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PERSISTENT INDUCTION OF CYTOCHROME P450 (CYP)1A ENZYMES BY 3-METHYLCHOLANTHRENE *IN VIVO* IN MICE IS MEDIATED BY SUSTAINED TRANSCRIPTIONAL ACTIVATION OF THE CORRESPONDING PROMOTERS

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Abstract

There is significant human exposure to polycyclic aromatic hydrocarbons (PAHs), many of which are potent carcinogens. Cytochrome P450 (CYP)1A enzymes play key roles in the metabolic activation of PAHs to carcinogenic metabolites. We previously showed persistent induction of CYP1A enzymes by 3-methylcholanthrene (MC) *in vivo* in rodents. In this study, we tested the hypothesis that MC elicits persistent induction of CYP1A1 and 1A2 *in vivo* by mechanisms entailing sustained transcriptional activation of the corresponding promoters. Adult male wild type (WT) (Cd-1) mice, transgenic mice expressing the human CYP1A1 promoter or the mouse CYP1A2 promoter were treated with the vehicle corn oil (CO) or the carcinogenic PAH, 3-methylcholanthrene (MC), once daily for 4 days, and luciferase reporter gene expression was determined at 1, 8, 15, and 22 days after MC withdrawal by bioluminescent imaging. Pulmonary and hepatic endogenous expression of CYP1A1 and 1A2 was also determined at the enzymatic, protein, and mRNA levels. The major findings were that MC elicited marked enhancement in the luciferase expression in the CYP1A1-luc as well CYP1A2-luc transgenic mice that was sustained for up to 22 days, the magnitude of induction being more pronounced in the CYP1A1-luc mice. MC also caused persistent induction of endogenous CYP1A1 and 1A2 expression in the WT, CYP1A1-luc, and 1A2-luc mice for up to 22 days. In conclusion, our results support the hypothesis that MC elicits sustained CYP1A1 and 1A2 expression by sustained transcriptional activation of the corresponding promoters. Thus, these novel transgenic models should be very useful for further understanding of the molecular mechanisms of persistent CYP1A induction, in relation to PAH-mediated carcinogenesis.

Keywords

Cytochrome P4501A1; CYP1A2; promoter; luciferase reporter; *in vivo*; mice; gene regulation; 3-methylcholanthrene; transgenic

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Introduction

3-Methylcholanthrene (MC) is one of the most potent polycyclic aromatic hydrocarbon (PAH) carcinogens ever known [1]. Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental contaminants, occurring as by-products of combustion processes such as coke ovens, cigarette smoke, diesel exhausts, and charcoal broiled meats, and many PAHs are suspected carcinogens [2]. Metabolism of MC by cytochrome P450 (P450) (CYP)1 enzymes leads to the formation of highly reactive intermediates that can bind covalently to DNA, a critical step in the initiation of carcinogenesis [3,4].

The CYP1A family comprises two proteins, CYP1A1 and 1A2, which are inducible by a number of chemicals, including MC and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and MC [5–11]. The molecular mechanisms by which MC or TCDD transcriptionally activate the CYP1A1 gene entail the binding of the ligand to the Ah-receptor (AHR), a cytosolic protein. The ligand-bound AHR complex translocates into the nucleus, where it dimerizes with the Ah receptor nuclear translocator (ARNT), and this heterodimer binds to aryl hydrocarbon responsive elements (AHREs), which are located in enhancer regions of CYP1A1, resulting in augmented expression of the CYP1A1 gene [12–16].

Although the molecular mechanisms of CYP1A1 induction have been investigated extensively, little is known regarding the decline of drug-metabolizing enzymes to pre-induction levels after termination of xenobiotic exposure. In rats, a single dose of MC (20–25 mg/kg) induces aryl hydrocarbon hydroxylase activity [5] and CYP1A1 mRNA expression [15] after 24 h, followed by a return of enzyme activity and mRNA levels within 5–8 days [17,18]. In mice, a single dose of MC (80 mg/kg) increases total P450 content and aryl hydrocarbon hydroxylase activities in mice for up to 9 days [19]. DePierre et al. [20] showed induction of total hepatic CYP contents that persists for 2 to 3 weeks.

