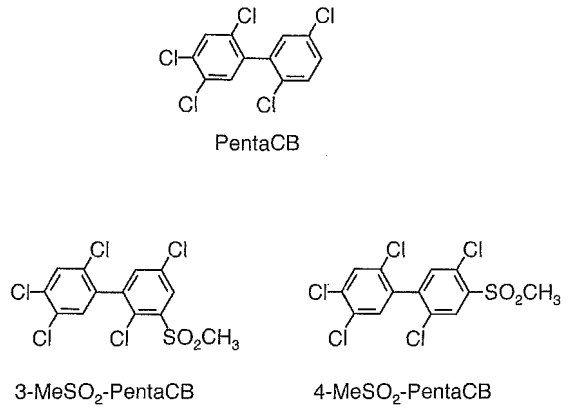

FIG. 1. Chemical structures of PentaCB and its $MeSO_2$ derivatives.

serum total T_4. The results demonstrate that there is a marked species difference in formation of $MeSO_2$ metabolites between rats and mice, whereas no species difference in PentaCB-induced decrease in level of serum total T_4 was observed, indicating that there is not necessarily correlation between formation of $MeSO_2$ metabolites and decrease in serum total T_4.

Materials and Methods

Chemicals. PentaCB was synthesized by using the Cadogan coupling reactions (Cadogan, 1962). 3-$MeSO_2$- and 4-$MeSO_2$-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 T_4. 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 T_4 was measured by radioimmunoassays using an Amerlex-MT4 assay system (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Hepatic Microsomal T_4-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 T_4-UDP-GT was determined by the method of Barter and Klaassen (1992).

Determination of PentaCB and Its $MeSO_2$ Metabolites. Amounts of PentaCB and its $MeSO_2$ 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

$MeSO_2$ 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-$MeSO_2$ metabolites detected in mice was

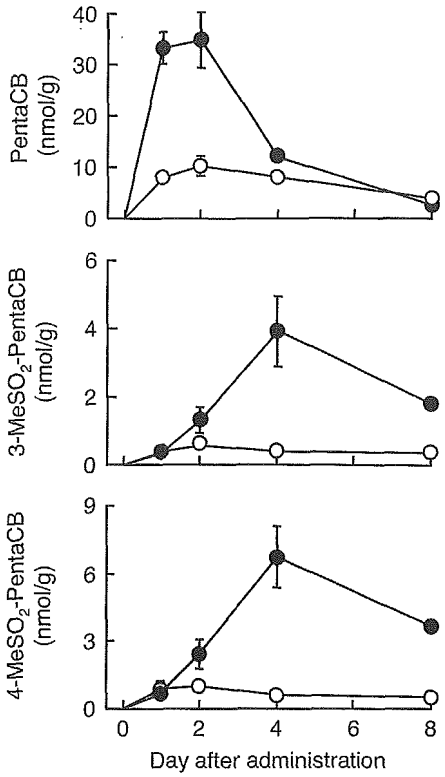

FIG. 2. Levels of parent PCB and its $MeSO_2$ metabolites in the liver of PentaCB-treated rats and mice. Animals were killed at the indicated times after treatment of PentaCB (342 μmol/kg i.p.). Each point represents the mean ± S.E. (vertical bars) for three to four animals. —○—, rats; —●—, mice.

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 $MeSO_2$ metabolites from PentaCB, we examined levels of the $MeSO_2$ metabolites in both the liver and feces from the PentaCB (342 μmol/kg)-treated rats and mice. Amounts of PentaCB and its $MeSO_2$ metabolites, 3- and 4-$MeSO_2$-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-$MeSO_2$-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-$MeSO_2$-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-$MeSO_2$ 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-$MeSO_2$-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-$MeSO_2$ metabolites of PentaCB were much higher in mice than in rats (Fig. 3). In addition, the 3-$MeSO_2$ metabolite was hardly detected in the rat feces.

Serum Thyroid Hormone Level. Effects of PentaCB on the level of serum thyroid hormone T_4 in rats and mice were next examined. Levels of serum total T_4 in both rats and mice were significantly decreased by treatment with PentaCB (Fig. 4). In either rats or mice, the level of serum total T_4 decreased to 40 to 60% of the corresponding control level at day 1 after PentaCB treatment, and the decrease

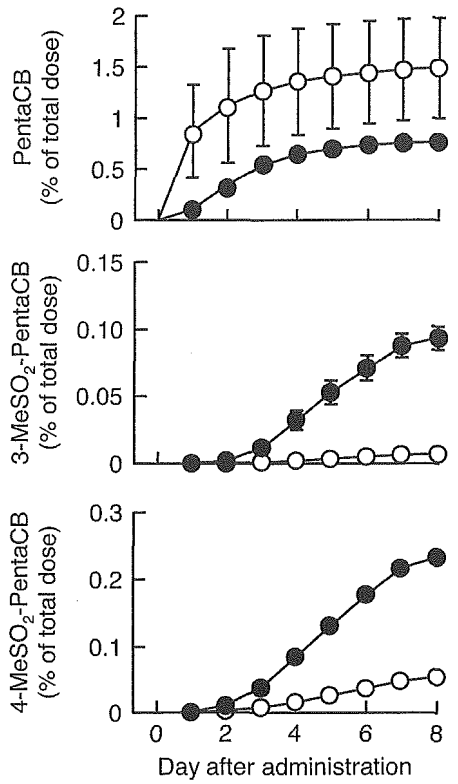

FIG. 3. Levels of parent PCB and its $MeSO_2$ metabolites in the feces of the PentaCB-treated rats and mice. Animals were given PentaCB (342 μmol/kg i.p., each). Each point represents the mean ± S.E. (vertical bars) for three to four animals. —○—, rats; —●—, mice.

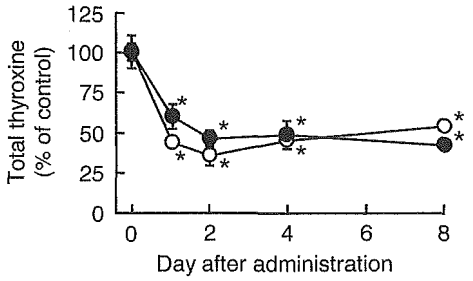

FIG. 4. Effects of PentaCB on levels of serum total T_4 in rats and mice. Levels of serum thyroid hormone T_4 after administration of PentaCB (342 μmol/kg i.p.) to animals were measured as described under *Materials and Methods*, and the data were represented as percentages of the corresponding controls (constitutive level). Constitutive levels: total T_4 (μg/dl), 1.53 ± 0.08 in rats and 1.17 ± 0.12 in mice. Each point represents the mean ± 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 T_4-UDP-GT Activity. We examined effects of PentaCB on hepatic T_4-UDP-GT activity in rats and mice. Hepatic activities of T_4-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).

TABLE 1

Effects of PentaCB on hepatic T_4-UDP-GT activity in rats and mice

Animals were given PentaCB (342 μmol/kg i.p.) and killed at day 4 after the treatment. Data represent the mean ± S.E. for three to six animals.

Animal	Activity	
	Control	PentaCB
	pmol/mg protein/min	
Rats	12.7 ± 1.2	25.7 ± 2.6*
Mice	21.2 ± 3.0	34.8 ± 4.8*

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

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 tissue distributions (Haraguchi et al., 2005). In the present study, a 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 $MeSO_2$ metabolites, including 3-$MeSO_2$- and 4-$MeSO_2$-PentaCBs, could reduce serum total T_4 level in rats (Kato et al., 1998, 1999a, 2000b). Accordingly, it has been expected that $MeSO_2$-PentaCB metabolites contribute to decrease in the level of serum T_4 in PentaCB-treated animals. However, despite levels of $MeSO_2$-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 T_4 in rats and mice were almost the same. The present findings indicate that PentaCB-induced decrease in serum T_4 level is not dependent on only the $MeSO_2$ metabolites formed.

