Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice

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Introduction

Arylhydrocarbon receptor (AhR) has been shown to play important roles in regulation and induction of CYP1A1, CYP1A2, and CYP1B1 and other drug-metabolizing enzymes including glutathione S-transferase, UDP-glucuronosyltransferase, aldehyde dehydrogenase, and NAD(P)H:oxidoreductase by a prototype inducer TCDD (1–5). PAHs such as carcinogenic B[a]P, 7,12-DMA, DB[a]P, and MC and co-planar PCB congeners, 3,4,3',4'-TCB, 3,4,3',4',5'-pentachlorobiphenyl, and 3,4,5,3',4',5'-hexachlorobiphenyl induce these enzymes through AhR-dependent mechanism (4–8). AhR(−/−) mice, as compared with AhR(+/+), are rather resistant to toxicity (9) and teratogenicity by TCDD (10) and to carcinogenicity by B[a]P (11). It is not clearly understood at present why TCDD does not cause such toxic responses in AhR(−/−) mice, although several possible mechanisms have been proposed (4,6,7,12,13). On the other hand, mechanisms underlying resistance to B[a]P carcinogenicity in AhR-deficient mice have been reported to be due to inability of these mice to express significant levels of CYP1A1 that catalyzes activation of B[a]P to ultimate carcinogenic metabolites (11,14,15). However, possibilities exist that CYP1B1, another enzyme that is also active in activating B[a]P, plays a role in the B[a]P carcinogenesis in mice. To understand the basis of roles of CYP1A1 and CYP1B1 in the activation of chemical carcinogens, we compared levels of induction of liver and lung CYP1A1, 1A2, and 1B1 by various polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls in AhR(+/-) and AhR(−/−) mice. Liver and lung CYP1A1 and 1B1 mRNAs were highly induced in AhR(+/-) mice by a single intraperitoneal injection of each of the carcinogenic PAHs, such as B[a]P, 7,12-dimethylbenz[a]anthracene, dibenz[a]pyrene, 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, benzo[b]fluoranthene, and benzo[a]anthracene and by a co-planar PCB congener 3,4,3',4'-tetrachlorobiphenyl. We also found that 6-aminochrysene, chrysene, benzo[e]pyrene, and 1-nitropyrene weakly induced the mRNA expression of CYP1A1 and 1B1, whereas anthracene, pyrene, and fluoranthene that have been reported to be non-carcinogenic in rodents, were very low or inactive in inducing these P450s. The extents of induction of liver CYP1A2 by these chemicals were less than those of CYP1A1 and 1B1 in AhR(±/±) mice. In AhR(−/−) mice, there was no induction of these P450s by PAHs and polychlorinated biphenyls. Liver microsomal activities of 7-ethoxyresorufin and 7-ethoxycoumarin O-deethylation and of mutagenic activation of (±)-trans-7,8-dihydroxy-7,8-dihydro-B[a]P to DNA-damaging products were found to correlate with levels of CYP1A1 and 1B1 mRNAs in the liver. Our results suggest that carcinogenicity potencies of PAHs may relate to the potencies of these compounds to induce CYP1A1 and 1B1 through AhR-dependent manner and that these induced P450s participate in the activation of B[a]P and related carcinogens causing initiation of cancers in mice.
B[a]P, 7,12-DMBA, and DB[a]P are well-known PAHs that cause lung and mammary tumors in experimental animals and have been suggested to be activated by CYP1A1 and CYP1B1 (21,33–40).

In this study, we determined effects of PAHs and PCBs on induction of mRNA levels of liver and lung CYP1A1, 1A2, and 1B1 in AhR(+/+) and AhR(−/−) mice. Potent PAH compounds examined in this study include B[a]P, 7,12-DMBA, DB[a]P, 3-MC, 1,2,5,6-dibenzanthracene, benzo[b]fluoranthene, and benzo[a]anthracene. Other compounds, such as 6-aminochrysene, chrysene, benzo[e]pyrene, 1-nitropyrene, anthracene, pyrene, and fluoranthene were also used. PCB mixtures, KC300 and KC500, and 3,4,3′,4′-TCB were compared to their abilities to induce CYP1A1, 1A2, and 1B1 in mice. Xenobiotic oxidations by liver microsomes of these mice treated with PAHs and PCBs were determined using substrates as 7-ethoxyresorufin, 7-ethoxycoumarin, MelIQ, Trp-P-1, (±)-B[a]P-7,8-diol, and 7,8-DMBA-3,4-diol.