We reported earlier that MC elicits a long-term induction of hepatic CYP1A enzymes in the rat for up to 45 days [21], and that the phenomenon of sustained induction is mediated by mechanisms other than the persistence of the parent MC [7]. We also reported that CYP1A2 plays an important role in the persistent induction of CYP1A1 by MC [8,9,22]. Recently, we demonstrated that MC causes coordinated induction of phase II enzymes by persistent activation of the AHR [23]. We also provided evidence for the presence of sequence-specific DNA adducts in the CYP1A1 promoter region, which might play a role in the regulation of CYP1A1 gene expression [24]. However, the mechanisms of persistent induction of CYP1A1 and 1A2 *in vivo* are not well understood. In this study, we tested the hypothesis that MC elicits persistent induction of CYP1A1 and 1A2 *in vivo* by mechanisms entailing sustained transcriptional activation of the corresponding promoters.

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). Polyvinylidene difluoride 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 was obtained from Bio-Rad Laboratories.

Animal studies and treatment protocol—Transgenic mice expressing the human 13.2 Kb CYP1A1 promoter to drive luciferase expression (CYP1A1-luc) or mice expressing the

8.4 kb mouse Cyp1a2 promoter (CYP1A2-luc) on CD-1 background were generated via pronuclear microinjection using Crl:CD-1 strain oocytes, and were obtained from Xenogen Corporation (Alameda, CA) [25]. Twelve week-old male wild type (WT) (CD-1), CYP1A1-luc, or CYP1A2-luc mice were treated with MC (100 μ mol/kg, i.p.) dissolved in corn oil (CO) (2 ml/kg), or CO alone as control once daily for 4 days, and mice were sacrificed at 1, 8, 15, or 22 days after CO or MC withdrawal. At least four animals were used for each group.

Bioluminescent imaging—The substrate luciferin was administered into the peritoneal cavity at a dose of 150 mg/kg (20 mg/ml) approximately 5 min prior to imaging. Mice were anesthetized with isoflurane/oxygen and placed on the imaging station. Ventral and dorsal images were collected for 1 second using the IVIS imaging system 100 (Xenogen Corporation) [25], and luciferase expression was determined *in vivo* by bioluminescent imaging (Xenogen Corporation (Alameda, CA) [25]. Photons emitted from the liver region were quantified using Series Livingimage software.

Enzyme assays—Ethoxyresorufin-O-deethylase (EROD) (CYP1A1) and methoxyresorufin O-deethylase (MROD) activities in the liver and lung microsomes were assayed essentially as described previously [7,8,26].

Electrophoresis and Western Blotting

Liver or lung microsomes (~ 20 μ g) were subjected to SDS-polyacrylamide gel electrophoresis in 10% acrylamide gel followed by Western blotting to detect CYP1A1 and 1A2 proteins, as reported in the recent articles from our laboratory [7,8,26].

Real-time reverse transcriptase polymerase chain reaction (RT-PCR)—Total RNA (500 ng) from MC treated HepG2 cells and DMSO treated controls was subjected to one step real time quantitative TaqMan RT-PCR. ABI PRISM 7700 Sequence Detection System was used for the RT-PCR reactions. Gene-specific primers in the presence of TaqMan reverse transcription reagents and RT reaction mix (Applied Biosystems) were used to reverse transcribe RNA, and TaqMan Universal PCR Master Mix and Assays-on-Demand Gene Expression probes (Applied Biosystems) were used for PCR amplification, as reported earlier [26,27]. The relative mRNA levels for P4501A1 were normalized to their 18S content, and relative expression levels of CYP1A1 and 1A2 genes were calculated as reported earlier [26, 27].

Statistical analyses—All statistical analyses were conducted by using Student's t-test or by two way ANOVA, followed by modified t-tests.

Results

Effect of MC on CYP1A1/1A2 promoter activation (luciferase reporter expression)

The major goal of this investigation was to investigate the mechanisms of persistent induction of CYP1A1 and 1A2 by MC *in vivo*. As shown in figure 1A–D MC treatment resulted in marked induction of CYP1A1- as well as CYP1A2-promoter driven luciferase expression *in vivo*. The induction was persistent for up to 22 days. CYP1A1-luc expression in the MC-treated mice was augmented by 15-, 10-, 7-, and 0.6-fold over controls at the 1, 8, 15, and 22 day time point, respectively (Figure 1B). In contrast, MC caused persistent CYP1A2-luc induction to a lesser extent than CYP1A1-luc. MC caused elevated CYP1A2-luc expression by 5-, 2.4-, 1.4-, and 0.6-fold increase over control at 1, 8, 15, and 22 day time points, respectively (Figure 1C,D).

