Differential Regulation of Expression of Hepatic and Pulmonary Cytochrome P4501A Enzymes by 3-Methylcholanthrene in Mice Lacking the CYP1A2 Gene

SUDHA R. KONDRAGANTI, WEIWU JIANG, and BHAGAVATULA MOORTHY
Department of Pediatrics, Baylor College of Medicine, Houston, Texas
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ABSTRACT
The cytochrome P4501A enzymes play important roles in carcinogen metabolism. We reported previously that 3-methylcholanthrene (MC) elicits a persistent induction of hepatic, pulmonary, and mammary microsomal cytochrome P450 (P450) 1A enzymes for several weeks after MC withdrawal. In this study, we tested the hypothesis that CYP1A2, a liver-specific P450 isozyme, plays an important role in the mechanisms governing persistent CYP1A1 induction by MC in liver but not in extra-hepatic tissues such as lung, which is devoid of endogenous CYP1A2. Administration of wild-type (WT) or CYP1A2-null mice with MC (100 μmol/kg i.p.) once daily for 4 days caused significant increases in hepatic CYP1A1/1A2 activities, apoprotein contents, and mRNA levels 1 day after carcinogen withdrawal compared with vehicle-treated controls. The induction persisted in the WT, but not CYP1A2-null animals, for up to 15 days. In the lung, MC caused persistent CYP1A1 induction for 15 days in both the genotypes. Since MC is almost completely eliminated by day 15, we hypothesize that CYP1A2 contributes to the up-regulation of CYP1A1 in liver, but not lung, by a novel mechanism, presumably involving a CYP1A2-dependent persistent metabolite. The studies demonstrate tissue-specific differences in the regulation of CYP1A by MC, a phenomenon that may have implications for human carcinogenesis caused by environmental chemicals.

3-Methylcholanthrene (MC) is one of the most potent polycyclic aromatic hydrocarbon (PAH) carcinogens known to date (Harvey, 1982). Metabolism of MC by cytochrome P450 (P450) enzymes leads to the formation of chemically reactive intermediates that can bind covalently to DNA, a critical step in the initiation of carcinogenesis (Guengerich, 1990).

The P450 enzymes are a very large multigene family of constitutive and inducible enzymes with a major role in the oxidative activation and/or inactivation of a wide range of xenobiotics, including environmental carcinogens and pollutants (Guengerich, 1990). The CYP1A family comprises two proteins CYP1A1 and 1A2, which play important roles in carcinogen activation (Guengerich, 1988). Recent studies have shown CYP1B1 also to be active in PAH-mediated carcinogenesis (Bowes et al., 1996; Shimada et al., 1996). The hepatic CYP1A1/1A2 enzymes are inducible by a number of chemicals, including the hepatocarcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Whitlock, 1987), MC (Thomas et al., 1983), benzo[a]pyrene (Conney, 1986), and cigarette smoke (Kawajiri et al., 1990). Moreover, MC induces CYP1A1 in extrahepatic tissues such as lung (Nebert and Gelboin, 1969) and mammary glands (Christou et al., 1995), which are target organs for carcinogenesis (Huggins et al., 1961; Rasmussen et al., 1984).

The mechanisms of induction of CYP1A1/IA2 by MC or TCDD have been investigated extensively (Safe, 1995). The inducer, upon entry into the cell, interacts with the Ah receptor (AhR), a cytosolic protein. The inducer-receptor complex is translocated into the nucleus, where it binds to the Ah receptor nuclear translocator, and this complex in turn interacts with specific regulatory elements, called AHRs, to the CYP1A1/IA2 genes, resulting in enhanced expression of the CYP1A enzymes (Safe, 1995).

The events occurring during the induction process have been investigated extensively, but relatively little is known regarding the decline of drug-metabolizing enzymes to pre-induction activities after termination of xenobiotic exposure. In animals exposed to persistent chemicals such as TCDD (Gasiwicz et al., 1986) and Aroclor 1254 (Beebe et al., 1992), induction of CYP1A1 and phase II enzymes persists for sev-

ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbons; P450, cytochrome P450; TCCD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AHR, Ah receptor; AHRre, Ah response elements; HRP, horseradish peroxidase; WT, wild-type; CO, corn oil; EROD, ethoxyresorufin O-deethylase; MROD, methoxyresorufin O-demethylase.
eral weeks after cessation of xenobiotic administration, which is attributed to the long biological half-life of these chemicals (Gasiawicz et al., 1986; Beebe et al., 1992).

