hand, methylated M-1 had a molecular weight of 388, which suggested that M-1 is a monohydroxylated and dechlorinated metabolite. From a comparison of the retention time and mass fragmentation

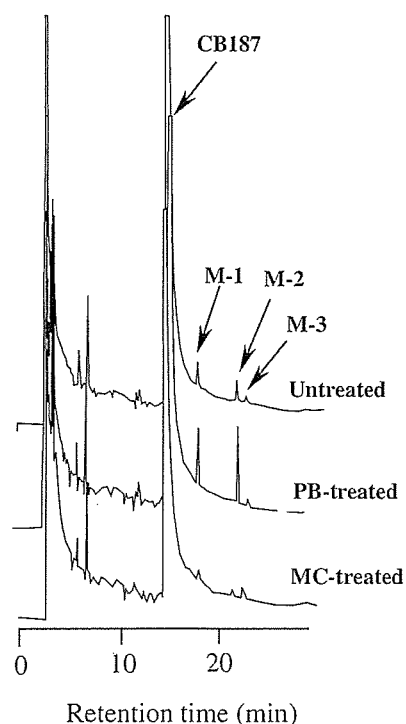


Figure 1. Gas chromatograms of the methylated derivatives of CB187 metabolites formed by guinea pig liver microsomes.

Table I. Mass spectral data and retention times of methylated derivatives of three CB187 metabolites and synthetic compounds.

Compound	Molecular weight	Mass spectral data							Retention time (min)
		[M ⁺]	[M ⁺ -15]	[M ⁺ -35]	[M ⁺ -43]	[M ⁺ -50]	[M ⁺ -70]	[M ⁺ -113]	
CB187	392	100	–	26	–	–	84	–	14.57
M-1	388	100	5	–	36	–	8	43	15.67
M-2	422	100	26	–	31	–	–	55	16.63
M-3	422	100	26	–	40	–	–	58	16.88
2'-MeO-hexaCB (CB165)	388	100	–	–	–	154	22	40	14.19
6-MeO-hexaCB (CB146)	388	100	–	–	–	134	29	39	14.35
5'-MeO-hexaCB (CB147)	388	100	20	–	27	–	7	50	15.29
3'-MeO-hexaCB (CB151)	388	100	–	–	24	–	9	46	15.34
6'-MeO-hexaCB (CB151)	388	100	–	–	–	86	43	36	15.62
4'-MeO-hexaCB (CB151)	388	100	5	–	34	–	7	43	15.67
5-MeO-hexaCB (CB146)	388	100	4	–	30	16	6	42	15.72
3'-MeO-heptaCB (CB187)	422	100	13	–	40	12	–	6	16.45
4'-MeO-heptaCB (CB178)	422	100	28	–	41	–	–	74	16.63
4-MeO-heptaCB (CB187)	422	100	15	–	46	–	–	54	16.88

pattern in GC/MS with synthetic monomethoxylated hexaCBs, M-1 was determined to be 4'-OH-CB151 (Table I).

Effect of CYP inducers on CB187 metabolism

The metabolic profile of CB187 was compared between liver microsomes of guinea pigs, rats and hamsters, and the metabolites were analyzed by GC/ECD. The formation rates of M-1, M-2 and M-3 were 18.1, 36.6 and 14.7 pmol h⁻¹ mg protein⁻¹ in untreated guinea pigs, whereas in untreated rats and hamsters, no metabolite was observed (Figure 2). The formation of M-1 and M-2 in guinea pigs increased by 1.9- and 3.4-fold of the control value by PB-treatment, respectively, but decreased to less than 50% following MC-treatment. In contrast, the formation of M-3 was not affected by PB- or MC-treatment. In rats, M-2 and M-3 was formed only by PB-treated liver microsomes at rates of 87.1 and 13.8 pmol h⁻¹ mg protein⁻¹, respectively, whereas M-1 was not found in any of the microsomes tested. In hamsters, PB-treated microsomes formed only M-2 but at a slower rate than those of guinea pigs and rats, but untreated and MC-treated microsomes formed no metabolite.

Inhibitory effect of antiserum against guinea pig CYP2B18

To elucidate the possible involvement of guinea pig CYP2B18 in CB187 metabolism, an inhibition study using antiserum against CYP2B18 was performed (Figure 3). When anti-CYP2B18 antiserum (300 µl) was preincubated with liver microsomes from PB-treated guinea pigs at 4°C for 16 h, the formation of M-1 was inhibited up to 80% of the control serum values, and the formation of both M-2 and M-3 was completely inhibited. These results suggested that guinea pig CYP2B18 is involved in CB187 metabolism to a major extent.