Decrease in the level of serum T_4 by PCB has been thought to occur through increase in hepatic T_4-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 T_4 by 2,2′,4,4′,5,5′-hexachlorobiphenyl is not well correlated with that of increase in activity of T_4-UDP-GT (Craft et al., 2002). Likewise, we have reported that treatment with Kanechlor-500 resulted in a significant decrease in serum T_4 level in both rats and mice, although a significant increase in the activity of T_4-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 T_4 level even in UGT1A1/1A6-deficient (Gunn) rats (Kato et al., 2004). These previous reports strongly propose that the decrease in serum total T_4 level

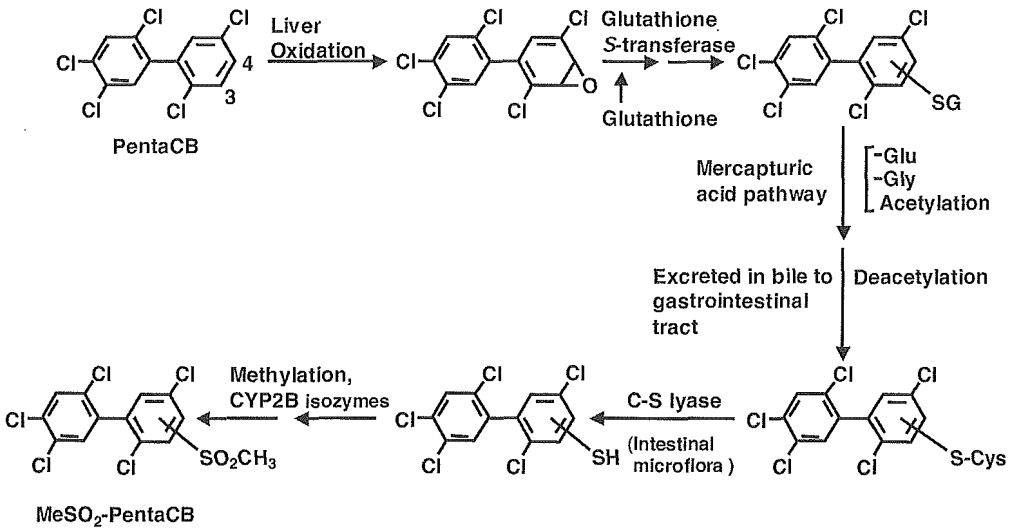

FIG. 5. A possible pathway for formation of $MeSO_2$ metabolites from PentaCB.

by PCBs including PentaCB does not occur only through increase in hepatic T_4-UDP-GT activity, although significant increase in T_4-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 T_4 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 T_4-transporting serum protein transthyretin. Therefore, PentaCB-induced decrease in the level of serum total T_4 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 $MeSO_2$ metabolites from PentaCB and further suggest that PentaCB-induced decrease in level of serum total T_4 is not necessarily dependent on the formation of the $MeSO_2$ metabolites. Additionally, the present findings demonstrate that PentaCB-induced decrease in serum total T_4 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 T_4-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 T_4-transporters in the liver and extrahepatic tissues would be necessary for the understanding of the exact mechanism.

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2,3',4,4',5-五塩素化ビフェニル（CB118）のモルモット肝ミクロゾームによる代謝

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Metabolism of 2, 3', 4, 4', 5-Pentachlorobiphenyl by Guinea Pig Microsomes

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Abstract The in vitro metabolism of 2,3',4,4',5-pentachlorobiphenyl (pentaCB) (CB118) was studied using liver microsomes of guinea pigs and the effect of cytochrome P450 inducers, phenobarbital (PB) and 3-methylcholanthrene (MC) on CB118 metabolism was also compared. After 30 min-incubation at 37°C with liver microsomes of guinea pigs, CB118 was hydroxylated to two metabolites (M-1 and M-2) with retention times of 15.84 min and 20.01 min in GC/ECD, respectively. GC/MS showed that the methylated derivative of a major metabolite M-2 had the molecular weight of 354 and an intense fragment ion of [M^+-50] which is a characteristic ion for PCBs possessing a methoxy-group at the 2 (2')- or the 6 (6')-position. By comparison of the mass fragmentation and the retention times in GC/MS with the synthetic authentic compounds, M-2 was identified as 2-hydroxy-3,3',4,4',5-pentaCB (CB126). On the other hand, the methylated derivative of a minor metabolite M-1 had the molecular weight of 320 and the similar fragment ion of [M^+-50] to the methylated M-2, assuming that M-1 was a dechlorinated monohydroxy-tetrachlorobiphenyl (tetraCB) possessing hydroxy-group at the 2 (2')- or the 6 (6')-position. However, the precise structure of M-1 could not be determined because its retention time in GC was in disagreement with that of the candidate 6-hydroxy-3,3',4,4'-tetraCB. PB-treatment increased the formation of M-1 and M-2 to 2.2- and 6.8-fold of untreated animals, whereas MC-treatment increased only M-2 to 2.6-fold of untreated ones. Addition of antiserum against a PB-inducible guinea pig cytochrome P450, CYP2B18, completely inhibited the formation of M-2. These results suggest that CB118 is principally metabolized by CYP2B18 to 2-hydroxy-CB126 which is formed via a 2,3-epoxide intermediate and the subsequent NIH-shift of a chlorine at the 2-position to the 3-position in guinea pig liver.

はじめに

2,3',4,4',5-Pentachlorobiphenyl (pentaCB) (CB 118) はカネミ油症の原因物質であるPCB製品 (Kanechlor 400) の主要な成分の1つである[28]とともに，油症患者血液中のPCB成分のうちで，健常者よりも濃度が低いPCB異性体として知られている[23]．PCBを含むダイオキシン類の毒性の強さを表す指標として毒性評価係数 (TEF) があるが，CB118に対しては，2,3,7,8-tetrachlorodibenzo-*p*-dioxinを1.0とした場合，0.00001の強さを有するとされている[34]．

PCB異性体は毒性の1つとして，肝チトクロムP450 (P450) を中心とした肝薬物代謝酵素に対する強い誘導能を有しており，その誘導様式の違いから，phenobarbital(PB)型，3-methylcholanth-

rene (MC) 型および混合型に分類されている[32]. PB型には, mono-ortho-およびdi-ortho-PCBが, また, MC型には高毒性のcoplanar PCBが含まれる. CB118はmono-ortho-PCBであるがcoplanar PCBの性質も有することから, 2,3,3',4,4'-pentaCB (CB105) とともに混合型に分類されている[37].

一方, PCB異性体のうち, 2,4,5-三塩素置換PCB, 例えばCB118, 2,2',3,4,4',5'-hexachlorobiphenyl (hexaCB) (CB138), 2,2',4,4',5,5'-hexaCB (CB 153), 2,2',3,4,4',5,5'-heptachlorobiphenyl (heptaCB) (CB180) および2,2',3,4',5,5',6-heptaCB (CB187) は, 肝, 脂肪組織および血液などへの残留性が非常に高いことが知られている[5)9)11)33)]. また, 最近, ヒト血液中において, 4-水酸化 (OH)-CB187や4-OH-2,2',3,4',5,5'-hexaCB (CB146) などの, 特にPCBの4位が水酸化された代謝物が高濃度で検出されている[5)9)33)]. さらに, これらのOH体は血液中の甲状腺ホルモン輸送タンパク質であるtransthyretinと高い親和性を有していることから, 結果的に血中甲状腺ホルモンの低下をもたらすといわれる[6)7)]. これらの事実は, PCBによるヒトへの生体影響を明らかにするためには, 2,4,5-三塩素置換PCBの代謝研究が急務であることを示している.