When induced by phenobarbital (Li et al., 1992) or β-naphthoflavone (Moorthy et al., 1994), the P450 enzyme activities return to control values within 5 days after cessation of treatment. In rats, a single dose of MC (20–25 mg/kg) induces aryl hydrocarbon hydroxylase activity (Poland and Glover, 1974) and CYP1A1 mRNA levels (Bresnick et al., 1981) after 24 h, followed by a return of enzyme activity and mRNA levels within 5 to 7 days. On the other hand, Boobis et al. (1977) showed that a single dose of MC (80 mg/kg) increases total P450 content and aryl hydrocarbon hydroxylase activities in mice for up to 9 days. We earlier reported that MC elicits a long-term induction of CYP1A enzymes for up to 45 days (Moorthy et al., 1993). Furthermore, we demonstrated that the sustained induction of CYP1A enzymes is mediated by mechanisms other than persistence of the parent compound (Moorthy, 2000). We recently postulated that a reactive metabolite of MC specifically targets the AHREs of the CYP1A1 promoter and up-regulates CYP1A1 expression by interacting covalently with AHREs (Moorthy, 2002). Since CYP1A2 is a major endogenous P450 isofrom in liver, we developed the hypothesis that CYP1A2 catalyzes the formation of the MC metabolite that may be responsible for sustained CYP1A1/A2 induction in liver.

Recent studies have demonstrated a role for CYP1A2 as a hepatic binding protein (DeVito et al., 1998; Diliberto et al., 1999). Using CYP1A2 (−/−) mice, it has been shown that CYP1A2 protein mediates the sequestration of TCDD and polychlorinated dibenzofurans (PCDFs) in liver (DeVito et al., 1998; Diliberto et al., 1999). CYP1A2, however, has no effect on the pharmacokinetic behavior of nondonixine-like compounds such as PCB 153 (DeVito et al., 1998). CYP1A2, through its role in the oxidation of uroporphyrinogen to uroporphyrin (Sinclair et al., 1998, 2000), contributes to the development of uroporphyria in mice by TCDD (Smith et al., 2001).

In view of the multiple functions of CYP1A2, we investigated whether CYP1A2 would play a role in the persistent induction of CYP1A1 by MC. To this end, using the CYP1A2-null mouse model, we studied the effect of MC on hepatic and pulmonary CYP1A expression in wild-type and CYP1A2-null mice and tested the hypothesis that the liver-specific CYP1A2 plays an important role in persistent induction by MC of CYP1A1 in liver, but not lung.

Materials and Methods

Chemicals. MC, calcium chloride, Tris, sucrose, NADP+, ethoxyresorufin, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). Methoxyresorufin was purchased from Molecular Probes (Eugene, OR). Polyvinylidene difluoride (PVDF) membranes and buffer components for electrophoresis and Western blotting were obtained from Bio-Rad Laboratories (Hercules, CA). The primary monoclonal antibody to CYP1A1 was a gift from Dr. P. E. Thomas (Rutgers University, Piscataway, NJ). Goat anti-mouse IgG conjugated with horseradish peroxidase (HRP) was obtained from Bio-Rad Laboratories (Hercules, CA). The primary monoclonal antibody to CYP1A2 (−/−) mouse of a mixed background (C57BL/6J/Sv129) were obtained from Dr. Frank J. Gonzalez. The antibodies were back-crossed with C57BL/6J mice for 12 generations to generate theoretically a >99.2% C57BL/6J background. The animals were acclimated for 7 days before the start of the experiment and randomized into three groups of 6 to 10 animals each. Tap water and food (Purina Rodent Lab Chow no. 5001; St. Louis, MO) were available ad libitum. A 12-h light/dark cycle was maintained.