### Materials and methods

**Chemicals**

B[a]P, 7,12-DMBA, DB[a]P, 3-MC, benzo[a]anthracene, benzo[b]fluoranthene, anthracene, 1,2,5,6-dibenzanthracene, benzo[e]pyrene, 6-aminochrysene, 1-nitropyrene, pyrene, and fluoranthene were purchased from Sigma Chemical Co. (St Louis, MO) and Kanto Kagaku (Tokyo). Other chemicals and reagents were purchased from Kanto Kagaku in AhR(–/–) female mice to give rise to CYP1A1, 1A2, and 1B1 in mice. Levels of mRNA expression of CYP1A1, 1A2, and 1B1 were calculated using mRNAs of CYP1A1, 1A2, and 1B1 were calculated using mRNA Selective PCR Kit (Takara Shuzo, Kyoto, Japan) under the following conditions for CYP1A1 and CYP1B2 (85°C for 30 s, 55°C for 30 s, 72°C for 1 min, and 72°C for 5 min), CYP1B1 (85°C for 30 s, 60°C for 30 s, 72°C for 1 min, and 72°C for 5 min), and β-actin (85°C for 30 s, 48°C for 30 s, 72°C for 1 min, and 72°C for 5 min). PCR amplifications were carried out for 28, 32, and 34 cycles for β-actin, CYP1A1, CYP1B1, respectively; the cycles used were in the linear phase in amplifying the respective cDNAs. The resultant products were analyzed by electrophoresis on 1.5% agarose gel.

The levels of mRNA expression of CYP1A1, 1A2, and 1B1 were calculated using obtaining ratios of staining intensities of β-actin mRNA and each of P450 mRNAs on the same agarose gels.

**Assay methods**

Standard incubation mixture for the assay of 7-ethoxyresorufin O-deethylation consisted of liver microsomes (10 μg of protein), substrate (at a concentration of 10 μM) and an NADPH-generating system consisting of 0.5 mM NADPH, 5 mM glucose 6-phosphate, and 0.5 unit of glucose 6-phosphate dehydrogenase/ml (40,44), in a final volume of 1.0 ml of 100 mM potassium phosphate buffer (pH 7.4) (40). 7-Ethoxycoumarin O-deethylation activities were determined by the method as described (45). Briefly, incubation mixtures consisted of liver microsomes (10 μg of protein), 7-ethoxycoumarin (80 μM), and an NADPH-generating system in a final volume of 0.25 ml of 100 mM potassium phosphate buffer (pH 7.4). The products thus formed were determined with high-performance liquid chromatography (45).

**Genotoxicity assay**

P450-dependent activation of procarrangons to reactive products that cause induction of umu gene expression in tester strain Salmonella typhimurium NM5000 was determined as described previously (19,21). Standard incubation mixtures contained microsomal protein (5 μg protein for the case of MeIQ and 10 μg protein for Trp-P-1, B[a]P-7,8-diol and 7,12-DMBA-3,4-diol) and procarrangons (0.1 μM for MeIQ and 2.5 μM for other cases) in a final volume of 1.0 ml of 100 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system and 0.75 ml of bacterial suspension. The induction of umu gene expression is presented as units of β-galactosidase activity/min/mg protein (46).