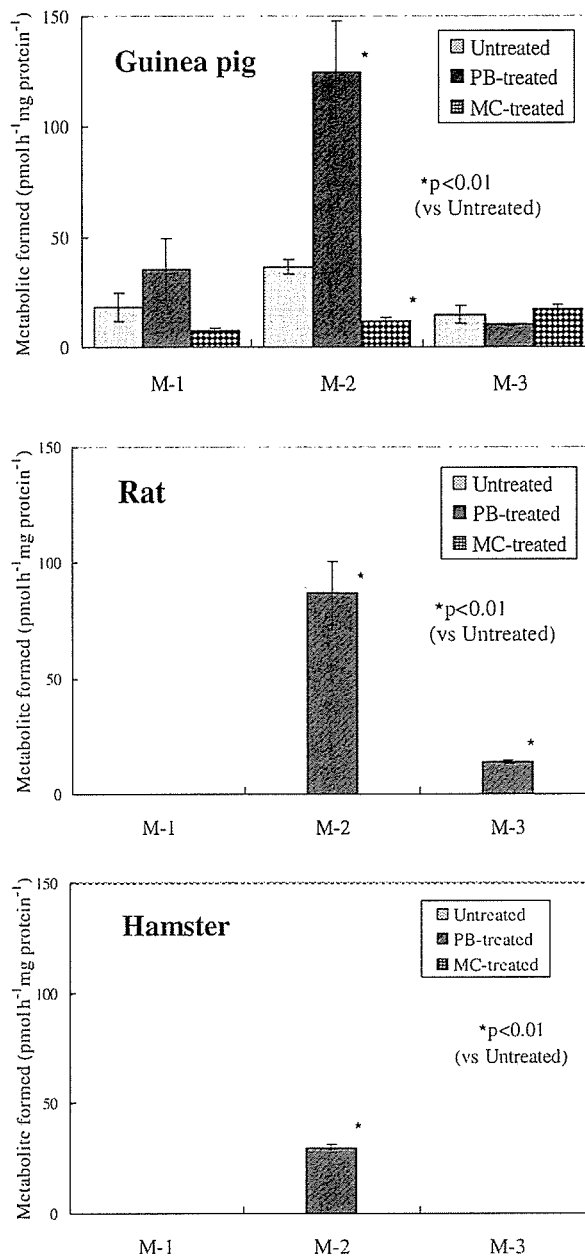


Figure 2. Effects of CYP inducers on CB187 metabolism with liver microsomes of guinea pigs, rats and hamsters. Each column represents the mean \pm SD (vertical bars) of three animals. *Significantly different from untreated animals ($p < 0.01$).

Discussion

In this study, we first found that CB187 was metabolized to three metabolites by guinea pig liver microsomes, and these corresponded to a monohydroxy-dechlorinated metabolite (M-1) and two monohydroxy-heptaCB (M-2 and M-3) from the GC/MS data.

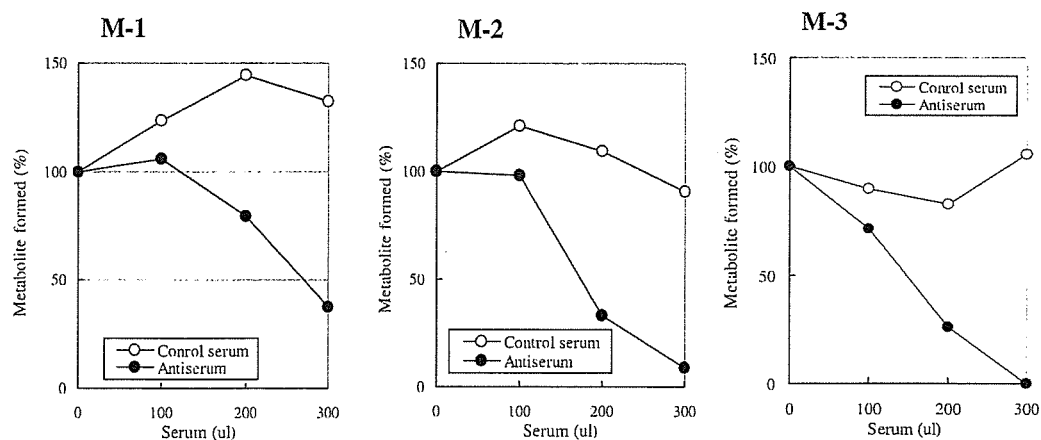


Figure 3. Inhibitory effects of anti-CYP2B18 antiserum on CB187 metabolism. Each point represents the mean of two determinations.

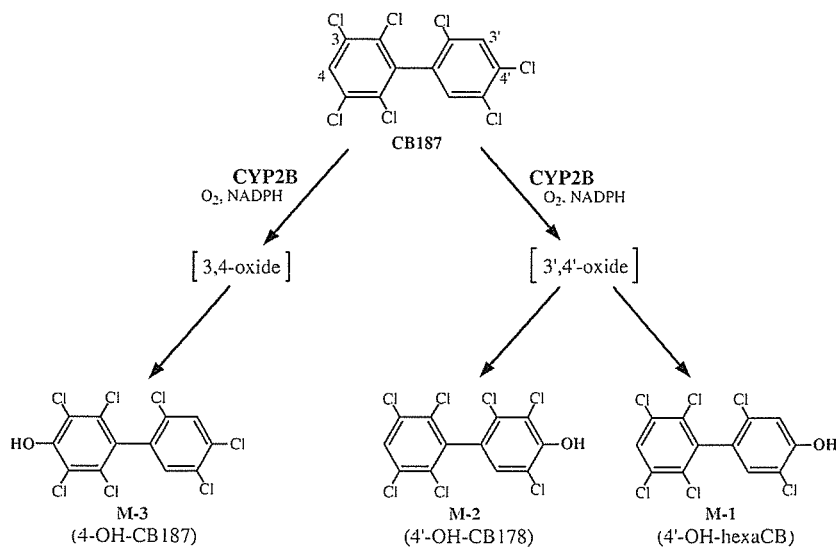


Figure 4. Postulated pathways of CB187 in laboratory animals.

By comparison with synthetic standards, M-1, M-2 and M-3 were determined to be 4'-OH-CB151, 4'-OH-CB178 and 4-OH-CB187, respectively, and the postulated metabolic pathways are shown in Figure 4. Our recent studies have demonstrated that 2,4,5-trichloro-substituted PCB congeners such as CB118, CB138 and CB153 are metabolized to their 2-OH metabolites as a major metabolite in dogs and guinea pigs and that 2-hydroxylation was catalyzed by PB-inducible CYP enzymes, dog CYP2B11 and guinea pig CYP2B18 (Ariyoshi et al. 1995, 1997, Koga et al. 2001, 2002). These facts suggest that the metabolism of 2,4,5-trichloro-substituted PCB congeners proceed mainly via a 2,3-oxide formation and subsequent NIH-shift of a chlorine at the 2-position to the 3-position on the 2,4,5-trichloro-substituted benzene ring. However, we observed the production of two 4'-OH metabolites,

M-1 and M-2, during CB187 metabolism, indicating that CB187 metabolism proceeds via the formation of 3',4'-oxide as an intermediate and subsequent NIH-shift of a chlorine at the 4'-position to the 3'-position or dechlorination on the 2,4,5-trichloro-substituted benzene ring. In addition, it is possible that M-3 may be produced by the formation of the 3,4-oxide as an intermediate or by direct hydroxylation (Preston et al. 1983). The reason why the metabolic profile of CB187 is different from that of CB118, CB138 and CB153 is unclear at present but the fact that CB187 is a tri-*ortho*-PCB and may be more bulky than mono- or di-*ortho*-PCB such as CB118, CB138 and CB153, needs to be considered.