Effect of MC on endogenous CYP1A1/1A2 enzyme activities—In order to determine if transgenic mice carrying the human or mouse CYP1A promoters will alter the modulation

of endogenous expression of CYP1A1 and/or 1A2 expression, we determined the activities of EROD and MROD, reflecting catalytic functionalities of CYP1A1 and 1A2, respectively (Figure 2). As shown in figure 2A, MC elicited a 14-fold induction of hepatic EROD activities in the WT (CD-1) mice at the 1 day time point. EROD activities declined by 15 days, albeit statistically significant increases were noticed at this time point. In the transgenic mice carrying the human CYP1A1 promoter, MC elicited marked induction of EROD activities at the 1 day time point (Figure 2A). The extent of induction was about 11-fold, being about 22% lesser than that observed in WT animals (Figure 2A). Similar to the WT mice, the induction of EROD activities declined by day 15 (Figure 2A). In mice carrying the CYP1A2 promoter, the extent of induction of hepatic EROD by MC was not different from that observed in the WT mice (Figure 2A).

MC also caused marked augmentation of hepatic MROD activities in the WT mice, being 9-, 3-, and 1-fold greater than corresponding controls at the 1, 8, and 15 day time points, respectively (Figure 2B). The EROD activities at the 22 day time point were similar to controls. In transgenic mice carrying the CYP1A1 or 1A2 luciferase constructs, the extent of induction of hepatic MROD by MC was markedly decreased (~ 4-fold at the 1 or 8 day time point). The EROD activities declined by 15 day in the transgenic mice, irrespective of whether they carried the CYP1A1-luc or the CYP1A2-luc (Figure 2B).

MC also elicited a robust persistent induction of EROD activities in the lungs of WT mice, being 380-, 370-, 180-fold at the 1, 8, and 15 day time points over vehicle controls (Figure 2C). This was in contrast to the liver, wherein the induction declined by day 15 (Figure 2A and B). The pulmonary EROD induction declined to control levels by day 22 (Figure 2C). In transgenic mice carrying the CYP1A1 promoter, MC caused induction of EROD to the extents that were similar to those observed in WT mice at the 1 or 8 day time points (Figure 2C). Similar to WT mice, MC elicited sustained induction of EROD activities in the CYP1A1-luc mice for up to 22 days. In mice carrying the CYP1A2-luc construct, MC caused significant induction of pulmonary EROD activities, being 400-, 320-, 280-, and 60-fold greater than the corresponding controls at the 1, 8, 15, and 22 days, respectively (Figure 2C).

Effect of MC on CYP1A1/1A2 apoprotein expression

In order to determine if persistent induction of EROD and MROD enzyme activities were paralleled by concomitant increases in the corresponding apoprotein contents of CYP1A1 and 1A2, we conducted Western blotting experiments using liver and lung microsomes from control and MC-treated WT, CYP1A1-luc, and CYP1A2-luc mice. As shown in figure, MC caused significant induction of hepatic CYP1A1 expression in the WT, CYP1A1-luc, and CYP1A2-luc mice at the 1 and 8 day time points (Figure 3A). Similar to EROD activities, the CYP1A1 apoprotein expression declined by 15 days (Figure 3A). MC also caused significant induction of CYP1A2 expression in WT as well as transgenic mice, which persisted for up to 15 days (Figure 3A). In lungs, MC caused persistent induction for up to 15 days in the WT mice and for up to 22 days in CYP1A1-luc and CYP1A2-luc mice (Figure 3B), correlating with EROD activities.