**Results**

Induction of liver and lung CYP1A1, 1A2, and 1B1 by B[a]P, 7,12-DMBA, DB[a]P, 3-MC, 3,4,3′,4′-TCB, KC300, and KC500 in AhR(+/+) and AhR(−/−) mice

We first determined levels of expression of mRNAs of β-actin, CYP1A1, 1A2, and 1B1 in liver and lung tissues of
AhR-dependent induction of CYP1A1, 1A2, and 1B1 by PAHs in mice

Effects of PAHs and PCBs on induction of liver microsomal P450-dependent drug oxidation activities in AhR(+/+) and AhR(−/−) mice

Xenobiotic oxidation activities were determined in liver microsomes of AhR(+/+) and AhR(−/−) mice that were the same animals used for the above experiments (Table I). The reactions determined were O-deethyllations of 7-ethoxyresorufin and 7-ethoxycoumarin and metabolic activation of MeIQ, Trp-P-1, (±)B[a]P-7,8-diol, and 7,12-DMBA-3,4-diol to DNA-damaging products in S. typhimurium NM2009. 7-Ethoxyresorufin O-deethylation activities in control mice were very low, and highly induced in AhR(+/+) mice by B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, and 3,4,3′,4′-TCB; the fold induction with these treatments was 105, 15, 80, 160, and 74, respectively, over control values (Table I). KC300 and KC500 also increased 7-ethoxyresorufin O-deethylation activities by 6- and 14-fold, respectively. However, in AhR(−/−) mice that were treated with these chemical inducers, there were no increases in 7-ethoxyresorufin O-deethylation activities. Although 7-ethoxycoumarin O-deethylation activities were also increased in AhR(+/+) mice treated with these P450 inducers, the extents of fold induction were lower than those seen in 7-ethoxyresorufin O-deethylation activities described above.

We also determined the activation of MeIQ, Trp-P-1, (±)B[a]P-7,8-diol, and 7,12-DMBA-3,4-diol to DNA-damaging products in S. typhimurium NM2009. It was found that these activities were also increased only in AhR(+/+) mice by these chemical inducers; the fold induction of activities was depending upon the inducers used (Table I). Of seven chemicals examined, 3-MC and 3,4,3′,4′-TCB were highly effective in inducing activities of activation of these procarcinogens, followed by B[a]P and DB[a,l]P. 7,12-DMBA was least effective particularly when (±)B[a]P-7,8-diol and 7,12-DMBA-3,4-diol were used as substrates.

To determine if P450 proteins are increased in liver microsomes of mice treated with these chemical inducers, we determined protein levels that are immunoreactive with goat anti-rat CYP1A1 serum, rabbit anti-rat CYP1A1 and 1A2 IgGs, and mouse anti-rat CYP1A2 monoclonal antibodies in liver microsomes. Of these antibodies used, goat anti-rat CYP1A1 serum was more sensitive to detect mouse CYP1 proteins. In AhR(−/−) mice, no immunoreactive proteins were detected in liver microsomes. The immunoreactive proteins with anti-rat CYP1A1 were found to be induced in AhR(+/+) mice very significantly by B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, and 3,4,3′,4′-TCB (data not shown). KC300 and KC500 also induced, but much lesser extents, the immunoreactive proteins with anti-CYP1A1 in the liver. When other antibodies were used for the immunodetection, basically similar, but not so sensitive, results were obtained.

Comparison of induction of liver mRNA levels and microsomal drug oxidation activities by 14 PAHs and 3 PCBs in AhR (+/−) mice

We further examined and compared abilities of 14 PAHs and 3 PCBs to induce mRNA levels of liver CYP1A1, 1A2, and 1B1, activities of liver microsomal 7-ethoxycoumarin

AhR(+/+) and AhR(−/−) mice that received a single intraperitoneal injection of each of B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, 3,4,3′,4′-TCB, KC300 and KC500 at a dose level of 100 mg/kg of body weight. Mice were killed after 72 h and the levels of liver and lung mRNAs of β-actin, CYP1A1, 1A2, 1B1 were determined (Figures 1 and 2). β-Actin mRNA was detected in all of the liver and lung samples of AhR(+/+) and AhR(−/−) mice examined (Figures 1 and 2). Liver CYP1A1 and CYP1B1 mRNAs were expressed constitutively at very low levels and increased markedly by B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, and 3,4,3′,4′-TCB only in AhR(+/+) mice (Figure 1). KC500 induced liver CYP1A1 mRNA in AhR (+/+) mice at significant levels, but KC300 was very weak in inducting mouse liver CYP1A1. Both KC300 and KC500 had little or no effects on induction of liver CYP1B1 in the AhR(+/+) mice. CYP1A2 mRNA was expressed constitutively in both AhR(+/+) and AhR(−/−) mice and was induced by PAHs and PCBs, particularly when 3-MC and KC500 were administered, in AhR(+/+) mice.