CB187 metabolism was also increased by PB treatment in all three species examined herein, which suggests the involvement of PB-inducible CYP enzymes such as guinea pig CYP2B18 (Oguri et al. 1991, Narimatsu et al. 1992, Koga et al. 1998a, b), rat CYP2B1/2B2 (Kaminsky et al. 1981, Ishida et al. 1991) and hamster CYP2B (HPB-1) (Koga et al. 1995). In fact, addition of antiserum against CYP2B18 showed almost complete inhibition of M-1, M-2 and M-3 formed by liver microsomes of PB-treated guinea pigs. Unexpectedly, as mentioned above, the metabolite formed by PB-treated animals was not a 2-OH metabolite but a 4- or 4'-OH metabolite. From these results, it is suggested that the substrate specificity of the CYP2B enzyme for 2,4,5-trichloro-substituted PCB congeners could be influenced by the degree of the angle where two phenyl rings in a PCB molecule rotate relative to each other.

The dechlorinated monohydroxy-metabolites have often been observed in the metabolism of PCB congeners such as CB77 (Yoshimura et al. 1987, Koga et al. 1989, Wehler et al. 1989), CB138 (Koga et al. 2001), CB153 (Kato et al. 1980, Ariyoshi et al. 1992) and CB155 (2,2',4,4',6,6'-hexaCB) (Ariyoshi et al. 1993). However, the formation mechanism still remains obscure at present although the oxidation by CYPs is required as an initial event. In the current study, guinea pig liver microsomes produced the dechlorinated monohydroxy-metabolite, M-1, at a relatively high rate, and the formation was increased by PB treatment. Thus, the enzyme system present in guinea pig liver microsomes may be useful to elucidate the reaction mechanism.

As described above, 4-OH-CB187 is the most abundant metabolite of all hydroxylated metabolites in human blood, whereas it was not a major metabolite formed by animal liver microsomes used in the present study. Recently, Malmberg et al. (2004) compared the half-lives of 4-OH-2,3,3',4',5-pentaCB (CB107), a main metabolite of CB118, and 4-OH-CB187 in rat blood after an intravenous injection of both compounds and demonstrated that the half-life of 4-OH-CB187 and 4-OH-CB107 was 15.4 and 4.3 days, respectively. These facts indicate that even if the amount of 4-OH-CB187 formed in animal liver is very small, it would be distributed preferably to blood and retained there selectively during the lifespan of the animals. On the other hand, 4-OH-CB187 could be produced from 2,2',3,4,4',5',6-heptaCB (CB183), which has also been detected as a minor component in PCB preparations (Ballschmiter et al. 1989, Mimura et al. 1999a). Further studies on CB183 metabolism are needed to elucidate the toxicological significance of 4-OH-CB187.

Acknowledgments

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DECREASE IN SERUM THYROXINE LEVEL BY PHENOBARBITAL IN RATS IS NOT NECESSARILY DEPENDENT ON INCREASE IN HEPATIC UDP-GLUCURONOSYLTRANSFERASE

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ABSTRACT:

We have previously reported that there is a poor correlation between increase in the levels of UDP-glucuronosyltransferases, UGT1A1 and UGT1A6, and decrease in the levels of serum total thyroxine (T_4) and free T_4 in phenobarbital (PB)-treated rats, although the PB-induced decrease in rats is generally thought to occur through induction of the UDP-glucuronosyltransferase (T_4 -UDP-GT: UGT1A1 and UGT1A6). In the present study, to clarify a relationship between the decrease in serum T_4 level and the increase in the T_4 -UDP-GT activity by PB in rats, we examined the relationship using Gunn rats, a mutant strain of Wistar rats deficient in UGT1A isoforms. Levels of serum total T_4 , free T_4 , and total triiodothyronine (T_3) were markedly decreased not only in Wistar rats but also in Gunn rats 1 day after the final administration of PB (80 mg/kg i.p., once daily for 4 days), and no significant difference in magnitude of the decrease between Wistar and Gunn rats was

observed. On the other hand, the level and activity of T_4 -UDP-GT were significantly increased by treatment with PB in Wistar rats but not in Gunn rats. Furthermore, significant decrease in the activity of hepatic type I iodothyronine deiodinase, which mediates the deiodination of T_4 and T_3 , by PB treatment was observed in both Wistar and Gunn rats. In addition, no significant change in the level of serum thyroid-stimulating hormone, the activity of hepatic sulfotransferase, and the binding of [125 I] T_4 to serum transthyretin and albumin by PB treatment was observed in either Wistar or Gunn rats. In conclusion, the present results demonstrate that the decrease in serum total T_4 level by PB in Gunn rats is not dependent on the increase in hepatic T_4 -UDP-GT activity and suggest that even in Wistar rats, the PB-induced decrease in serum T_4 level does not occur only through increase in hepatic T_4 -UDP-GT.

Phenobarbital (PB) is well known to decrease the level of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats and mice (O'Connor et al., 1999; Hood et al., 2003). Furthermore, PB increases levels of serum thyroid-stimulating hormone (TSH) and thyroid gland growth in rats (Hood et al., 1999). As a possible mechanism for the PB-induced decrease in the level of serum thyroid hormone, enhancement of thyroid hormone metabolism by PB is considered (McClain, 1989; Capen, 1997). Especially, the decrease in the level of serum thyroxine (T_4) by PB in rats is thought to occur mainly through the induction of T_4 -UDP-glucuronosyltransferase (T_4 -UDP-GT), responsible for glucuronidation of T_4 (Barter and Klaassen, 1992a; Liu et al., 1995). This hypothesis is supported by the previous reports that a number of T_4 -UDP-GT inducers, such as polychlorinated biphenyl (PCB), 3-methylcholanthrene, and pregnenolone-16 α -carbonitrile, show the ability to decrease serum thyroid

hormone (Saito et al., 1991; De Sandro et al., 1992; Barter and Klaassen, 1994). However, the magnitude of decrease in the level of serum total T_4 by PB is not necessarily correlated with that of increase in T_4 -UDP-GT activity (Saito et al., 1991; Hood et al., 2003). Likewise, our preliminary study (Suzuki et al., 2004) has indicated that there is a poor correlation between increase in the levels of UGT1A1 and UGT1A6 and decrease in the levels of serum total T_4 and free T_4 in PB-treated rats.