Animal Treatment. WT and CYP1A2-null mice were treated with MC (100 µmol/kg) in corn oil (CO) (8 ml/kg) or were given equal volumes of CO (i.p.) once for 4 days. At selected time points, animals were sacrificed, and microsomes were isolated immediately from portions of liver and lung and stored at −80 °C until analysis. The remainder of the livers were stored at −80 °C for later isolation of RNA.

Preparation of Microsomes. The livers were excised, weighed, and homogenized in 10 mM Tris-HCl, pH 7.4, containing 0.25 M sucrose, and microsomes were prepared by the calcium chloride precipitation method (Cinti et al., 1972). Microsomes from lungs were prepared by differential centrifugation (Matsubara et al., 1974). Lungs were perfused with cold saline before excision and subsequent preparation of microsomes. Protein concentrations were estimated by the Bradford dye-binding method (Bradford, 1976).

Enzyme Assays. Ethoxyresorufin O-deethylation (EROD) and methoxyresorufin O-demethylation (MROD) activities in microsomes were assayed essentially as described previously (Moorthy, 2000).

Electrophoresis and Western Blotting. Liver (10 µg) or lung microsomes (20 µg of protein) were subjected to SDS-polyacrylamide gel electrophoresis in 7.5% acrylamide gels followed by Western blotting, as reported in a recent articles from our laboratory (Moorthy, 2000; Moorthy et al., 2000). Detection of liver CYP1A1/A2 proteins on the Western blots was accomplished by the use of a monoclonal antibody to CYP1A1 that cross-reacts with CYP1A2 as the primary antibody and goat anti-mouse IgG conjugated with HRP as the secondary antibody. The blots were developed with the HRP color reagent 4-chloro-1-naphthol (Moorthy, 2000; Moorthy et al., 2000). Lung CYP1A1 proteins were detected on the blots with 4-chloro-1-naphthol and diaminobenzidine in the presence of hydrogen peroxide (Moorthy et al., 2000). Apoprotein levels were determined by scanning the photographic negatives of the Western blots by laser densitometry, as described by Moorthy et al. (2000).

Northern Hybridization. Total liver RNA was isolated using a modification of the procedure of Chomczynski and Sacchi (1987). The RNA (20 µg/sample) was loaded on to 1% agarose/formaldehyde denaturing gel, separated by electrophoresis, and transferred to nitrocellulose filters. The filters containing separated RNA were prehybridized to exclude prehybridization to 20 µM Na2HPO4, pH 7.6, 10× Denhardt’s reagent, 100 µg/ml of heat-denatured salmon sperm DNA, and 7% SDS for approximately 3 h at 55 °C. After prehybridization, the solutions were discarded, and nitrocellulose filters were hybridized at 55°C for 16 h by using the same solution used for prehybridization and adding 10% dextran and the 32P-labeled CYP1A1 cDNA probe (20 × 106 cpm), which was prepared by the random prime technique (Hamburg et al., 1994). The hybridized filters were washed 3 times for 20 min with 3× sodium chloride sodium citrate (1× sodium chloride sodium citrate, 0.15 M sodium chloride, 0.015 M sodium citrate), and 1% SDS at 68°C to decrease nonspecific binding of radioactive transcripts to the filter. Following these washes, the membranes were exposed to autoradiography. Relative levels of CYP1A1/A2 mRNAs were quantitated by phosphor-imager analysis. GAPDH cDNA probe was used as a RNA transfer and loading control. For this, the nitrocellulose membranes were stripped and hybridized with GAPDH cDNA probe, followed by exposure to autoradiography.

Statistical Analysis. Data obtained from individual animals are expressed as means ± S.E.M. Statistical significance between con-
control and treated groups for each time point was assessed by Students’ t tests, with P < 0.05 being considered significant.