In the lung, CYP1A2 mRNA was not detected in AhR(+/+) and AhR(−/−) mice with or without treatments of mice with PAHs and PCBs (Figure 2). CYP1B1 was expressed in the lung of control mice and was induced only in AhR (+/+) mice by B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, and 3,4,3′,4′-TCB. KC300 and KC500 had little effect on CYP1B1 induction. CYP1A1 was constitutively expressed in AhR (+/−), but not AhR(−/−), mice and was found to be increased by B[a]P, 7,12-DMBA, DB[a,l]P, 3-MC, and 3,4,3′,4′-TCB in AhR(+/+) mice. Again KC300 and KC500 were very weak in inducing lung CYP1A1 in the mice.

Fig. 1. mRNA expression of CYP1A1, CYP1A2, CYP1B1, and β-actin in livers of AhR(+/+) (left part) and AhR(−/−) (right part) mice. Liver samples from control mice (A and a) and mice treated with B[a]P (B and b), 7,12-DMBA (C and c), DB[a,l]P (D and d), 3-MC (E and e), 3,4,3′,4′-TCB (F and f), KC300 (G and g), and KC500 (H and h) were analyzed.

Fig. 2. mRNA expression of CYP1A1, CYP1A2, CYP1B1, and β-actin in lungs of AhR(+/+) (left part) and AhR(−/−) (right part) mice. Other details are the same as in the legend to Figure 1.
Table 1: Effects of B[a]P, 7,12-DMBA, DB[a]P, 3-MC, 3,4,3′,4′-TCB, KC300, and KC500 on the metabolism of xenobiotics by liver microsomes of AhR(+/-) and AhR(-/-) mice

<table>
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<tr>
<th>AhR genotype</th>
<th>Treatment of animals</th>
<th>Xenobiotic oxidation (nmol/min/mg protein)</th>
<th>Activation of procarcinogens (umu units/min/mg protein)</th>
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<tr>
<td></td>
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<td>7-Ethoxyresorufin (5 μM)</td>
<td>7-Ethoxyresorufin (80 μM)</td>
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<td>AhR(+/-)</td>
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<td></td>
<td>B[a]P</td>
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<td>0.60 ± 0.11</td>
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<td>DB[a]P</td>
<td>3.13 ± 0.46</td>
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<td>3-MC</td>
<td>6.50 ± 0.86</td>
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<td>3,4,3′,4′-TCB</td>
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<td>KC 300</td>
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<td>KC 500</td>
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<td>B[a]P</td>
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<td>KC500</td>
<td>0.28 ± 0.08</td>
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Data are means from two animals and SE (range). Parentheses indicate substrate concentrations used for the assays.

O-deethylation, and levels of liver microsomal proteins immunoreactive with anti-rat CYP1A1 antibodies, activities of 7-ethoxyresorufin O-deethylation in liver microsomes, and levels of mRNA expression of CYP1A1, 1A2, and 1B1 in the liver of AhR(+/-) mice. Liver samples from control mice (A) and mice treated with furanoxanthene (B), antracene (C), pyrene (D), 1-nitropyrene (E), benzo[e]pyrene (F), 7,12-DMBA (G), 6-aminochrysene (H), KC300 (I), KC500 (J), chrysene (K), benzo[a]antracene (L), 3,4,3′,4′-TCB (M), DB[a]P (N), B[a]P (O), 3-MC (P), 1,2,5,6-dibenzoanthracene (Q), and benzo[b]fluoranthe (R) were used. Purified rat liver CYP1A1 (0.5 pmol) acted as a control (1).