In the present study, therefore, we examined a relationship between the decrease in serum total T_4 level and the increase in hepatic T_4 -UDP-GT (UGT1A1 and UGT1A6) by PB using UGT1A-deficient Wistar rats (Gunn rats) and demonstrated that the PB-induced decrease in serum total T_4 level in rats was not necessarily dependent on the increase in hepatic T_4 -UDP-GT activity.

Materials and Methods

Chemicals. PB was purchased from Nakakita Yakuhin Co., Ltd. (Aichi, Japan). The 125 I-reverse triiodothyronine (T_3) and [125 I] T_4 , radiolabeled at the 5'-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (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.

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ABBREVIATIONS: PB, phenobarbital; ID-I, iodothyronine deiodinase; PCB, polychlorinated biphenyl; T_3 , triiodothyronine; T_4 , thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase.

(Shizuoka, Japan). Male Wistar and Gunn rats 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-8:00 PM light) in an air-controlled room (temperature, $24.5 \pm 1^\circ\text{C}$; humidity, $55 \pm 5\%$), and handled with humane care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Rats received four consecutive intraperitoneal injections of PB (80 mg/kg) dissolved in 0.9% saline (5 ml/kg). Control animals were treated with a vehicle alone (5 ml/kg). All rats were killed by decapitation 1 day after the final administration. The liver was removed, and hepatic microsomes and cytosols were prepared according to the method of Kato et al. (1995) and stored at -85°C until used. 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 used.

Analysis of Serum Hormones. Levels of serum total T₄, free T₄, total T₃, and TSH were measured by radioimmunoassay using the Total T4 and Free T4 kit (Diagnostic Products Corporation, Los Angeles, CA), the T-3 · RIABEAD (Abbott Japan Co., Ltd., Tokyo, Japan), and the rTSH [¹²⁵I] Biotrak assay system (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK), respectively.

Hepatic T₄-Metabolizing Enzyme Assays. Amounts of proteins of hepatic subcellular fractions, microsomes and cytosols, were determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. The activity of microsomal UDP-GT toward T₄ was determined by the method of Barter and Klaassen (1992b). The activity of microsomal type I iodothyronine deiodinase (ID-I) was determined by the method of Hood and Klaassen (2000). The activity of cytosolic sulfotransferase toward T₄ was determined by the method of Kaptein et al. (1997).

Western Blot Analysis. Polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, and UGT2B1 were used (Ikushiro et al., 1995, 1997). Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). Isolated proteins corresponding to UGT1A1, UGT1A6, and UGT2B1 were detected using chemical luminescence (ECL detection kit; Amersham Biosciences Inc., Piscataway, NJ), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Analysis of [¹²⁵I]T₄ Binding to Serum Proteins. At 1 day after a consecutive 4-day treatment with PB, rats 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 (polyethylene tube SP31; Natume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml). Fifteen minutes later, rats were given 1 ml of [¹²⁵I]T₄ i.v., at 15 μCi/ml in 10 mM NaOH saline + 1% normal rat serum. After the administration of [¹²⁵I]T₄, a portion (0.3 ml) of blood was sampled from the artery at the indicated time, and serum was collected and stored at -50°C for assay. Serum was diluted in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels PAG Mid "Daiichi" 4/20 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan), using 0.025 M Tris (pH 8.4) containing 0.192 M glycine as running buffer, for 11 h at 20 mA at 4°C. Human albumin and transthyretin (TTR), which were incubated with [¹²⁵I]T₄, were also applied on the gel as templates. After the electrophoresis, a gel was dried and radioautographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd.). The levels of [¹²⁵I]T₄-albumin and [¹²⁵I]T₄-TTR complexes were determined by counting the gel fractions identified from a Bio Imaging Analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

Statistics. The data obtained were statistically analyzed according to Dunnett's test after the analysis of variance.

Results

Serum Hormone Levels. Constitutive levels of serum total T₄, free T₄, total T₃, and TSH were more than 1.8-fold higher in Gunn rats than in Wistar rats. Effects of PB on levels of serum thyroid hormones were next examined in Wistar and Gunn rats (Fig. 1). In both Wistar and Gunn rats, PB treatment resulted in decreases of serum total T₄, free T₄, and total T₃, and the magnitude of the decrease in each serum thyroid hormone was almost the same in both rats. On the other hand,