Results

**Hepatic CYP1A1 and 1A2 Enzyme Activities.** Hepatic EROD and MROD activities were assayed as indicators of CYP1A1 and 1A2 activities, respectively (Sinclair et al., 1998; Moorthy, 2000). On the first day following 4 daily doses of MC, EROD activities of WT mice were 6 times greater than those in animals treated with vehicle not containing MC (Fig. 1). The EROD activities in the MC samples were persistently higher, being 5- and 4.8-fold greater than those of controls at 8 and 15 days, respectively (Fig. 1). In CYP1A2-null animals, MC treatment led to a 5.7-fold increase in EROD activities over those of vehicle-treated animals. By day 8, the induction declined to 4.5-fold, and by day 15, the EROD activities decreased further and were approximately double of those of control animals (Fig. 1).

MC administration also resulted in persistent elevation of hepatic MROD activities in the WT animals (Fig. 2). The hepatic CYP1A2 activities in the MC-treated animals were 5.4-, 5.8-, and 4.3-fold higher than the corresponding controls at the 1-, 8-, and 15-day time points, respectively (Fig. 2). In the CYP1A2-null animals, MROD activities were barely detectable in the vehicle-treated mice. In MC-treated animals, MROD activities were slightly higher than control at each of the time points, but this was not statistically significant (Fig. 2).

**Hepatic CYP1A1/1A2 Apoproteins.** As shown in Fig. 3, liver microsomes from WT animals treated with vehicle displayed a band corresponding to CYP1A1/CYP1A2 proteins (52 kDa). Treatment of animals with MC led to a 16-fold increase in the hepatic expression of CYP1A1 and 1A2 at the 1-day time point (Table 1). The induction of these proteins continued through 15 days (Fig. 3), correlating with enzyme activities (Figs. 1 and 2). In the CYP1A2-null animals, MC treatment led to a strong induction of CYP1A1 and 1A2 mRNA species. The MC-mediated expression of CYP1A1 markedly declined by 15 days, being 50% of the 1 day value (Table 1).

**Hepatic CYP1A1/1A2 mRNA Levels.** To determine whether the sustained induction of CYP1A1/1A2 enzyme activities and apoprotein contents was accompanied by a corresponding modulation in mRNA levels, we performed Northern hybridization analysis on total RNA from livers isolated from control and MC-exposed WT and CYP1A2-null animals with cDNA probes for CYP1A1, which cross-hybridizes with CYP1A2 mRNA. Vehicle-treated WT animals did not display mRNA signals corresponding to either CYP1A1 (23S) or CYP1A2 (18S) (Fig. 4). MC treatment led to a strong induction of CYP1A1 as well as CYP1A2 mRNA species. The induction continued through 8 days, and even after 15 days, the expression of CYP1A1 and 1A2 mRNAs was elevated in MC-treated samples. In the CYP1A2-null animals, MC exposure led to substantial induction of CYP1A1 mRNA at the 1-day time point. CYP1A2 mRNA was not detectable (Fig. 4). In contrast to the WT animals, which displayed persistent induction of CYP1A1, the expression of this gene in the CYP1A2-null animals declined by 8 days and was barely detectable at the 15-day time point (Fig. 4). GAPDH mRNA levels were not altered by MC treatment at any time point, and the expression of this gene was similar in the WT and CYP1A2-null animals (Fig. 4).

Quantification of mRNA levels by a phosphor-imaging technique revealed that MC induced CYP1A1 mRNAs in the WT
animals by 20.4-, 23.2-, and 20.6-fold, respectively, over the corresponding controls (Fig. 5) at the 1-, 8-, and 15-day time points. In the CYP1A2-null animals, the extent of induction of CYP1A1 mRNA was 31-fold over control at the 1-day time point. The induction declined at the later time points, being 12.7- and 8.5-fold higher than control at 8 and 15 days, respectively (Fig. 5). MC treatment also led to induction of CYP1A2 mRNA in the WT animals at the 1-day time point; however, the extent of induction was less than that observed for CYP1A1. The induction continued through day 15. The induction at the 1-, 8-, and 15-day time points was 25-, 12-, and 11-fold higher, respectively, than the corresponding controls (Fig. 6).