O-deethylation, and levels of liver microsomal proteins immunoreactive with anti-rat CYP1A1 in AhR(-/-) mice (Figure 3). P450 mRNA levels were represented as a ratio of mRNA expression of P450s to that of β-actin. PAH compounds examined were B[a]P, 7,12-DMBA, DB[a]P, 3-MC, 1,2,5,6-dibenzoanthracene, benzo[b]fluoranthe, benzo[a]antracene, 6-aminochrysene, chrysene, benzo[e]pyrene, 1-nitropyrene, antracene, pyrene, and fluoranthe (Scheme 1). We also used 3,4,3′,4′-TCB, KC300, and KC500 for comparison. It should be mentioned that all of these compounds, 14 PAHs and 3 PCBS, did not cause any increases in liver mRNAs of CYP1A1, 1A2, and 1B1, liver microsomal proteins immunoreactive with anti-rat CYP1A1), and activities to catalyze 7-ethoxyresorufin O-deethylation in AhR(-/-) mice (data not shown).

Immunoblotting analysis with anti-rat CYP1A1 antibodies showed that at least two bands were increased by the treatments of P450 inducers in AhR(+/-) mice (Figure 3). Upper band (‘a’ in the figure) was highly induced in liver microsomes of AhR(+/-) mice treated with 3,4,3′,4′-TCB, DB[a]P, B[a]P, 3-MC, 1,2,5,6-dibenzoanthracene, and benzo[b]fluoranthe. It was also found that 7,12-DMBA, chrysene, and benzo[a]antracene weakly induced this protein band, whereas other chemicals tested were not so effective or inactive in increasing this protein band. On the other hand, a lower protein band (b in the figure) was increased by several chemicals, being very significant with 3,4,3′,4′-TCB, DB[a]P, B[a]P, 3-MC, 1,2,5,6-dibenzoanthracene, and benzo[b]fluoranthe. Patterns of 7-ethoxyresorufin O-deethylation activities were found to be similar to the extents of the levels of an upper protein band and mRNAs of liver CYP1A1 and 1B1. The extents of lower protein band that may corresponds to CYP1A2 were similar to the levels of mRNA of CYP1A2 and activities of activation of MeIQ to active metabolites by mouse liver microsomes (data not shown).

Dose–response studies of induction of CYP1A1, 1A2, and 1B1 by 7 PAHs and 3,4,3′,4′-TCB in AhR(+/-) mice

Dose–response studies of induction of P450 proteins and mRNAs in the liver were examined with eight chemicals, B[a]P, 7,12-DMBA, 3-MC, 3,4,3′,4′-TCB, benzo[b]fluoranthe, benzo[a]antracene, benzo[e]pyrene, and 6-aminochrysene, in AhR(+/-) mice. These mice received a single intraperitoneal injection of these chemicals at levels of 0, 0.01, 0.1, 1, 10, and 100 mg/kg body weight and were killed after 72 h of the treatments. Levels of induction of an upper protein band (a in the figure) in liver microsomes were similar to the extents of induction of mRNAs of liver CYP1A1 and 1B1 in mice treated with 3-MC and 3,4,3′,4′-TCB (Figure 4). The lower protein band (b in the figure) was increased by these.
AhR-dependent induction of CYP1A1, 1A2, and 1B1 by PAHs in mice

Scheme 1. Chemical structure of PAH compounds used in this study.

Fig. 4. Dose–response studies of expression of liver microsomal proteins (that are immunoreactive with anti-rat CYP1A1) and mRNA levels of liver CYP1A1, 1A2, and 1B1 in AhR(+/+) mice treated with different doses of 3-MC and 3,4,3',4'-TCB.