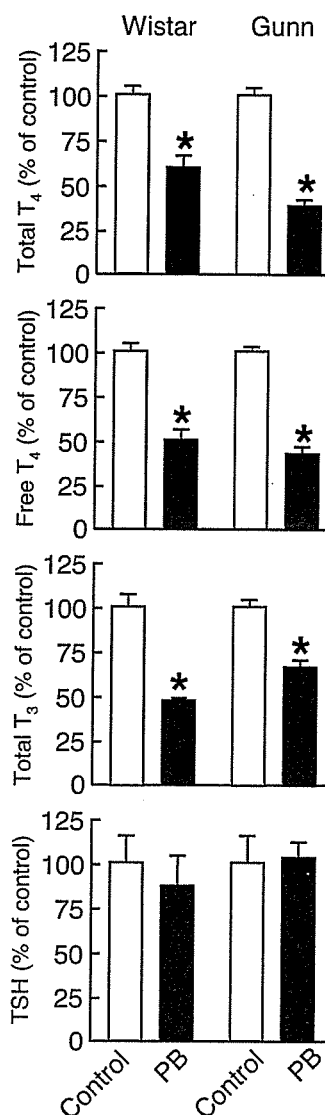


FIG. 1. Effect of PB on levels of serum total thyroxine (T₄), free T₄, total triiodothyronine (T₃), and TSH. Animals were killed 1 day after the final administration of PB (80 mg/kg i.p., once daily for 4 days), and levels of serum thyroid hormones were measured as described under *Materials and Methods*. Constitutive levels: total T₄, 3.32 ± 0.21 (Wistar, $N = 7$) and 7.99 ± 0.30 μg/dl (Gunn, $N = 4$); free T₄, 1.54 ± 0.07 (Wistar, $N = 7$) and 2.77 ± 0.07 ng/dl (Gunn, $N = 4$); total T₃, 0.34 ± 0.03 (Wistar, $N = 6$) and 0.96 ± 0.05 ng/ml (Gunn, $N = 4$); TSH, 8.37 ± 1.25 (Wistar, $N = 6$) and 20.85 ± 1.79 ng/ml (Gunn, $N = 4$). Each column represents the mean \pm S.E. (vertical bars) for four to seven animals. *, $P < 0.01$, significantly different from each control.

no significant change in the level of serum TSH by PB treatment was observed in either Wistar or Gunn rats.

Hepatic T₄-Metabolizing Enzyme Activities. It has been reported that T₄ glucuronidation is primarily mediated by UGT1A enzymes, UGT1A1 and UGT1A6, in the rat liver (Visser, 1996). Therefore, we examined effects of PB on hepatic microsomal T₄-UDP-GT activity in Wistar and Gunn rats. Constitutive activity of T₄-UDP-GT was more than 2.1-fold higher in Wistar rats than in Gunn rats. PB treatment resulted in a significant increase of T₄-UDP-GT activity in Wistar rats but not in Gunn rats (Fig. 2).

Hepatic microsomal ID-I activity in both Wistar and Gunn rats was significantly decreased by PB (Fig. 3). On the other hand, no significant change in activity of hepatic sulfotransferase by PB treatment was observed in either PB-treated Wistar or Gunn rats (data not shown).

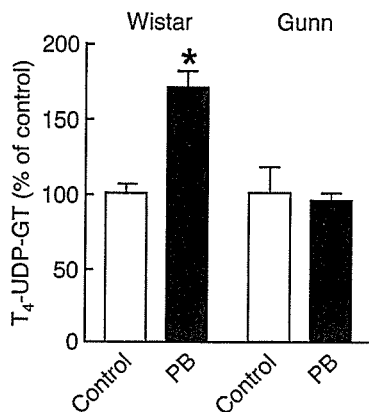


FIG. 2. Effect of PB on the activity of hepatic microsomal UDP-glucuronyltransferase. Each column represents the mean \pm S.E. (vertical bars) for four animals. Constitutive levels: T₄-UDP-GT, 12.60 \pm 0.69 (Wistar) and 5.95 \pm 1.06 pmol/mg protein/min (Gunn). *, $P < 0.01$, significantly different from each control.

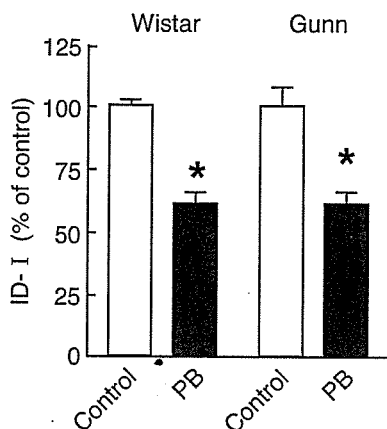


FIG. 3. Effect of PB on the activity of hepatic microsomal type I iodothyronine deiodinase (ID-I). Each column represents the mean \pm S.E. (vertical bars) for four animals. Constitutive levels: ID-I, 161.8 \pm 3.1 (Wistar) and 126.3 \pm 9.5 pmol/mg protein/min (Gunn). *, $P < 0.01$, significantly different from each control.

Immunoblot Analysis for UGT1As. Levels of immunoreactive proteins responsible for UGT1A isoforms, UGT1A1 and UGT1A6, were increased by PB 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. On the other hand, the level of UGT2B1 was significantly increased by PB in both Wistar and Gunn rats, and the magnitude of the increase was higher in Gunn rats than in Wistar rats (Figs. 4 and 5).

Serum Protein Binding of [¹²⁵I]T₄. The effect of PB on the binding of [¹²⁵I]T₄ to serum proteins, TTR and albumin, was examined in Wistar rats. No significant change in the binding level of [¹²⁵I]T₄ to each serum protein by PB treatment was observed with the exception of a decrease in the level of [¹²⁵I]T₄-TTR complex at 120 min after [¹²⁵I]T₄ administration (Fig. 6).

Discussion

In the present study, we found that treatment with PB resulted in a drastic decrease in serum total T₄ and free T₄ levels in both Wistar and Gunn rats, although significant increase in the activity of T₄-UDP-GT occurred only in Wistar rats and not in Gunn rats. The present findings demonstrate that in Gunn rats, PB-induced decrease in the level of serum T₄ occurs in a hepatic T₄-UDP-GT-independent fashion. In addition, constitutive levels of serum total T₄ and T₃ were more than 1.8-fold higher in Gunn rats than in Wistar rats, suggesting that the

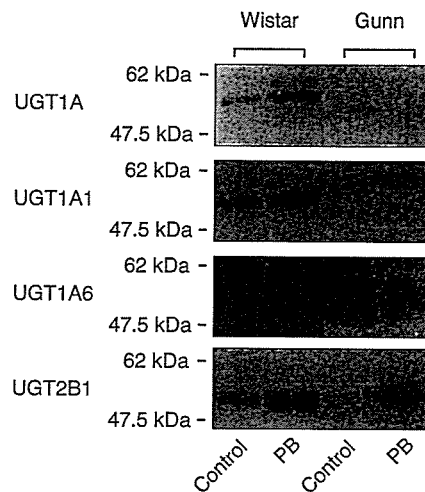


FIG. 4. Representative Western blot patterns for hepatic microsomal UGT isoforms.