Effect of MC on CYP1A1 and 1A2 mRNA expression

Our goal was to determine if induction of CYP1A1 and 1A2 protein expression was preceded by corresponding augmentation of their mRNAs, as determined by real time RTPCR. In WT mice, MC caused a 3,000-, 1,000-, 50-fold induction of hepatic CYP1A1 mRNA levels at the 1, 8, and 15 day time points, respectively (Figure 4A). In CYP1A1-luc mice, MC caused a much greater extent of induction, being 5400-, 3100-, and 50-fold more than control at the 1, 8, and 15 days, respectively (Figure 4A). In contrast, in the CYP1A2-luc mice, the degree of induction was less than that seen in WT or CYP1A1-luc mice, being 1750-, 750-, and 180-

fold, respectively at the 1, 8, and 15 day time points, respectively (Figure 4A). The induction declined at the 22 day time point (Figure 4A).

MC elicited induction of hepatic CYP1A2 mRNA expression, but the extent of induction was several orders of magnitude less than that of CYP1A1 (Figure 4B). In WT mice, MC caused a 11-, 6-, and 1.2-fold enhancement of CYP1A2 mRNA expression at the 1, 8, and 15 day time points, respectively (Figure 4B). Similar trends were observed in the CYP1A1-luc and CYP1A2-luc mice (Figure 4B).

Similar to our observations in liver, MC caused a much greater induction in the levels of pulmonary CYP1A1 mRNA in the CYP1A1-luc compared to that of WT mice (Figure 4C). In WT mice, MC-treated samples showed an induction of 50-, 37-, and 10-fold compared to controls at the 1, 8, and 15 day time points, respectively, whereas in the CYP1A1-luc mice the fold induction was 95-, 65-, 10-, and 9 at the 1, 8, 15, and 22 day time points, respectively (Figure 4C). In CYP1A2-luc mice, the results were similar to that observed in liver in that the extent of induction was lesser compared to WT mice, the fold induction being 32-, 28-, and 5-fold, respectively (Figure 4C).

Discussion

In the present study, we investigated the mechanisms of sustained CYP1A induction by MC and tested the hypothesis that persistent induction of CYP1A1 and 1A2 gene expression by MC is mediated by sustained transcriptional activation of the corresponding promoters *in vivo*.

Our results showing marked induction of luciferase expression in transgenic mice expressing CYP1A1-luc or 1A2-luc (Figure 1A–1D), which persisted for up to day 22, supported the hypothesis that MC elicited persistent CYP1A induction by sustained transcriptional activation of the corresponding promoters. The observation that MC elicited greater degree induction of luciferase expression in the CYP1A1-luc mice compared to that of CYP1A2-luc mice was consistent with the previous reports from other laboratories [28,29] including our own [7] that PAHs induce CYP1A1 to a greater extent than CYP1A2. In fact, our observations in this study show for the first time that persistent transcriptional activation of the CYP1A promoters occurs *in vivo*. Thus, these transgenic models can be very useful in determining the mechanisms of persistent induction of CYP1A *in vivo*.

The finding that MC caused marked increases of hepatic EROD (Figure 2A) and MROD activities (Figure 2B) in the WT mice indicated induction CYP1A1 and 1A2 expression, as EROD and MROD activities are known to reflect CYP1A1 and 1A2 activities, respectively [26]. The fact that induction was still noticed at day 15 was consistent with our earlier published findings that MC causes persistent induction of CYP1A1 and 1A2 in C57BL/6J mice [26].

The fact that MC elicited prolonged induction of the endogenous CYP1A1 and 1A2 activities in the transgenic mice suggests that introduction of the promoters into the mice did not affect the phenomenon of persistent induction, and that the cellular and molecular properties that influence gene expression of the CYP1A-luc mice and the endogenous CYP1A genes are similar [16]. Similar results were reported by Operana [28].

Our observation that MC also caused persistent induction of CYP1A1 and 1A2 apoproteins in liver (Figure 3A–B) CYP1A1 alone in the WT and transgenic mice was in agreement with our enzyme activity data [7,26]. The results in Figure 4A showing a robust induction of hepatic CYP1A1 mRNA in the WT mice was consistent with the hypothesis that MC induced CYP1A1 activity and protein expression by inducing its message [7,26]. Our observation that MC caused a much higher fold induction of CYP1A1 mRNA in the livers and lungs of CYP1A1-luc mice

was probably due to increased accessibility of the MC-AHR-ARNT complex and other co-activators to the endogenous CYP1A1 enhancer elements, presumably through alteration in chromatin remodeling as a result of incorporation of the CYP1A1 promoter transgene. But the contrasting results in regard to CYP1A1 or 1A2 induction in the CYP1A2-luc mice may have been due to decreased accessibility of the MC-AHR-ARNT complex to regions of the CYP1A1/1A2 enhancer(s) that are responsible for induction of the CYP1A1 or 1A2 mRNA (Figure 4A,B).