Liver microsomal activities of 7-ethoxyresoruﬁn and 7-ethoxycoumarin O-deethylations and of metabolic activation of MeIQ to active metabolites were determined in AhR(+/+) mice that were treated with different doses of B[a]P, 7,12-DMBA, 3-MC, 3,4,3',4'-TCB, benzo[b]ﬂuoranthene, benzo[a]anthracene, benzo[e]pyrene, and 6-aminochrysene (Figure 5). 7-Ethoxyresoruﬁn O-deethylation activities were very low in AhR(+/+) mice at lower doses of these chemicals and were increased markedly at higher doses of benzo[b]ﬂuoranthene, 3-MC, B[a]P, 3,4,3',4'-TCB, and benzo[a]anthracene. Activities of 7-ethoxycoumarin O-deethylation and metabolic activation of MeIQ by liver microsomes were detected at signiﬁcant levels in control mice and mice treated with lower doses of these chemicals and were highly induced by these chemicals at high levels.

mRNA levels of liver CYP1A1 and 1B1 were also very low in control mice and mice injected at low doses of these chemicals and were highly induced in these mice by treatments at higher doses of B[a]P, 7,12-DMBA, 3-MC, 3,4,3',4'-TCB, benzo[b]ﬂuoranthene, and benzo[a]anthracene (data not shown). CYP1A2 mRNA was detected at significant levels in control mice and mice administered low doses of these chemicals at higher doses and intensities of this band were essentially similar to the levels of CYP1A2 mRNA.
chemicals and was induced with increasing the dose levels, particularly those with 3-MC, 3,4,3’,4’-TCB, and benzo[β]fluoranthene.

**Discussion**

Shimizu et al. (11) have recently reported that AhR(--/--) mice are resistant to B[a]P-induced carcinogenesis when compared with those in AhR(+/+) mice and have concluded that such ineffectiveness in AhR(--/--) mice to B[a]P may be due to the inability of these mice to express significant amounts of CYP1A1 that activate B[a]P to carcinogenic metabolites (11,14,15). However, they did not determine whether levels of expression of CYP1B1 are also affected by B[a]P in AhR(+/+) and AhR(--/--) mice. It is very important to know the expression levels of CYP1B1 expression in these mice, since CYP1B1 has recently been shown to be one of the major enzymes in activating a number of PAHs including B[a]P to ultimate carcinogenic metabolites (21,43,52). In fact, Buters et al. (53) have reported that the cultured embryonic fibroblast cells isolated from CYP1B1-null mice, which are inactive in catalyzing metabolism of 7,12-DMA, are found to be resistant to the 7,12-DMA toxicity. Since these cells express CYP1B1, but not CYP1A1, at significant levels, the toxicity caused by 7,12-DMA in the cells are suggested to be due to the activation of 7,12-DMA by CYP1B1 to the highly toxic metabolites (53). They also found that CYP1B1-null mice are resistant to the formation of malignant lymphomas and other tumors initiated by 7,12-DMA, when the carcinogen was dosed orally to mice at 200 μg/kg for 3 days (53). In this study, we found that 7,12-DMA induced CYP1A1 and CYP1B1 that catalyzed activation of (+)B[a]P-7,8-diol and 7,12-DMA-3,4-diol to DNA-damaging products in mice through AhR-dependent mechanism (Table I).

In this study we found that PAH compounds that are known to be environmental carcinogens in experimental animal models and possibly in humans induced very significantly mRNAs of liver and lung CYP1A1 and CYP1B1 in genetically engineered C57BL/6J mice through AhR-dependent mechanism(s). Active compounds that induce CYP1A1 and 1B1 were B[a]P, 7,12-DMA, DB[a]fl, 3-MC, 1,2,5,6-dibenzanthracene, benzo[β]fluoranthene, and benzo[a]anthracene. It was also found that 6-aminohidrosyrene, chrysene, benzo[β]pyrene, and 1-nitropyrene increased slightly the expression of CYP1A1 and 1B1 in AhR(+/+) mice, whereas anthracene, pyrene, and fluoranthene that have been shown to be weak or inactive in producing tumors in rodents (14,22,23), were very low or inactive in inducing these P450 enzymes in the mice. Liver CYP1A2 mRNA that was detected in control livers of AhR(+/+) and AhR(--/--) mice was induced by treatments with carcinogenic PAH compounds only in AhR(+/+) mice. The extents of induction of CYP1A2 were lesser than those of CYP1A1 and 1B1. These results suggest the possibility that CYP1B1 as well as CYP1A1 may play important roles in the activation of PAHs to carcinogenic metabolites in mice.