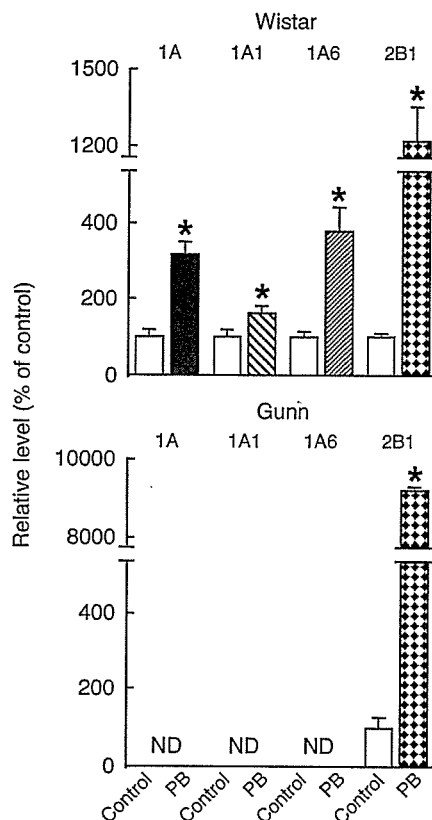


FIG. 5. Effect of PB on levels of hepatic microsomal UGT isoforms. After Western blot as shown in Fig. 4, the isolated bands responsible for UGT isoforms were densitometrically quantified as described under *Materials and Methods*. The data are represented as the mean \pm S.E. (vertical bars) for four animals. *, $P < 0.05$, significantly different from each control. ND, not detectable.

deficit of T₄ glucuronidation results in higher T₄ serum levels. Similar results and suggestions have been obtained by Benathan et al. (1983).

In general, T₄-UDP-GT inducers, including PB, clobazam, PCB, 3-methylcholanthrene, and pregnenolone-16 α -carbonitrile, have been considered to decrease a level of serum T₄ through an increase in hepatic T₄-UDP-GT (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Miyawaki et al., 2003). However, it has been reported that the difference between rats and mice in the magnitude of decrease in the level of serum total T₄ by an inducer of T₄-UDP-GT is not well

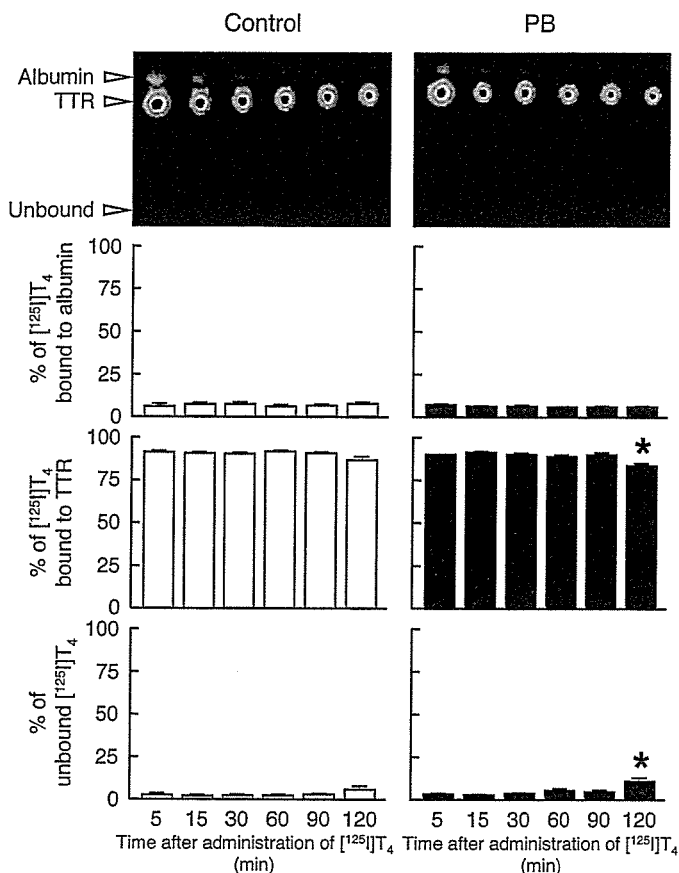


FIG. 6. Effect of PB on the binding of [¹²⁵I]T₄ to the serum proteins in Wistar rats. The binding of [¹²⁵I]T₄ to the serum proteins was assessed by nondenaturing polyacrylamide gel electrophoresis, as described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for three animals. *, *P* < 0.05, significantly different from each control.

correlated with that of the increase in activity of T₄-UDP-GT (Craft et al., 2002; Hood et al., 2003). Furthermore, we have reported previously that the PCB-induced decrease in serum T₄ level might occur not only through the increase in hepatic T₄-UDP-GT activity but also via formation of hydroxylated PCB metabolites in rats (Kato et al., 2004) and that in PB-treated rats, there was a poor correlation between the increase in the levels of UGT1A1 and UGT1A6 and the decrease in the levels of serum total T₄ and free T₄ (Suzuki et al., 2004). These previous reports strongly support a possibility that the decrease in serum total T₄ level by PB does not occur only through an increase in hepatic T₄-UDP-GT activity.

As possible mechanisms for the PB-induced decrease in serum T₄ level, changes in hepatic ID-I activity and serum TSH level might be considered. However, a significant decrease in activity of hepatic ID-I, which mediates the deiodination of T₄ and T₃, by PB treatment was observed in both Wistar and Gunn rats. Similar results have been reported in PB-treated Sprague-Dawley rats (O'Connor et al., 1999; Hood and Klaassen, 2000). Accordingly, the PB-induced decrease in serum T₄ level is thought to occur through an ID-I-independent pathway. Furthermore, in the present study, levels of serum TSH in both Wistar and Gunn rats were not significantly changed by PB, indicating that TSH is not related to the PB-induced decrease in serum T₄ level, although a significant increase in serum TSH level has been reported to occur in PB-treated rats (Hood et al., 1999; O'Connor et al., 1999). The difference between the previous results and our present results might be attributed to the difference in the dose examined.