モルモットはダイオキシン類などの毒性に対して, 最も感受性の高い動物として知られているが[17], 一方, PCBに対する代謝活性に関してもラットとかなり異なっている. 例えば, 3,3',4,4'-tetrachlorobiphenyl (tetraCB) (CB77) などのcoplanar PCBに対する代謝能はラットに比べ, かなり低い[22]のに対し, 2,4,5-三塩素置換PCBに対する代謝能は逆にラットよりかなり高い[2)12)]. 当研究室では, 高蓄積性PCBのCB153やCB138などのin vitro代謝を調べ, その結果, モルモットではラットより数倍高い代謝活性を有すること, NIH転位代謝物である2-OH体が主代謝物であること, さらに, これらの代謝にPB誘導性P450であるCYP2B18が強く関与することを報告した[3)19)].

しかしながら, 2,4,5-三塩素置換PCBの原型ともいえるCB118の代謝に関する報告はほとんどない. Haraguchiら[10]はCB118投与ラットの糞中に, 4種類のOH体, すなわち, 4-OH-2,3',4',5-tetraCB (CB70), 4-OH-2,3,3',4',5-pentaCB (CB107), 4'-OH-2,3',4,5,5'-pentaCB (CB120) および5'-OH-CB118と2種類のメチルチオ (MeS) 体, すなわち5'-および6'-MeS-CB118を検出した. また, 4-OH-CB107および4'-OH-CB120は特に血液中および肝, 肺, 腎などの臓器に高濃度分布していることも明らかにした. さらに, 最近, Haraguchiら[12]はKanechlor 500投与のラット, ハムスターおよびモルモットにおいて, 代謝物の組織分布を比較したところ, ラットおよびハムスター血液中ではCB118由来と考えられる4-OH-CB107が高濃度検出されたが, モルモットではラットやハムスターとは全く異なり3-OH-CB118のみが検出されたと報告した. これらの結果はCB118のin vivo代謝において, 大きな動物種差があることを示しているが, われわれがモルモット肝を用いて行ったCB153やCB138のin vitro代謝で報告した2-OH体について, CB118では全く検出されていない. そこで本研究では, この点を明らかにするため, モルモット肝ミクロゾームを用いてCB118のin vitro代謝を調べた. また, 代謝に関与するP 450分子種を明らかにするため, 代表的なP 450誘導剤として知られているPBおよびMCで前処理した動物でも同様に検討した.

実験方法

1. 実験材料

(1) CB 118の合成

CB 118はCadoganの方法[8]で合成した. まず, 1,2-dichlorobenzeneおよび2,4,5-trichloroanilineをtetrachloroethyleneで溶解し, さらに亜硝酸イソペンチルを加えて, 110°Cで24時間反応させた. 反応物はアルミナカラム (100 g, Merck) およびシリカゲル60カラム (65 g, Merck) で精製した後, 不純物を除くため, 高速液体クロマトグラフィー (HPLC) に付した. HPLC条件は次の通りである. カラム, ODS (250 × 20 mm i.d., 5 μm, YMC); 移動相, acetonitrile; 流速, 4 ml/min; 検出波長, 254 nm. CB118の純度を電子捕獲型検出器付ガスクロマトグラフィー (GC/ECD) により調べた結果, 最終的に99.0 %以上であった.

CB 118：MS (EI) m/z (relative intensity) 324 (100) [M^+], 326 (163) [M^++2], 328 (99) [M^++4], 330(32) [M^++6], 254(40) [M^+-CH_3Cl].

（2）代謝物の合成

2-OH-CB 126の合成は，3,4-dichloroaniline と2,3,4-trichlorophenol を合成原料として用い，Hutzingerらの方法[13]で行った．反応後，シリカゲル60カラム（65 g，Merck）およびHPLCで精製した．なお，メチル化は，アルカリ性条件下，ジメチル硫酸の添加により行った[24]．一方，3-methoxy (MeO)-CB118および4-MeO-2,3,3',4',5-pentaCB (CB107) の合成は，Cadoganの方法[8]で行った．合成原料として3,4-dichloroaniline と2,3,6-trichloroaniline を用い，これらをtetrachloroethyleneで溶解し，さらに亜硝酸イソペンチルを加えて，110°Cで24時間反応させた．精製は上記と全く同様に，アルミナカラム(100 g，Merck)，シリカゲル60カラム（65 g，Merck）およびHPLCで行った．また，6-MeO-CB77，5-MeO-2,3,3',4'-tetraCB (CB56)および6-MeO-CB56の合成は，3,4-dichloroaniline と3,4-dichlorophenol を合成原料として用い，Hutzingerらの方法[13]で行った．反応後，シリカゲル60カラム（65 g，Merck）およびHPLCで精製した．なお，メチル化は，diazomethaneにより行った．

2-OH-CB126：^{1}H-NMR（500 MHz，chloroform-*d*）δ 7.52(1H, d, J=8.3 Hz, 5'-H), 7.47 (1H, d, J=2.0 Hz, 2'-H), 7.28 (1H, s, 6-H), 7.22 (1 H, dd, J=8.3 Hz, 2.0 Hz, 6'-H), 3.95 (3H, s, 4-MeO).

2-MeO-CB126：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(160) [M^++2], 358(109) [M^++4], 360 (38) [M^++6], 304 (103) [M^+-CH_3Cl], 284(26) [M^+-Cl_2], 241(35) [M^+-$COCH_3Cl_2$].

3-MeO-CB118：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(156) [M^++2], 358(93) [M^++4], 360 (35) [M^++6], 311 (53) [M^+-$COCH_3$], 241 (40) [M^+-$COCH_3Cl_2$].

4-MeO-CB107：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(120) [M^++2], 358(82) [M^++4], 360 (35) [M^++6], 339 (48) [M^+-CH_3], 311 (45) [M^+-$COCH_3$], 241 (21) [M^+-$COCH_3Cl_2$]. ^{1}H-NMR(500 MHz, chloroform-*d*) δ 7.52 (1H, d, J=8.1 Hz, 5'-H), 7.47 (1 H, d, J=2.2 Hz, 2'-H), 7.28 (1H, s, 6-H), 7.22 (1H, dd, J=8.3 Hz, 2.0 Hz, 6'-H), 3.95 (3H, s, 4-MeO).

5-MeO-CB 56：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(127) [M^++2], 324(43) [M^++4], 326 (8) [M^++6], 277 (14) [M^+-$COCH_3$], 207 (16) [M^+-$COCH_3Cl_2$].

6-MeO-CB 56：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(132) [M^++2], 324(63) [M^++4], 326(13) [M^++6], 270(103) [M^+-CH_3Cl], 207 (37) [M^+-$COCH_3Cl_2$].

6-MeO-CB 77：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(132) [M^++2], 324(67) [M^++4], 326(15) [M^++6], 270(120) [M^+-CH_3Cl], 207 (34) [M^+-$COCH_3Cl_2$].

2．薬物投与

Hartley系雄性モルモット（体重約300 g）に，P 450誘導剤としてPB（80 mg/kg/day）あるいはMC (20 mg/kg/day) を3日間腹腔内に投与した．PBは生理食塩水に，MCはコーン油に溶解した．モルモット肝ミクロゾームは，PBおよびMCの最終投与日の翌日に屠殺した後，肝を摘出し，常法により調製した．

3．代謝物の分析

モルモット肝ミクロゾームによるCB118の代謝は既報[20]に準じて行った．すなわち，40 μM CB118, NADPH生成系(0.33 mM NADP, 5 mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase 1.0 unit)，6 mM $MgCl_2$およびモルモット肝ミクロゾーム（1 mg protein）を100 mM HEPES緩衝液（pH 7.4）とともに合計1 mlとして，37°Cで30 minインキュベート後，代謝物をchloroform-methanol（2：1）1 mlと*n*-hexane 3 mlでそれぞれ3回ずつ抽出した．抽出液はdiazomethaneでメチル化後，GC / ECDに付した．GC/ECDの条件は次の通りである．分析機器，ECD付HP 5890 Series IIガスクロマトグラフ（Hewlett-Packard製）；カラム，DB-1 fused silica capillary column（30 m × 0.25 mm

i.d., 0.25 μm膜厚, J & W Scientific製)；オーブン温度, 220°C；注入口温度, 250°C；検出器温度, 250°C；キャリアーガス, N_2 (1 ml/min).