Pathways for activation of B[a]P to ultimate carcinogenic metabolites by drug-metabolizing enzymes have extensively been studied by Conney and Jerina and their associates around 1980 (14,36,54,55). B[a]P is reported to be first oxidized by P450s to form intermediate metabolites, B[a]P-7,8-epoxide, and these epoxides are hydrolyzed by epoxide hydrolase to form B[a]P-7,8-diol metabolites (14). Finally, B[a]P-7,8-diols are oxidized again by P450s to form highly reactive diol-epoxides that cause initiation of cancers in several organs (14,15,26,29,36,54,56,57). Our recent studies using recombinant human P450 enzymes expressed in *Escherichia coli* have suggested that both CYP1A1 and 1B1 are equally active in catalyzing B[a]P-7,8-diol to DNA-damaging products in *S.typhimurium* NM2009 tester strain (3,43,52). These findings are of interest since our present studies showed that treatments of AhR(+/+) mice with carcinogenic PAHs, such as B[a]P, caused induction of liver microsomal activation of (+)B[a]P-7,8-diol to DNA-damaging products in *S.typhimurium* NM2009 and that these increases in the activities were correlated with levels of induction of mRNAs of CYP1A1 and 1B1 in the liver. We also found that activities of metabolic activation of (+)B[a]P-7,8-diol by liver microsomes were related to the upper protein band with anti-rat CYP1A1 antibodies in liver microsomes. Further work is required using antibodies raised against mouse CYP1A1, 1A2, and 1B1 in order to better understand the basis of roles of these P450 proteins in the activation of B[a]P and other PAHs in mice.

In 1974, Poland and Glover (58) have reported that TCDD is about 3 x 10^3 times more potent than 3-MC in inducing arylhydrocarbon hydroxylation activities in livers of rats. The estimated dose levels of TCDD and 3-MC to cause half-maximal induction of the activities were calculated to be ~0.3 μg/kg and ~8 mg/kg, respectively, of body weight (58). The value (~8 mg/kg) for the induction by 3-MC in rats seems to be semi-quantitatively similar to those obtained in the present AhR(+/+) mice. In this study we found that 3-MC at dose level of 100 mg/kg caused significant induction of 7-ethoxyresorufin O-deethylation, 7-ethoxycoomarin O-deethylation and metabolic activation of MeIQ in AhR(+/+) mice (Figure 5). At 10 mg/kg of 3-MC, these activities were 15%, 35%, and 41%, respectively, of those obtained at 100 mg 3-MC/kg body weight. The levels of mRNAs of liver CYP1A1, 1A2, and 1B1 at 10 mg/kg of 3-MC were 35%, 48%, and 39%, respectively, of those at 100 mg/kg of 3-MC in AhR(+/+) mice.

In this study, we measured liver microsomal activities of P450-catalyzed xenobiotic oxidations as well as mRNA levels of liver and lung P450s, in order to determine the levels of induction of P450s in mice treated with various PAHs and PCBs. 7-Ethoxyresorufin O-deethylation was found to be a good marker for the detection of induction of CYP1A1 and 1B1 by these compounds in mice. The basal 7-ethoxyresorufin O-deethylation activities of liver microsomes in control mice were very low and were highly induced in mice particularly when potent P450 inducers, such as B[a]P, 7,12-DMA, DB[a]fl, 3-MC, 1,2,5,6-dibenzanthracene, benzo[β]fluoranthene, and benzo[a]anthracene were administered. For example, treatment of AhR(+/+) mice with B[a]P, 7,12-DMA, DB[a]fl, 3-MC, and 3,4,3’,4’-TCB caused increases in 7-ethoxyresorufin O-deethylation activities by about 105-, 15-, 80-, 160-, and 74-fold, respectively, over control values. Considerable levels of 7-ethoxyoumarin O-deethylation activities were found in control mice, probably due to the fact that 7-ethoxyoumarin has been reported to be catalyzed by different P450s, such as CYP1A2, 2B, and 2E1 enzymes as well as CYP1A1 and 1B1 in liver microsomes (59–61).