**Pulmonary CYP1A1 Expression.** The effect of MC on EROD activities in lung of WT and CYP1A2-null animals at selected time points is shown in Fig. 7. Comparison of EROD activities between MC-exposed and control WT animals revealed a 21-fold induction of EROD by MC at 1 day (Fig. 7). On days 8 and 15, the EROD activities were persistently induced, being 21 and 17 times higher than control. In the CYP1A2-null animals, MC caused a 22-fold increase in EROD activities at the 1-day time point. At the 8 and 15-day time points, EROD activities continued to remain elevated, being about 24 times higher than control (Fig. 7). CYP1A1 apoprotein expression was analyzed in the lung microsomes by Western blotting. As shown in Fig. 8, MC caused a 17-fold induction of CYP1A1 apoprotein in the WT animals the 1-day time point (Table 1). The augmented expression was maintained at the 8- and 15-day time points, being about 10-fold higher than control (Fig. 8, Table 1).

**Discussion**

In the present study using the CYP1A2-null mouse model, we investigated the mechanisms of sustained CYP1A1 induction by MC, and tested the hypothesis that the liver-specific CYP1A2 would play a regulatory role in the long-term induction of CYP1A1 in the liver by MC. The marked increases in hepatic EROD activities in WT animals exposed to MC for up

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**Table 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Time (days)</th>
<th>Pixel Density Liver CYP1A1/1A2 (mean ± S.E.)</th>
<th>Pixel Density Lung CYP1A1 (mean ± S.E.)</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>CO</td>
<td>1</td>
<td>80 ± 20</td>
<td>190 ± 40</td>
</tr>
<tr>
<td>WT</td>
<td>MC</td>
<td>1</td>
<td>1275 ± 180&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3243 ± 350&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KO</td>
<td>CO</td>
<td>1</td>
<td>N.D.</td>
<td>100 ± 32</td>
</tr>
<tr>
<td>KO</td>
<td>MC</td>
<td>1</td>
<td>956 ± 110</td>
<td>3996 ± 320&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WT</td>
<td>CO</td>
<td>8</td>
<td>92 ± 10</td>
<td>390 ± 36</td>
</tr>
<tr>
<td>WT</td>
<td>MC</td>
<td>8</td>
<td>825 ± 150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3898 ± 408&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KO</td>
<td>CO</td>
<td>8</td>
<td>N.D.</td>
<td>360 ± 40</td>
</tr>
<tr>
<td>KO</td>
<td>MC</td>
<td>8</td>
<td>775 ± 80</td>
<td>3386 ± 318&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WT</td>
<td>CO</td>
<td>15</td>
<td>96 ± 12</td>
<td>402 ± 44</td>
</tr>
<tr>
<td>WT</td>
<td>MC</td>
<td>15</td>
<td>960 ± 100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3980 ± 412&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KO</td>
<td>CO</td>
<td>15</td>
<td>N.D.</td>
<td>440 ± 69</td>
</tr>
<tr>
<td>KO</td>
<td>MC</td>
<td>15</td>
<td>474 ± 80</td>
<td>4164 ± 560&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N.D., not detectable; KO, knockout.

<sup>a</sup> Statistically significant differences between vehicle and MC-treated mice at *P* < 0.05.
to 15 days (Fig. 1) suggests that MC elicited a persistent induction of CYP1A1 enzyme in mice. This finding was in agreement with our previous findings showing sustained induction of CYP1A enzymes by MC in the rat model (Moorthy et al., 1993; Moorthy, 2000). The lack of a persistent effect of MC on hepatic EROD activities in the CYP1A2-null mice (Fig. 1) provides strong evidence for an important role for CYP1A2 in the maintenance of CYP1A1 induction by MC.

That MC elicited induction of hepatic MROD activities in WT mice for up to 15 days (Fig. 2) supported the hypothesis that this PAH persistently induces CYP1A2 in mice. The very low activities of MROD in vehicle-treated CYP1A2-null animals was most likely due to the absence of a functional CYP1A2 in these mice.