一方, 代謝物の質量分析(GC/MS)は質量分析計付ガスクロマトグラフ(Agilent 5973 inert MSD, Agilent製)を用いて, EIモードで行った. 分析条件は次の通りである. カラム, HP-5 fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm膜厚, Agilent製)；オーブン温度, 70°C (1.5 min) - 20°C/min - 230°C(0.5 min) - 4°C/min - 280°C (5 min)；注入口温度, 250°C；検出器温度, 230°C；キャリアーガス, He (1 ml/min).

^{1}H-NMRスペクトルの測定は日本電子製のJEOL GSX-500 (500 MHz)を用いて行った. 試料は, chloroform-d に溶解し, tetramethylsilaneを内部標準物質として用いた.

4. 抗体阻害実験

モルモット肝P450(CYP2B18)に対する抗血清をウサギで調製した[21]. 抗血清を100 mM HEPES緩衝液 (pH 7.4) 中で, 肝ミクロゾームとともに, 室温で30 min放置した. 抗血清の添加量は, 50, 100および150 μlとした. 次に, CB118および$MgCl_2$を添加後, NADPH生成系を加えることにより, インキュベートを開始した. 代謝物の分析は, 上記の通りである.

5. その他

肝ミクロゾームのタンパク質の定量は, Lowryらの方法[25]に従い行った. なお, 標準タンパク質としてウシ血清アルブミンを用いた.

実験結果

1. 肝ミクロゾームによるCB118の代謝

CB118を, 未処理, PB前処理あるいはMC前処理モルモットから調製した肝ミクロゾームとともに, NADPH存在下, 37°C, 30 minインキュベートした. 有機溶媒抽出物をメチル化後, GC/ECDに供した結果, いずれの肝ミクロゾームによっても, 未変化体(保持時間12.80 min)以外に, 代謝物と思われる2本のピークが保持時間15.84 minおよび20.01 minに検出された(Fig. 1). これを以下, それぞれM-1およびM-2とする. PB前処理ミクロゾームの場合, M-2が未処理ミクロゾームの6.8倍に著しく増加した. また, M-1も2.2倍に増加した. 一方, MC前処理ミクロゾームの場合にも, M-2は未処理ミクロゾームの2.6倍に増加したが, M-1は逆に50 %以下に減少した.

2. 代謝物の化学構造

両代謝物の化学構造を明らかにするために, PB前処理ミクロゾームを用いて100倍量 (100 ml)でインキュベーションを行った後, 代謝物を抽出し, さらにメチル化後, GC/MSにより, M-1およびM-2の分子量を調べた. その結果, M-1およびM-2はそれぞれ分子量320および354を有することが明らかとなった(Table 1). これらの結果から, M-1は塩素が1個脱離したOH-tetraCBであること, 一方, M-2はCB118のOH体であることが明らかとなった. さらに, 両代謝物のメチル誘導体のマススペクトルにおいて, いずれもフラグメントイオン [M^+-50] が非常に多いことから, ortho位すなわち2 (6) 位または2' (6') 位にMeO基を有していることが示唆された. これまでの報告[1)18)20)]によると, ビフェニル骨格のpara位すなわち4 (4') 位にMeO基が置換されている場合にはフラグメントイオン [M^+-15] が, またmeta位すなわち3 (5) 位あるいは3' (5') 位にMeO基が置換されている場合にはフラグメントイオン [M^+-43] が多く検出されることが知られている. そこで, M-1およびM-2がそれぞれ6-OH-CB 77と2-OH-CB 126であると予想し, これらを別途合成した. その結果, 主代謝物M-2 (メチル化体) のGC保持時間15.43 minは, 2-MeO-CB126のそれと完全に一致した(Table 1). また, マスフラグメンテーションもほとんど一致した. なお, 合成標品3-MeO-CB 118および4-MeO-CB107は, GC保持時間がそれぞれ15.45 minと15.51 minであり, また, マスフラグメンテーションもM-2 (メチル化体) と異なっていた. 次に, M-1 (メチル化体) について, 合成標品の6-MeO-CB77と比較したところ, 分子量およびマスフラグメンテーションはほぼ一致したが, GC保持時間では0.03 min遅く検出され完全には一致しなかった. 以上の結果から, M-2は2位の塩素が3位にNIH転位をした2-OH-CB 126

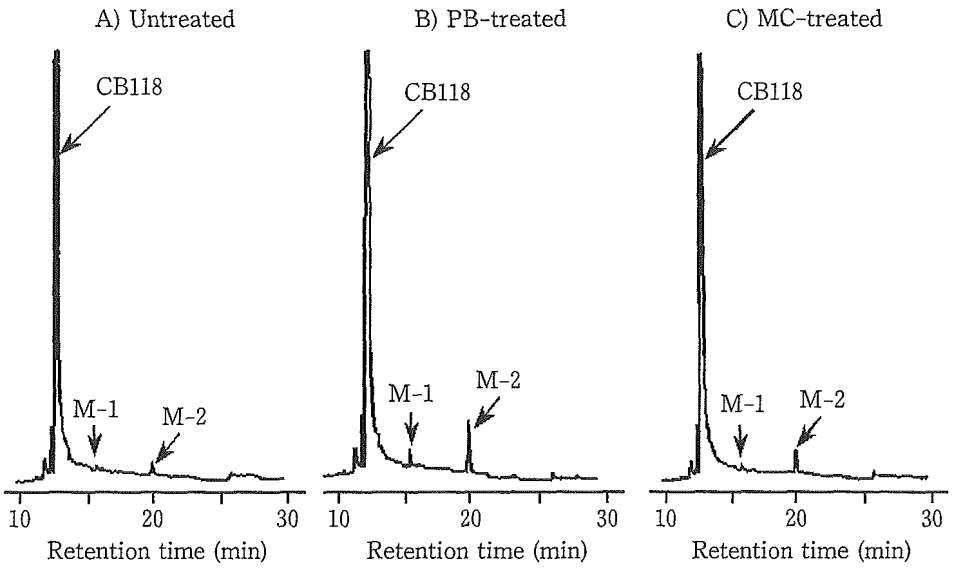

Fig. 1 Gas chromatograms of methylated derivatives of CB118 metabolites formed by liver microsomes of untreated (A), PB-treated (B) and MC-treated (C) guinea pigs.
Column for GC/ECD used was DB-1 capillary column (30 m long).