Earlier we observed in *in vitro* experiments entailing incubation of liver microsomes from wild type mice, MC, NADPH, and plasmids carrying CYP1A1 promoter, followed by ³²P-postlabeling, that some MC-DNA adducts are targeted to the CYP1A1 promoter region [24]. The adducts were dramatically decreased when incubations were conducted using microsomes from Cyp1a2-null mice, suggesting that CYP1A2 might have possibly catalyzed the formation of an MC metabolite, which may have complexed with the AHR and got translocated to the nucleus, wherein it covalently bound to AHREs on the CYP1A1 gene promoter and maintained CYP1A1 gene expression *in vivo* [7,22,24]. In fact, our major finding was that CYP1A2 plays a key role in the sequence-specific formation of DNA adducts on the CYP1A1 promoter that may in turn modulate gene expression. It is also possible that DNA adducts *in vivo* were formed in the regions containing negative regulatory elements [24], a phenomenon that may have contributed to persistent CYP1A1 induction.

Alternatively, persistent CYP1A1 induction by MC may have occurred through DNA damage response of other genes [29] involved in the downregulation of CYP1A1 expression, resulting in sustained expression of CYP1A1. Another plausible mechanism of the sustained induction by MC in the absence of direct binding of the AHR may have been through the action of tyrosine kinases, as has been postulated for the induction of CYP1A1 and 1A2 by the proton pump inhibitor, omeprazole [30] or via cAMP-dependent protein kinase A signaling of the AHR pathway [31]. Since recent work has shown the involvement of promoter methylation [32] and histone modifications in the chromatin [33] to contribute to CYP1A1 induction, it is tempting to speculate that these epigenetic mechanisms may have contributed to the sustained CYP1A1 induction.

Regardless of the mechanism of persistence induction of CYP1A1 by MC, this phenomenon may have important implications for human carcinogenesis by PAHs, as CYP1A1 appears to play a critical role in MC-mediated pulmonary carcinogenesis, based upon evidence that very few lung tumors are formed in Cyp1a1-null mice [34].

In conclusion, these results support the hypothesis that persistent induction of CYP1A1 and 1A2 *in vivo* by MC was mediated by sustained transcriptional activation of the corresponding gene promoters, a phenomenon that may be of relevance to carcinogenesis mediated by PAHs.

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Abbreviations

CYP, cytochrome P450
AHR, Ah receptor
EROD, ethoxyresorufin O-demethylase
ANOVA, analyses of variance

AHREs, Ah response elements
 ARNT, Ah receptor nuclear translocator
 SDS-PAGE, SDS-polyacrylamide gel electrophoresis
 MC, 3-methylcholanthrene
 PAH, polycyclic aromatic hydrocarbons
 RT-PCR, reverse transcriptase-Polymerase chain reaction
 TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