Our finding that sustained induction of the enzyme activities in the WT animals was paralleled by persistent induction of the apoproteins for CYP1A1 and 1A2 (Fig. 3) supports the idea that the phenomenon of persistent induction of CYP1A1/1A2 activities is a result of long-term induction of their corresponding apoproteins. The decline of CYP1A1 apoprotein in the CYP1A2-deficient animals exposed to MC was in agreement with our results with regard to EROD activities, which also declined by day 15. The lack of CYP1A2 expression in the CYP1A2-null animals was expected as these animals are devoid of CYP1A2. The sustained increase in CYP1A2 by MC in WT mice supports the notion that MC also elicits persistent induction of CYP1A2 in mice, albeit to a lesser extent than CYP1A1.

Our Northern hybridization experiments showing sustained induction of hepatic CYP1A1 and 1A2 mRNAs by MC in the WT animals (Figs. 4–6) indicates that the persistent induction of CYP1A1 enzyme was preceded by prolonged up-regulation of the corresponding gene. The possible contribution of message stabilization to the persistent augmentation of CYP1A1/1A2 mRNA levels has not been excluded, and it is possible that post-transcriptional mechanisms may have also contributed to the sustained induction.

The substantial induction of hepatic and pulmonary CYP1A1 by MC at the 1-day time point in mice deficient in CYP1A2 was analogous to the findings of Dalton et al. (2000), who demonstrated that disruption of the CYP1A1 gene does not alter hepatic constitutive expression of other genes of the Ah gene battery. The marked suppression of hepatic CYP1A1 mRNA expression by MC in the CYP1A2-null animals (Figs. 4 and 5) at the later time points strongly suggests that attenuation of CYP1A1 expression in the CYP1A2-null animals was due to down-regulation at the transcriptional or post-transcriptional level. That the extent of decline of CYP1A1 mRNA (Figs. 4 and 5) in the MC-exposed CYP1A2-null animals at 15 days was much greater than that of CYP1A1 protein (Fig. 3; Table 1) may have been due to differences in their corresponding half-lives. Shiraki and Guengerich (1984) have reported the half-life of β-naphthoflavone-inducible CYP1A1 apoprotein in rats to be 37 h. On the other hand, the half-life of human CYP1A1 mRNA, which is conserved across species, is only 2.4 h (Lekas et al., 2000).

The observation that MC elicited sustained induction of EROD (CYP1A1) (Fig. 7) and apoprotein contents (Fig. 8) in the lungs of WT and CYP1A2-null animals suggested that this phenomenon is of relevance to carcinogenesis as lung is a target organ for carcinogenesis mediated by MC and other PAHs (Huggins et al., 1961; Rasmussen et al., 1984). The findings of Beebe et al. (1992) demonstrating a correlation between persistent induction of CYP1A1 enzyme in lungs of mice exposed to the tumor promoter Aroclor 1254 indicates the significance of sustained CYP1A1 induction to tumors...
genesis. We had earlier reported that MC did not elicit sustained induction in kidney, a non-target organ for PAH-mediated carcinogenesis (Li et al., 1992), suggesting that the prolonged induction in the lung may have important implications for human carcinogenesis.

Although the mechanisms underlying the role of CYP1A2 in the sustained induction of CYP1A1 in the liver have not yet been determined, we postulate the following mechanism. CYP1A2 might catalyze the formation of a MC metabolite, which may complex with the AHR and get targeted to the nucleus, wherein it could covalently bind to the AHR-ES on the CYP1A1 promoter and maintain CYP1A1 gene expression. We recently provided experimental evidence that MC, upon metabolic activation in vitro, yields metabolite(s) that covalently bound to plasmids containing CYP1A1 promoter, suggesting that some of the MC-DNA adducts are sequence-specific, being targeted toward the CYP1A1 promoter (Moorthy, 2002). Furthermore, we demonstrated that polymerase chain reaction amplification of sequences containing AHR-Es was inhibited in MC-treated but not vehicle-treated DNA, supporting the hypothesis that MC-DNA adducts were present on AHR-Es, resulting in inhibition of Taq polymerase activity (Moorthy, 2002). MC treatment did not inhibit polymerase chain reaction amplification of sequences that lacked the AHR-Es, lending further credence to the hypothesis that MC-DNA adducts were targeted to the AHR-Es of the CYP1A1 gene (Moorthy, 2002).