であると推定されたものの，M-1についての正確な化学構造は未定となった．

3．抗CYP2B18抗血清による代謝阻害

モルモット肝P450のCYP2B18は，PB誘導性P450としてだけでなく，未処理肝ミクロゾームにおいて主要なP450としても知られている[19)30)]．そこで，CB118代謝におけるCYP2B18の寄与を明らかにするために，このP450に対する抗血清を用いて，主代謝物M-2の生成阻害を試みた．なお，肝ミクロゾームは最もM-2生成活性が高かったPB前処理モルモット肝ミクロゾームを用いた．その結果，抗血清150 μlの添加で，M-2の生成はほとんど完全に阻害された（Fig. 2）．これらの結果から，モルモット肝におけるM-2の生成にはCYP2B18が強く関与していることが明らかとなった．

考　　察

今回，CB118のモルモット肝ミクロゾームによるin vitro代謝を調べたところ，2種類の代謝物（M-1およびM-2）が生成された．さらに，GC/

Table 1 Mass spectral data and retention times of methylated derivatives of two metabolites and six synthetic compounds in GC/MS

Compound	Molecular weight	Mass spectral data				Retention time (min)
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	$[M^+-50]$	
M-1	320	100	—	—	108	14.40
M-2	354	100	—	—	103	15.43
6-MeO-CB 56	320	100	—	—	103	13.72
5-MeO-CB 56	320	100	—	13	—	14.32
6-MeO-CB 77	320	100	—	—	120	14.37
2-MeO-CB 126	354	100	—	—	103	15.43
3-MeO-CB 118	354	100	—	53	—	15.45
4-MeO-CB 107	354	100	48	45	—	15.51

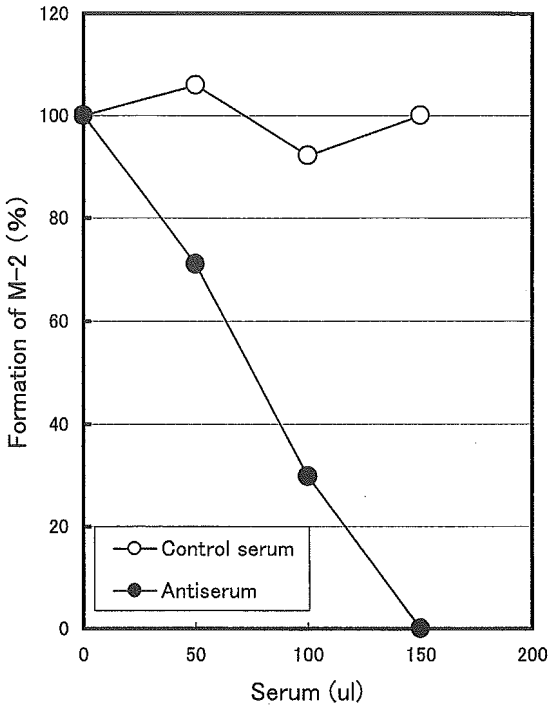

Fig. 2 Effect of antiserum against CYP2B18 on CB118 metabolism by liver microsomes from PB-treated guinea pigs.
Open and closed circles indicated control serum and antiserum against CYP2B18, respectively. Each point represents the mean of duplicate determinations.

MSにより，M-1は塩素が1個脱離し，かつortho位に水酸基が置換されたOH-tetraCBであること，またM-2は2-OH-CB126であることが明らかとなった．これまでに，われわれはCB153やCB138などの2,4,5-三塩素置換PCBの代謝をモルモットで調べ，主代謝物としてNIH転位代謝物の2-OH体が生成されることを報告している[2)20)]．今回のCB118の場合も，NIH転位代謝物の2-OH体が主代謝物であったことから，モルモット肝におけるCB 118の代謝は，CB153やCB138と同様に，まず中間体として2,3-epoxideの生成，さらには2位の塩素の3位へのNIH転位を経て進行することが示唆された（Fig. 3）．

前述のように，Haraguchiらは CB 118投与ラットにおいて，4-OH-CB107, 4'-OH-CB120および3-OH-CB118が生成されること，このうち前2者は主に血液に局在することを報告している[10)]．さらに，Kanechlor 500を腹腔内投与したモルモット血中では，3-OH-CB118のみが局在していることも報告した[12)]．これらの代謝物のうち，4-OH-CB107は，CB118からだけではなくCB 105からも生成されること[10)16)]，また血中での半減期が比較的長い主要なPCB代謝物の1つであることが明らかにされている[26)29)]．さらには，ヒト

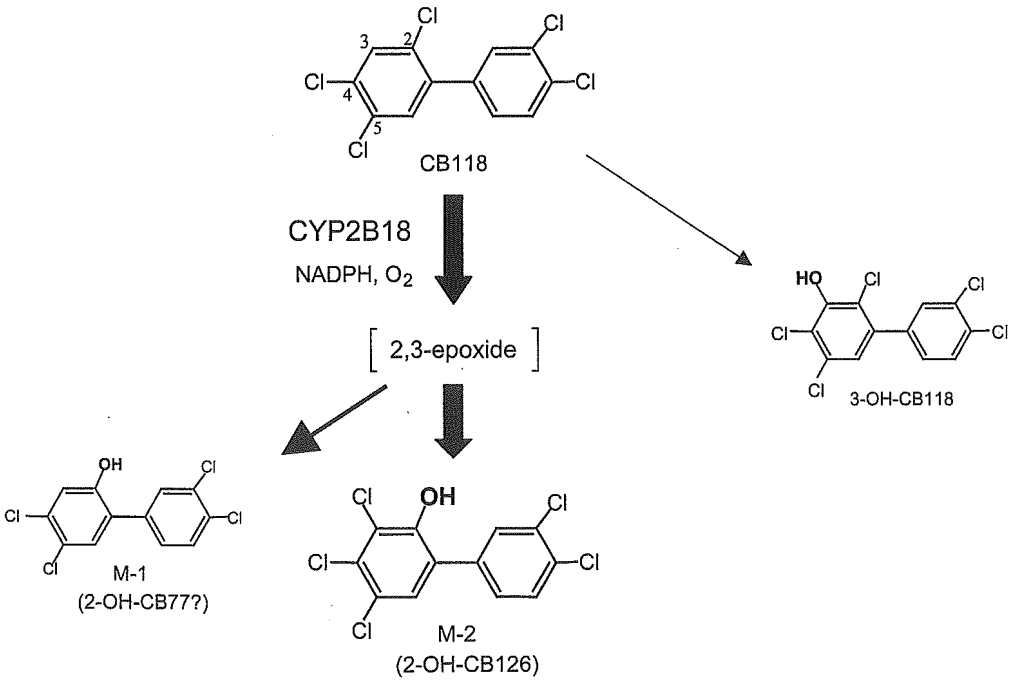

Fig. 3 Postulated metabolic pathways of CB118 in guinea pig liver.

エストロゲン硫酸転移酵素に対し強い阻害活性を有する[15]ことやラット胎児への神経毒性を示す[27]ことからも，注目されている．しかしながら，今回モルモット肝ミクロゾームを用いたin vitro代謝系では，4-OH-CB107および4'-OH-CB120は全く生成されなかった．ただ，3-OH-CB118についてはlarge scale (100 ml) で行ったときに，痕跡程度検出されたにすぎなかった(データ未掲載)．このように，in vitro代謝で生成された2-OH体がなぜin vivo代謝でみられなかったのか現在不明であるが，代謝物の局在性が異なっていることによるのかもしれない．すなわち，2-OH体はそのほとんどが血液以外の組織に分布したり，糞中へと排泄されることによるのかもしれない．今後，CB118投与モルモットにおける代謝物の生体内分布と糞中への排泄を調べることが必要であろう．

PCB代謝物として，脱塩素化されたOH体がしばしば報告されている．Di-ortho-PCBのCB 138[20]，CB 153[1)~3)]および2,2',4,4',6,6'-hexaCB (CB155)[4)14)]，またtri-ortho-PCBのCB187[31]だけではなく，coplanar PCBのCB77[35)36)]でもみられている．しかしながら，この生成機構に関しては，P450による酸化反応に伴い生成されるという事実を除き，ほとんど不明のままである．本研究のCB118代謝においても，同様に脱塩素化された代謝物M-1が生成された．マススペクトルより，ortho位にOH基が入ったtetraCBであることから，6-OH-CB77であろうと予想された．そこで，これを合成しM-1と比較したが，期待に反してGC保持時間が一致しなかった．以上のことから，M-1の正確な化学構造については現在不明であるが，モルモット肝ミクロゾームに存在する酵素系は，この酸化的脱塩素化機構を検討するのに有益であろう．