Although it has been reported that the binding of PCB and its

hydroxylated metabolites to TTR, a major T₄-transporting protein, might be attributed, in part, to a decrease in the level of serum T₄ in PCB-treated rats (Lans et al., 1993; Brouwer et al., 1998; Kato et al., 2004), displacement of T₄ from serum TTR by PB did not occur in PB-treated rats, with the exception of the slight displacement in Wistar rats at 120 min after PB treatment. Accordingly, the decrease in the level of serum T₄ in PB-treated rats occurs in a TTR-independent pathway. In addition, no change in activity of hepatic sulfotransferase, which efficiently catalyzes the sulfation of iodothyronines (Kester et al., 1999), by PB treatment was observed in either Wistar or Gunn rats.

In conclusion, the present findings demonstrate that the decrease in serum total T₄ level by PB in Gunn rats occurs without increases in hepatic T₄-metabolizing enzymes (T₄-UDP-GT, ID-I, and sulfotransferase), the binding of PB to serum TTR, and the level of serum TSH, although an exact mechanism for the PB-induced decrease remains unclear. In Wistar rats, PB-induced T₄-UDP-GT might also contribute, in part, to the decrease in serum T₄ level. To clarify the exact mechanisms for the PB-induced decrease in serum thyroid hormones, further studies involving T₄ transporters, nonhepatic T₄-UDP-GT, and exchangeable thyroid hormone pools in nonhepatic tissues would be necessary.

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

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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 8 days after PentaCB-treatment. However, there was a marked difference between rats and mice in the amount of the metabolites formed, and the cumulative amount of the either MeSO₂ metabolite for 8 days after PentaCB treatment in

the liver was 4 to 15 times higher in mice than in rats. On the other hand, a 40 to 60% decrease in level of serum total T₄ occurred in both rats and mice at 1 day after PentaCB treatment, and the decrease was retained up to 8 days after PentaCB treatment. Thus, there was a marked difference between rats and mice in the formation of MeSO₂ metabolites from PentaCB but not a significant difference between rats and mice in PentaCB-induced decrease in the level of serum total T₄, indicating that PentaCB-induced decrease in the level of serum total T₄ is not necessarily dependent on the MeSO₂ metabolites formed.

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; Fångström et al., 2002).

A number of methylsulfonyl (MeSO₂) 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, MeSO₂-PCBs were identified in human milk, blood, liver, and adipose tissue (Norén et al., 1996; Weistrand and Norén, 1997; Weistrand et al., 1997; Guvenius et al., 2002). The main MeSO₂-PCBs found are 3- and 4-MeSO₂ derivatives of nonplanar

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-MeSO₂-PCBs derived from nonplanar 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), whereas the 4-MeSO₂-PCBs have no such capacity (Kato et al., 1995). More recently, we have found that some of MeSO₂ metabolites, including 3-MeSO₂- and 4-MeSO₂-PentaCBs (Fig. 1), were able to reduce level of serum thyroxine (T₄) and/or to increase level of serum thyroid-stimulating hormone (TSH) in rats (Kato et al., 1998, 1999a, 2000b). Furthermore, the MeSO₂ metabolite-induced decrease in serum T₄ level 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., 2000a). Thus, MeSO₂ metabolites of nonplanar 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

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ABBREVIATIONS: PCB, polychlorinated biphenyl; MeSO₂, methylsulfonyl; PentaCB, 2,2',4,5,5'-pentachlorobiphenyl; T₄, thyroxine; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase; AUC, area under the curve.

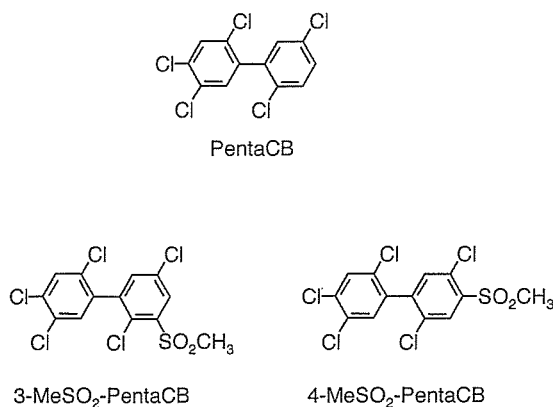


FIG. 1. Chemical structures of PentaCB and its MeSO₂ derivatives.

serum total T₄. The results demonstrate that there is a marked 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 to 200 g, and male ddY mice, weighing 27 to 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 AM-8:00 PM 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 the 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 AM. After clotting at room temperature, serum was separated by centrifugation and stored at -50°C until used. The level of serum total T₄ was measured by radioimmunoassays using an Amerlex-MT4 assay system (GE Healthcare, Little Chalfont, Buckinghamshire, 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), the sum of hepatic 3- and 4-MeSO₂ metabolites detected in mice was

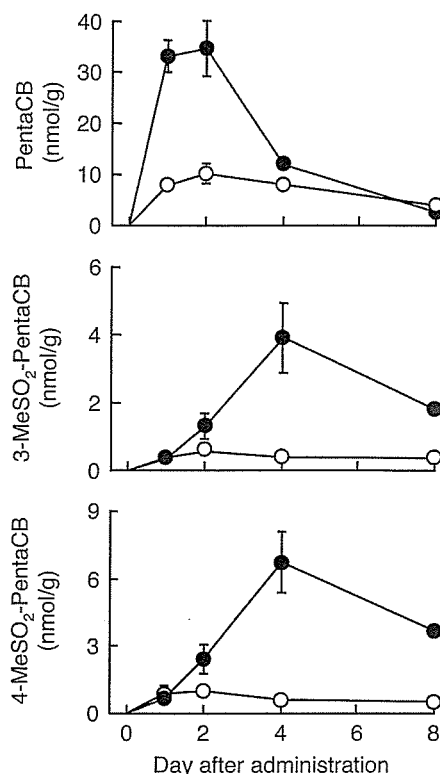


FIG. 2. Levels of parent PCB and its MeSO₂ metabolites in the liver of PentaCB-treated rats and mice. Animals were killed at the indicated times after treatment of PentaCB (342 μmol/kg i.p.). Each point represents the mean ± S.E. (vertical bars) for three to four animals. —○—, rats; —●—, mice.