Our data pertaining to the uptake, distribution, and elimination of $[^{3}H]$MC in vivo has revealed rapid metabolism and clearance of the carcinogen from the rat (Moorthy et al., 1993; Moorthy, 2000). We found that only 0.94% of the administered dose is recovered in liver 1 day after termination of MC treatment, suggesting that MC is metabolized and eliminated rapidly from liver. Furthermore, the finding that of a total recovery of 93% greater than 92.3% of radioactivity is recovered in feces and urine suggests that MC is almost entirely excreted from the body and was not sequestered into different storage sites of the body (Moorthy et al., 1993; Moortho, 2000). Thus, mechanisms other than persistence of the parent compound were responsible for the sustained CYP1A1 induction by MC.

Recent studies have demonstrated a role for CYP1A2 as a hepatic binding protein (DeVito et al., 1998; Diliberto et al., 1999). Using CYP1A2 (−/−) mice, it has been shown that CYP1A2 protein mediates the sequestration of TCDD and polychlorinated dibenzofurans in liver (DeVito et al., 1998; Diliberto et al., 1999). It is unlikely that CYP1A2 contributed to the hepatic sequestration of MC in the WT animals by acting as a hepatic binding protein for MC (DeVito et al., 1998; Diliberto et al., 1999) because, if this were to be the case, then we would have also observed a persistent induction of hepatic CYP1A1 expression in the CYP1A2-null mice, as the lack of CYP1A2 in these mice would have led to increased intrahepatic concentration of MC, resulting in sustained CYP1A1 expression. This idea is supported by the studies of DeVito et al. (1998) demonstrating that CYP1A2 has no effect on the pharmacokinetic behavior of nonoestrogen-like compounds such as PCB 153.

The observation that MC elicited persistent induction of pulmonary CYP1A1 in WT as well as CYP1A2-null mice (Figs. 7 and 8) is intriguing. The mechanisms underlying the differential regulation of CYP1A1 expression by MC in the pulmonary and hepatic tissues of CYP1A2-null mice are not understood. Although a putative CYP1A2-catalyzed MC metabolite might have contributed to the sustained induction of CYP1A1 in the liver, it is conceivable that the metabolite responsible for the persistent effect in the lung may have been produced by a closely related isoform (e.g., CYP1A1). Alternatively, as yet unidentified tissue-specific factors may have contributed to the differential regulation of pulmonary and hepatic CYP1A1. That about 60-fold interindividual variation in hepatic CYP1A2 levels has been reported in humans (Smith et al., 2001) suggests that CYP1A2 expression may strongly influence the susceptibilities of humans to carcinogenesis mediated by environmental chemicals.

Regardless of the mechanism of persistent induction of CYP1A1/1A2 by MC, the phenomenon of sustained induction by MC may have significant implications for carcinogenesis by PAHs. First, since CYP1A1/1A2 are involved in the metabolic activation of PAHs to DNA-binding metabolites, exposure to a persistent inducer may modulate the genotoxic risk upon subsequent exposure to other genotoxic carcinogen(s). Second, persistent induction may enhance metabolism of PAHs, leading to generation through redox cycling of increasing amounts of reactive oxygen species that may elicit oxidative DNA damage (Ichinose et al., 1997). Third, as CYP1A2 enzymes are also involved in estrogen metabolism (Safe, 1995), persistent induction of CYP1A2 enzymes may enhance the production of estrogenic metabolites, some of which are tumor promoters.

In conclusion, the results of the present study strongly suggest that hepatic CYP1A2 plays a critical role in the sustained induction of CYP1A1 by MC in liver, but not lung. The recent study of Dey et al. (1999) suggesting an important role for CYP1A1 in regulating critical life processes warrants further in-depth investigations to elucidate the molecular mechanisms of persistent CYP1A1/1A2 induction, a phenomenon that potentially has important implications for carcinogenesis as well as other important physiological processes.

Acknowledgments

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Address correspondence to: Dr. Bhagavatula Moorthy, Associate Professor of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. E-mail: bmoorthy@bcm.tmc.edu