CB153やCB138の代謝に関与するP450分子種として，モルモット肝ではPB誘導性のCYP2B18が最も重要であることが明らかになっている[2)3)20)]が，抗2B18抗血清を用いてCB118の代謝阻害を試みたところ，M-2の生成がほぼ完全に阻害された．この結果から，CB118を含め2,4,5-三塩素置換PCBの代謝では，CYP2B18が強く関与していることが示唆された．一方，MC前処理ミクロゾームにより，M-2の生成が未処理ミクロゾームの約2倍に増加したが，この事実はMC誘導性のP450，特にCYP1A1あるいはCYP1A2の関与を示唆している．しかしながら，モルモット肝において，これらのMC誘導性P450がPCB代謝に関与するかどうかは全く報告がなく，今後の研究課題である．

総　　括

1．未処理，PBおよびMC前処理モルモット肝ミクロゾームによるCB118の代謝を調べた．その結果，2種類の代謝物 (M-1およびM-2) が生成された．これらのうち，主代謝物のM-2は合成標品との比較により，2-OH-CB126であることが明らかとなった．また，M-1はマススペクトルより，ortho位置換OH-tetraCBであることが明らかとなった．

2．CB118代謝に及ぼすP450誘導剤の影響を調べたところ，PB前処理によりM-1は未処理の2.2倍，M-2は6.8倍に増加した．また，MC前処理でもM-2は2.6倍に増加したが，M-1は逆に50％以下に減少した．

3．抗モルモットCYP2B18抗血清を用いて，主代謝物M-2の代謝阻害を試みたところ，抗血清150 μlの添加で，M-2の生成はほとんど完全に阻害された．

以上の結果から，モルモットにおいて，CB118は主にCYP2B18によって代謝され，中間体として2，3-epoxideを経由した後，NIH転位を経て，主代謝物2-OH-CB126へと変換されることが示唆された．

謝　　辞

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Original

Electron Microscopical Evidence of the Protective Function of Thioredoxin (TRX/ADF) Transgene against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced Cellular Toxicity in the Liver and Brain

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Abstract: The present study was performed to assess the protective role of thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF) on the liver and brain cell damages induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in ADF wild-type (WT) and transgenic (Tg) mice. The ADF WT and Tg mice were intraperitoneally injected with a single dose of TCDD (150 μg/kg body weight). One day after the treatment, the liver and brain tissues were examined electron microscopically to evaluate the cellular toxicity. In the ADF WT mice, marked reduction of subcellular components, such as mitochondria, rough endoplasmic reticula, and glycogen granules, as well as swelling of the remaining mitochondria, were evident in the liver cells. However, attenuation of these changes was evident in TCDD-treated TRX/ADF mice. Similar subcellular changes noted in the neuronal cells of TCDD-treated WT mice were also attenuated in Tg mice. The results suggest that oxidative cellular damage contributes to the acute toxicity induced by TCDD and that TRX/ADF protects against it. (J Toxicol Pathol 2005; **18**: 41–46)

Key words: Ah receptor, brain, liver, 2,3,7,8-tetrachlrodibenzo-*p*-dioxin (TCDD), thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF), transgenic (Tg) mouse

Introduction

As one of the aromatic hydrocarbons, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a widely spread environmental pollutant that has a broad spectrum of toxic effects on a variety of tissues such as the thymus, liver, testes and central nervous system in mammals[1–6]. Although a number of studies have shown that the toxic effects of TCDD are mediated by intracytoplasmic aromatic hydrocarbon receptor (AhR)[7–9], the toxic mechanism of TCDD on the target organs is still not fully understood. Among the toxic events, oxidative stress is considered to play a major role in the toxic mechanism of TCDD, as characterized by marked increases of lipid peroxidation, the formation of reactive oxygen species, and DNA single-strand break[9–14].

Exogenous xenobiotics, such as aromatic hydrocarbons, result in profound induction of cytochrome P450 enzymes in the liver, resulting in the generation of reactive oxygen species[15,16]. On the other hand, the brain is rich in peroxidizable fatty acids and has relatively low catalase activity[17]. Therefore, these organs are considered to be highly susceptible to oxidative stresses[18]. In fact, the contribution of oxidative stress in TCDD-induced cellular damage of the liver and brain has been suggested in previous studies[13,18–22].

Adult T-cell leukemia-derived factor (ADF) is a human thioredoxin (TRX) associated with the reduction/oxidation (redox) regulation of the cellular environment[23]. TRX/ADF is a stress-inducible protein and its expression is up-regulated after viral infection as well as in cellular stress conditions induced by oxidative agents such as hydrogen peroxide or diamide, irradiation with X-rays and ultraviolet

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light, or ischemic reperfusion[23]. Previous studies have shown that TRX/ADF plays a role in the cellular defense mechanism against oxidative cellular damage via the regulation of intracellular redox status, since exogenously administered TRX/ADF protected cells from oxidative cellular injury[24,25].

We recently reported for the first time the protective function of TRX/ADF against TCDD-induced hematotoxicity in ADF transgenic (Tg) mice, indicating oxidative stress contributes to the hematotoxic mechanism of TCDD[26]. We hypothesized in the present study that overexpression of TRX/ADF might also be effective for protection against the toxic effects of TCDD on the liver and brain tissues in which oxidative stress has also been implicated in the toxic mechanism. For this purpose, we injected TCDD with a dosage capable of inducing oxidative stress in the liver following acute exposure[21], to ADF wild-type (WT) and transgenic (Tg) mice, and then compared subcellular changes electron microscopically in the liver and brain tissues.

Materials and Methods

Animals

TRX/ADF overexpressed mice (ADF Tg mice), originally produced by Dr. A. Mitsui[27], were maintained in a laboratory facility with a 12:12-hour light-dark cycle at an ambient temperature of 21 ± 2°C at the National Institute of Health Sciences (NIHS) of Japan by breeding ADF WT and Tg mice. Animals were screened by PCR of their tail DNA to determine their genotypes. At 8 weeks of age, male ADF WT and Tg mice (23.5–24.8 g) were transferred to a vinyl isolator established in a hazard room designed to prevent contamination from the outside environment and randomly allocated within the same genotype to housing with 6 animals per cage. A pelleted basal diet (CRF-1; Funabashi Farm, Funabashi, Japan) and tap water were provided *ad libitum* throughout the study.

Chemical

TCDD was obtained from Radian International, Cambridge Isotope Laboratories, Inc. (Andover, MA, USA; purity: 98 %). TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to the concentration of 10 μg/ml in olive oil.

Experimental design

ADF WT and Tg mice were divided into vehicle controls and TCDD treatment groups, each consisting of 6 animals. After one week of acclimation, TCDD at 150 μg/kg was intraperitoneally injected once to animals of treatment groups, and the corresponding volume of olive oil was similarly injected to vehicle controls. The dosage of TCDD was selected based on previous study results that showed oxidative stress in the liver was induced by a single bolus injection to mice[21]. One day after the treatment, the animals were sacrificed by decapitation and then examined grossly. The liver and brain were then excised and their weights were measured.

The animal protocol was reviewed and approved by the Animal Care and Use Committee of the NIHS, Japan.

Morphological assessment

For histological examination, liver tissues in all animals were fixed in 10% neutral buffered formalin (pH 7.4). After routine processing, the paraffin-embedded sections were stained with hematoxylin and eosin and then examined histopathologically under a light microscope.

For electron microscopical examination, tissue specimens from the liver and cerebral cortex were respectively prepared from three animals each of the control and treatment groups of ADF WT and Tg mice. Small tissue blocks, sized 1 mm^3, were fixed with 2.5% glutaraldehyde in 0.2 M Sorenson's sodium phosphate buffer, pH 7.2, for 8 hours at 4°C. After washing with 0.1 M PBS (pH 7.4), the tissues were post-fixed with 1% osmium tetroxide for 90 minutes. After washing in 0.1 M PBS, the tissues were dehydrated with ethanol and propylene oxide and then embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate. The sections were examined with JEOL-1200 EX II electron microscope (JEOL, Tokyo, Japan).