Among 14 PAHs determined in this study, 1,2,5,6-dibenzanthracene and benzo[β]fluoranthene were found to be more potent than B[a]P, 7,12-DMA, DB[a]fl, 3-MC, and benzo[a]anthracene in inducing CYP1A1, 1A2, and 1B1 mRNAs and proteins in AhR(+/+) mice. The former two
compounds, as in the cases of 2[α]P, 7,12-DMBA, DB[a,j]P and B[a]A, have been shown to be potent carcinogens and to be catalyzed by P450 enzymes and epoxide hydrolase to form ultimate carcinogenic diol-epoxide metabolites (11,14,15). Benzo[α]pyrene and chrysene that have also been shown to be carcinogenic in rodents were found in this study to be weak inducers of CYP1A1, 1A2, and 1B1 in AhR(+/-) mice. These two compounds have also been shown to be activated by P450s and epoxide hydrolase to ultimate diol-epoxide metabolites (14). The three PAHs, anthracene, pyrene, and fluoranthene, that have been shown to be weak or inactive in producing tumors in rodents (14) induced weakly CYP1A1, 1A2, and 1B1 in AhR(+/-) mice. Striking differences were obtained in the expression of these enzymes through AhR-dependent mechanisms, although CYP1B1 was expressed at very low levels in AhR(+/-) and AhR(-/-) mice, whereas CYP1A1 was not detected in lung of AhR(-/-) mice. It is not known at present whether constitutive expression of CYP1A1 in the lung is regulated by AhR in mice.

TCDD has been shown to be a prototype ligand for AhR and to induce very effectively numerous enzymes in mammalian tissues (6,13,68). Structurally related co-planar PCBs, such as 3,4,3',4'-TCB, 3,4,3',4',5'-pentachlorobiphenyl, and 3,4,5,3',4',5'-hexachlorobiphenyl are also known to induce these enzymes through AhR-dependent mechanisms, although the extents of the effects by these PCBs are lower than those by TCDD (6,13,68). In this study we used 3,4,3',4'-TCB as a prototype co-planar PCB congener and compared activities to induce CYP1A1, 1A2, and 1B1 with those by PAHs in AhR(+/-) mice very effectively and found that the potencies to induce P450 enzymes in AhR(+/-) mice were roughly similar to those by B[a]P and DB[a,j]P. PCB mixtures, KC300 and KC500, on the other hand, were weak in inducing P450 enzymes in AhR(+/-) mice, probably reflecting that these PCB mixtures contain small amounts of co-planar PCB congeners (69,70).

In conclusion, present results showed that potent carcinogenic PAH compounds such as B[a]P, DMBA, DB[a,j]P, 3-MC, 1,2,5,6-dibenzoanthracene, benzo[b]fluoranthene, and benzo[a]anthracene significantly induced liver and lung CYP1A1 and 1B1 mRNAs and protein catalytic activities in C57BL/6J mice through AhR-dependent mechanism. Liver CYP1A2 was constitutively expressed in control mice and was induced by potent carcinogenic PAHs through AhR-dependent mechanism, whereas the extents of induction of CYP1A2 were lower than those seen in CYP1A1 and 1B1. 7-Ethoxyresorufin O-deethylation activities in liver microsomes were found to be a good indicator in detecting induction of CYP1A1 and 1B1 in mice treated with PAHs and PCBs. Striking differences in the expression of CYP1A1 and 1B1 in the lungs of AhR(-/-) mice were noted that CYP1A1 was not detected in control mice, although CYP1B1 was expressed at significant levels. Our present results suggest that potent carcinogenic PAH compounds induce CYP1A1, 1A2, and 1B1 through AhR-dependent mechanism, and these induced P450 enzymes participate in the toxicity and tumor formation caused by these environmental chemicals. We also reported that AhR(+/-) and AhR(-/-) mice are of use to determine levels of induction of mRNAs of CYP1A1, 1A2, 1B1 by PAHs and PCBs through AhR-dependent manner and that 7-ethoxyresorufin O-deethylation is one of the sensitive determinants in detecting extents of induction of CYP1A1 and 1B1 in liver microsomes of mice.

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References


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