2-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. The level of PentaCB in the liver of either rats or mice reached the maximum at 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 4 days 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, the 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 level 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, the level of serum total T₄ decreased to 40 to 60% of the corresponding control level at day 1 after PentaCB treatment, and the decrease

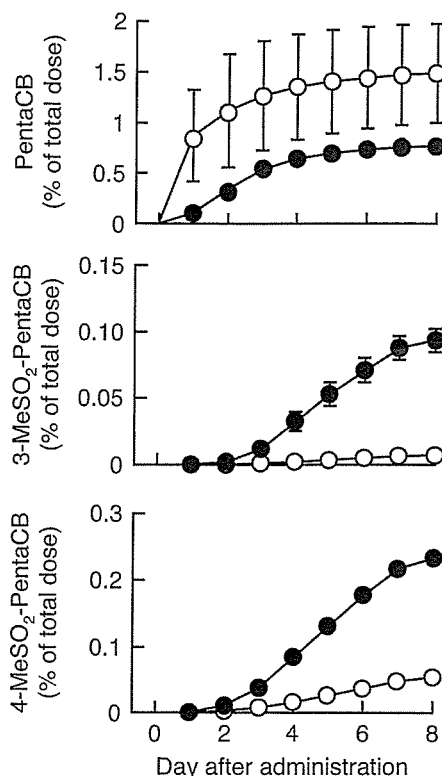


FIG. 3. Levels of parent PCB and its MeSO₂ metabolites in the feces of the PentaCB-treated rats and mice. Animals were given PentaCB (342 $\mu\text{mol/kg}$ i.p., each). Each point represents the mean \pm S.E. (vertical bars) for three to four animals. —○—, rats; —●—, mice.

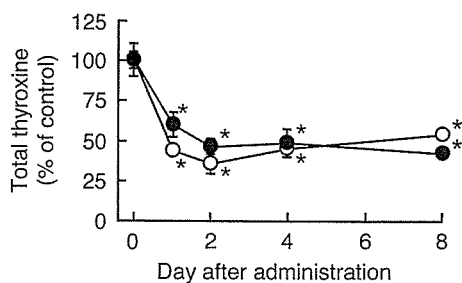


FIG. 4. Effects of PentaCB on levels of serum total T₄ in rats and mice. Levels of serum thyroid hormone T₄ after administration of PentaCB (342 $\mu\text{mol/kg}$ i.p.) to animals were measured as described under *Materials and Methods*, and the data were represented as percentages of the corresponding controls (constitutive level). Constitutive levels: total T₄ ($\mu\text{g/dl}$), 1.53 \pm 0.08 in rats and 1.17 \pm 0.12 in mice. Each point represents the mean \pm S.E. (vertical bars) for three to eight animals. *, $P < 0.05$, significantly different from the corresponding controls (0 h). —○—, rats; —●—, mice.

was maintained up to 8 days 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 at day 4 after treatment with PentaCB in rats and mice increased to 2- 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 levels of PentaCB and its metabolites, including methylsulfonylated and hydroxylated PentaCBs, and in their

TABLE 1

Effects of PentaCB on hepatic T₄-UDP-GT activity in rats and mice

Animals were given PentaCB (342 $\mu\text{mol/kg}$ i.p.) and killed at day 4 after the treatment. Data represent the mean \pm S.E. for three to six animals.

Animal	Activity	
	Control	PentaCB
	<i>pmol/mg protein/min</i>	
Rats	12.7 \pm 1.2	25.7 \pm 2.6*
Mice	21.2 \pm 3.0	34.8 \pm 4.8*

* $P < 0.05$, significantly different from the corresponding controls.

tissue distributions (Haraguchi et al., 2005). In the present study, a clear difference in the formation of MeSO₂ 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 (MeSO₂ 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 MeSO₂ metabolites, difference between rats and mice in the amount of hepatic MeSO₂ 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-MeSO₂ 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, 2000b). 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 that 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; Van Birgelen et al., 1995; Schuur et al., 1997). 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

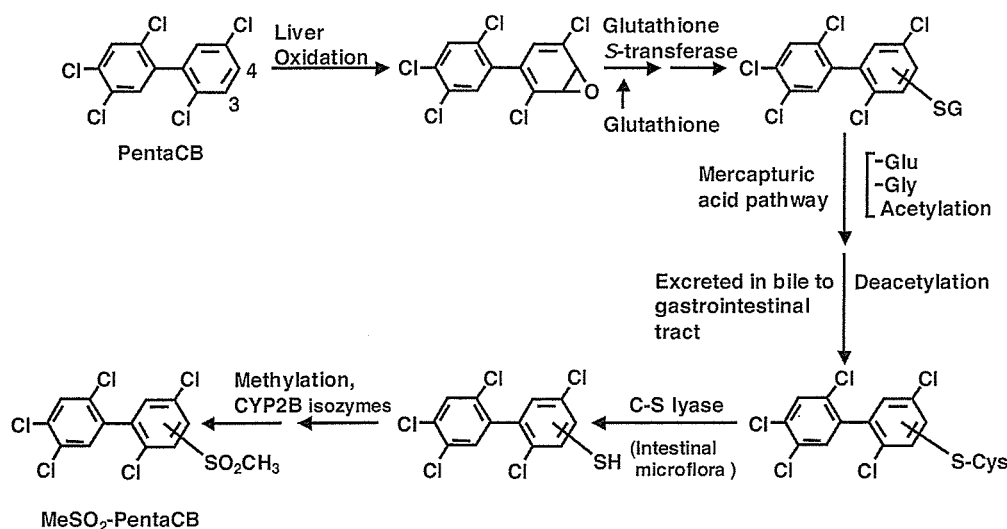


FIG. 5. A possible pathway for formation of MeSO₂ metabolites from PentaCB.

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 levels. 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 both the liver and serum (Haraguchi et al., 2005). Mono- and dihydroxylated PCB derivatives (Lans et al., 1993; Meerts et al., 2002), 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. 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 transthyretin.

In conclusion, we demonstrate herein that there is a marked 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, the 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 the understanding of the exact mechanism.

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