References

- [1]. Harvey RG. Polycyclic aromatic hydrocarbons and cancer. *Am. Sci* 1982;70:386–393. [PubMed: 7149435]
- [2]. Cooper, CS.; Grover, PL., editors. *Handbook of Experimental Pharmacology*. Springer Verlag; New York: 1990. Hemminki, Environmental carcinogens; p. 33-61.
- [3]. Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* 1988;48:2946–2954. [PubMed: 3284639]
- [4]. Xu M, Nelson GB, Moore JE, McCoy TP, Dai J, Manderville RA, Ross JA, Miller MS. Induction of Cyp1a1 and Cyp1b1 and formation of DNA adducts in C57BL/6, Balb/c, and F1 mice following in utero exposure to 3-methylcholanthrene. *Toxicol. Appl. Pharmacol* 2005;209:28–38. [PubMed: 15885734]
- [5]. Poland A, Glover E. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol* 1974;10:349–359. [PubMed: 4851117]
- [6]. Postlind H, Vu TP, Tukey RH, Quattrochi LC. Response of human CYP1-luciferase plasmids to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polycyclic aromatic hydrocarbons. *Toxicol. Appl. Pharmacol* 1993;118:255–262. [PubMed: 8442004]
- [7]. Moorthy B. Persistent expression of 3-methylcholanthrene-inducible cytochromes P4501A in rat hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther* 2000;294:313–322. [PubMed: 10871328]
- [8]. Kondraganti SR, Jiang W, Moorthy B. Differential regulation of expression of hepatic and pulmonary cytochrome P4501A enzymes by 3-methylcholanthrene in mice lacking the CYP1A2 gene. *J. Pharmacol. Exp. Ther* 2002;303:945–951. [PubMed: 12438513]
- [9]. Moorthy, B. The CYP1A Subfamily. In: Ioannides, C., editor. *Role of Cytochromes P450 in Drug Metabolism and Toxicity of Drugs and other Xenobiotics*. RSC Publishing; Cambridge: 2008. p. 97-135.
- [10]. Conney AH. Induction of microsomal cytochrome P-450 enzymes: the first Bernard Brodie lecture at Pennsylvania State University. *Life Sci* 1986;39:2493–2518. [PubMed: 3540505]
- [11]. Kawajiri, K.; Nakachi, K.; Imai, K.; Hayashi, S.; Watanabe, J. Individual differences in lung cancer susceptibility in relation to polymorphisms of P-4501A1 gene and cigarette dose. Ernstner, L.; Gelboin, HV.; Sugimura, T., editors. *Xenobiotics and Cancer Japan Society*; London: 1991. p. 55-61.
- [12]. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J. Biol. Chem* 2004;279:23847–23850. [PubMed: 15028720]
- [13]. Fujii-Kuriyama Y, Mimura J. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem. Biophys. Res. Commun* 2005;338:311–317. [PubMed: 16153594]
- [14]. Nukaya M, Moran S, Bradfield CA. The role of the dioxin-responsive element cluster between Cyp1a1 and Cyp1a2 loci in aryl hydrocarbon receptor biology. *Proc. Natl. Acad. Sci. USA* 2009;106:4923–4928. [PubMed: 19261855]
- [15]. Wang S, Hankinson O. Functional involvement of the Brahma/SWI2-related gene I protein in cytochrome P4501A1 transcription mediated by the aryl hydrocarbon receptor complex. *J. Biol. Chem* 2002;277:11821–11827. [PubMed: 11805098]