Results

After one day of TCDD treatment, absolute liver weight had decreased to 71.4% of the vehicle control group in ADF WT mice and 83.2% in ADF Tg mice (data not shown).

Histologically, apoptotic liver cell debris and also focal liver cell necrosis were sparsely observed in the centrilobular areas of both TCDD-treated WT and ADF Tg mice, without showing apparent difference in the severity between genotypes (data not shown). Vehicle control animals did not show such liver cell changes in either genotype.

Electron microscopically, liver cells of the WT mice treated with TCDD exhibited a prominent decrease of cytoplasmic glycogen granules and rough endoplasmic reticula (RERs) and an increase of smooth endoplasmic reticula (SERs) (Fig. 1B). The number of mitochondria was also decreased and the remaining mitochondria showed swelling with disorganized cristae and lucent matrix. Increased fat droplets were also evident in the cytoplasm of less affected hepatocytes. On the other hand, transgene of Trx/ADF notably attenuated these morphological changes following TCDD treatment (Fig. 1C). In the cerebral cortex, neuronal cells showed a decrease in the number of RERs, ribosomes and mitochondria in WT mice treated with TCDD (Fig. 2B) but not in ADF Tg mice treated similarly with TCDD (Fig. 2C). Vehicle control animals did not show such neuronal cell changes in either genotype.

Discussion

In the present study, acute treatment with TCDD

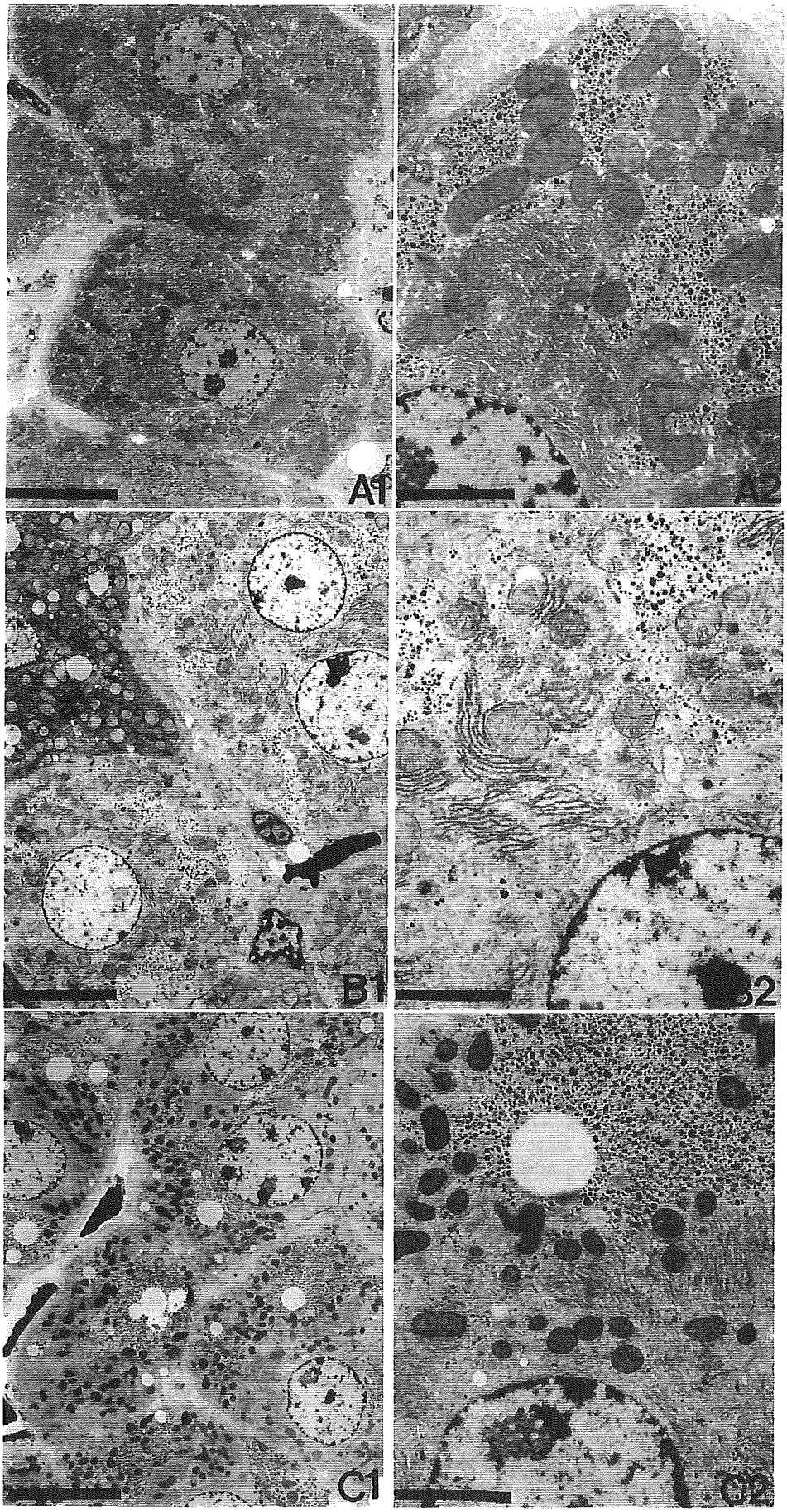

Fig. 1. Electron micrographs of liver cells from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note cytoplasmic swelling associated with a profound decrease of glycogen granules, RERs and mitochondria in the liver cells of the TCDD-treated ADF WT mouse (B). Swelling of the remaining mitochondria with disorganized cristae and lucent matrix is also evident (B). Attenuation of these morphological changes is evident in the TCDD-treated ADF Tg mouse (C). Uranyl acetate and lead citrate. Bar=10 μm (A1, B1, C1), Bar=3 μm (A2, B2, C2).

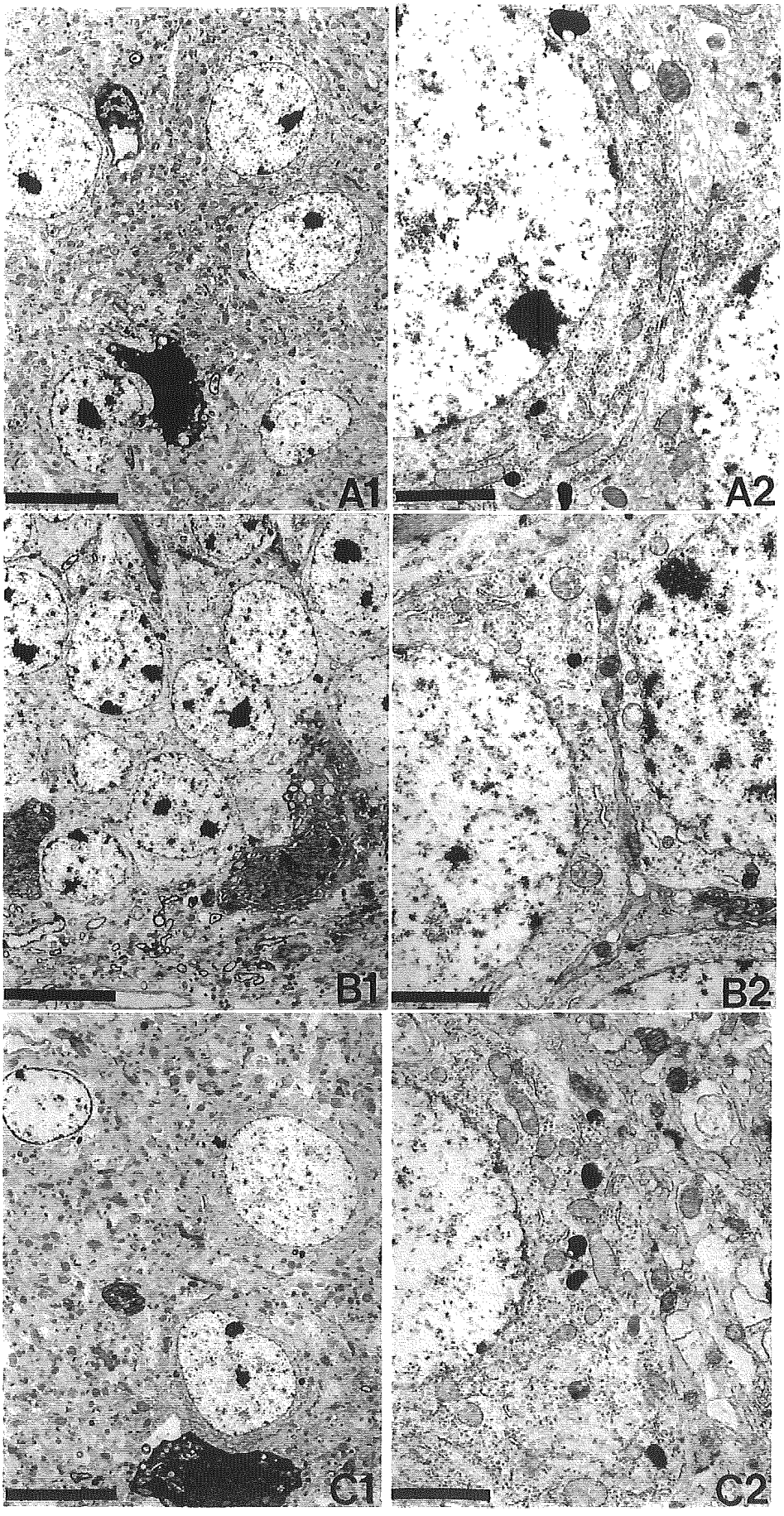

Fig. 2. Electron micrographs of neuronal cells in the cerebral cortex from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note the decrease of RER, ribosome and mitochondria in the cytoplasm of neuronal cells of the TCDD-treated ADF WT mouse (B). In the TCDD-treated ADF Tg mouse, mitochondrial swelling is also evident, but attenuation of the morphological changes can be seen, too. (C). Uranyl acetate and lead citrate. Bar=10 μm (A1, B1, C1), Bar=2 μm (A2, B2, C2).

induced ultrastructural alterations in the cytoplasmic components of liver cells characterized by prominent decrease of glycogen granules and RERs, proliferation of SERs, decrease and degradation of mitochondria, and increase of lipid droplets. These subcellular alterations were mostly consistent with those noted in the guinea pig liver following TCDD treatment[28], but concentric membrane arrays in the liver cells were not evident in the present study, presumably due to the different experimental protocol or the different species used in the studies. In the cerebral neuronal cells in the present study, alterations in subcellular components by TCDD were also evident, despite the changes being less profound than those in the liver cells. These subcellular changes in the liver and neuronal cells may represent the cytotoxic outcome of TCDD due to oxidative cellular damage and also cellular adaptation including detoxification.

Effective prevention of TCDD-induced toxicity by administration of antioxidants such as oltipraz[5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] or butylated hydroxyanisole, or by pretreatment with vitamins A and E further supports the hypothesis that oxidative processes are involved in TCDD-induced toxicity[29,30]. Attenuation of subcellular changes in the liver and neuronal cells by transgene of TRX/ADF in the present study indicates the critical role of oxidative stress in the toxic events induced by TCDD, and also the protective function of ADF/TRX in these organs, as in our previous study of TCDD-induced bone marrow toxicity[26]. The protective effect of TRX/ADF against oxidative cellular damage is believed to be achieved by free radical scavengers[31], activation of DNA repair enzymes, such as activator protein endonuclease (Ref-1; redox factor-1)[32], and activation of nuclear factor-kappa B (NF-kB)[33].

Taken together, the results of our present study strongly suggest that the acute toxic effect induced in the liver and brain by a single large dose of TCDD is due to oxidative cellular damage, and that TRX/ADF plays a role in protection against TCDD-induced acute toxicity. Considering the routes and concentrations of TCDD exposed to humans, research on the effect of extremely low doses of TCDD by oral ingestion on the oxidative cellular damage of target organs is clearly warranted.

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BENZENE-INDUCED HEMATOPOIETIC TOXICITY TRANSMITTED BY AHR IN THE WILD-TYPE MOUSE WAS NEGATED BY REPOPULATION OF AHR DEFICIENT BONE MARROW CELLS.

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Introduction

Recent studies have shown that the aryl hydrocarbon receptor (AhR) in primitive cells transmits negative signals for the proliferation of such cells[1, 2]. As we previously reported, primitive hemopoietic progenitor cells increases in number in AhR-knockout (KO) mice; on the other hand, relatively mature progenitor cells on the other hand, decreases in number in a homeostatic manner[1].

We have reported that benzene-induced hemopoietic toxicity is transmitted by AhR[3]. We also found that cytochrome P450 2E1 (CYP2E1) related to benzene metabolism is also up regulated in the bone marrow by benzene exposure in the bone marrow[4]. Therefore, it is of interest to hypothesize a greater role of bone marrow cells in hemopoietic toxicities rather than the hepatic metabolism. Accordingly, in the present study, benzene-induced hemopoietic toxicity was evaluated in wild type (Wt) mice after a lethal dose of whole-body irradiation followed by repopulation of bone marrow cells that lack AhR or, *vice versa,* in AhR KO mice after repopulation of Wt bone marrow cells.

As results, benzene-induced hemopoietic toxicity seems to have been transmitted through AhR, and benzene was transformed by *de novo* metabolism with CYP2E1 in the bone marrow.

Materials and Methods

Animals. The establishment of homozygous AhR KO ($AhR^{-/-}$) mice, the 129/SvJ strain, is described elsewhere[3, 5]. The breeding of heterozygous AhR KO ($AhR^{+/-}$) males with $AhR^{+/-}$ females generated wild-type ($AhR^{+/+}$), $AhR^{+/-}$, and $AhR^{-/-}$mice. The neonates were genotyped by PCR screening of DNA from the tail. Female mice (12 weeks old) were used in the study. Eight-week-old C57BL/6 male mice from Japan SLC (Shizuoka, Japan) were used as recipients for the repopulation assay and the assay of CFU in the spleen. All the mice were housed under specific pathogen-free conditions at 24 ± 1°C and 55 ± 10%, using a 12-hr light-dark cycle. Autoclaved tap water and food pellets were provided *ad libitum.*

Blood and bone marrow (BM) parameters. Peripheral blood was collected from the orbital sinus. Peripheral blood leukocyte (WBC), red blood cell (RBC) and platelet (PLT) counts were determined using a blood cell counter (Sysmex M-2000, Sysmex Co., Kobe, Japan). Bone marrow (BM) cellularity was evaluated by harvesting BM cells from the femurs of each mouse[6]. The animals were sacrificed. Then a 27-gauge needle was inserted into the femoral bone cavity through the proximal and distal edges of the bone shafts, and BM cells were flushed out under pressure by injecting 2 ml of a-MEM. A single-cell suspension was obtained by gently triturating the BM cells through the 27-gauge needle, and cells were counted using Sysmex M-2000.

Irradiation. Recipient mice were exposed to a lethal radiation of 800.1 cGy, at a dose rate of 124 cGy/min, using a ^{137}Cs-gamma irradiator (Gamma Cell 40, CSR, Toronto, Canada) with a 0.5-mm aluminum-copper filter.

CFU-S Assay. The Till and McCulloch method[7]was used to determine the number of colony-forming units in the