- [16]. Galijiatovic A, Beaton D, Ngyuyen N, Chen S, Bonzo J, Johnson R, Maeda S, Karin M, Guengerich FP, Tukey RH. The human CYP1A1 gene is required is regulated in a developmental and tissue-specific fashion in transgenic mice. *J. Biol. Chem* 2004;279:23969–23976. [PubMed: 15037607]
- [17]. Bresnick E, Brosseau M, Levin W, Reik L, Ryan DE, Thomas PE. Administration of 3-methylcholanthrene to rats increases the specific hybridizable mRNA coding for cytochrome p-450c. *Proc. Natl. Acad. Sci. USA* 1987;78:4083–4087. [PubMed: 6945571]
- [18]. Masaki R, Matsuura S, Tashiro Y. A biochemical and electronic microscopic study of changes in the content of cytochrome P-450 in rat livers after cessation of treatment with phenobarbital, β -naphthoflavone or 3-methylcholanthrene. *Cell Struct. Funct* 1984;9:53–66. [PubMed: 6373027]
- [19]. Boobis AR, Nebert DW, Felton JS. Comparison of beta-naphthoflavone and 3-methylcholanthrene as inducers of hepatic cytochrome(s) P-448 and aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity. *Mol. Pharmacol* 1997;13:259–268. [PubMed: 854023]
- [20]. DePierre JW, Lundqvist G, Ernster L. Return of drug-metabolizing systems to control levels after induction with 3-methylcholanthrene. *Acta. Chem. Scand. B* 1982;36:497–498. [PubMed: 6815948]
- [21]. Moorthy B, Chen S, Li D, Randerath K. 3-methylcholanthrene-inducible liver cytochrome(s) P450 in female Sprague-Dawley rats: possible link between P450 turnover and formation of DNA adducts and I-compounds. *Carcinogenesis* 1993;14:879–886. [PubMed: 8504481]
- [22]. Moorthy B. 3-Methylcholanthrene-inducible hepatic DNA adducts: a mechanistic hypothesis linking sequence-specific DNA adducts to sustained cytochrome P4501A1 induction by 3-methylcholanthrene. *Redox. Rep* 2002;7:9–13. [PubMed: 11981449]
- [23]. Kondraganti SR, Jiang W, Jaiswal AK, Moorthy B. Persistent induction of hepatic and pulmonary phase II enzymes by 3-methylcholanthrene in rats. *Toxicol. Sci* 2008;102:337–344. [PubMed: 18203689]
- [24]. Moorthy B, Muthiah K, Fazili IS, Kondraganti SR, Wang L, Couroucli XI, Jiang W. 3-Methylcholanthrene elicits DNA adduct formation in the CYP1A1 promoter region and attenuates reporter gene expression in rat H4IIE cells. *Biochem. Biophys. Res. Commun* 2007;354:1071–1077. [PubMed: 17276403]
- [25]. Zhang W, Moorthy B, Chen M, Muthiah K, Coffee R, Purchio AF, West D. A CYP1a2-luciferase transgenic CD-1 mouse model: Responses to aryl hydrocarbons similar to the humanized AhR mice. *Toxicol Sci* 2004;82:297–307. [PubMed: 15329440](2004)
- [26]. Kondraganti S, Jiang W, Moorthy B. Differential regulation of expression of hepatic and pulmonary cytochrome P4501A enzymes by 3-methylcholanthrene in mice lacking the CYP1A2 gene. *J Pharmacol Exp Ther* 2002;303:945–951. [PubMed: 12438513]
- [27]. Jiang W, Welty SE, Couroucli XI, Barrios R, Kondraganti SR, Muthiah K, Yu L, Moorthy B. Disruption of the Ah receptor alters the susceptibility of mice to oxygen-mediated regulation of pulmonary and hepatic cytochrome P4501A expression and exacerbates hyperoxic lung injury. *J. Pharmacol. Exp. Ther* 2004;310:512–519. [PubMed: 15123765]
- [28]. Operana TN, Nguyen N, Chen S, Beaton D, Tukey RH. Human CYP1A1^{GFP} expression in transgenic mice serves as a biomarker for environmental toxicant exposure. *Toxicol. Sci* 2007;95:98–107. [PubMed: 17065433]
- [29]. Hockley SL, Arlt VM, Brewer D, Poele RT, Workman P, Giddings I, Phillips DH. AHR- and DNA-damage-mediated gene expression responses induced by benzo(a)pyrene in human cell lines. *Chem. Res. Toxicol* 2007;20:1797–1810. [PubMed: 17944540]
- [30]. Yoshinari K, Ueda R, Kusano K, Yoshimura T, Nagata K, Yamazoe Y. Omeprazole transactivates human CYP1A1 and CYP1A2 expression through the common regulatory region containing multiple xenobiotic-responsive elements. *Biochem. Pharmacol* 2008;76:139–145. [PubMed: 18502397]
- [31]. Oesch-Bartimowicz B, Oesch F. Role of cAMP in mediating AHR signaling. *Biochem. Pharmacol* 2009;77:627–641. [PubMed: 19013136]
- [32]. Wu L, Whitlock JP. Mechanism of dioxin action: Ah receptor-mediated increase in promoter accessibility *in vivo*. *Proc. Natl. Acad. Sci. USA* 1992;89:4811–4815. [PubMed: 1317573]

- [33]. Schnekenburger M, Peng L, Puga A. HDAC1 bound to the *Cyp1a1* promoter blocks histone acetylation associated with Ah receptor-mediated *trans*-activation. *Biochem. Biophys. Acta* 2007;1769:569–678. [PubMed: 17707923]
- [34]. Moorthy B, Wang L, Fazili I, Kondraganti S, Zhou G, Jiang W. Mice deficient in gene for cytochrome P450 (CYP) 1A1 or 1A2 display differential susceptibilities to CYP1A induction and lung tumorigenesis by 3-methylcholanthrene (MC). *Proc. Am. Assoc. Cancer Res* 2009;288 (Abstract #1202)

Figure 1.

Bioluminescent imaging of CYP1A1-luc (A, B) or CYP1A2-luc (C,D) mice following MC treatment. Adult male CYP1A1-luc (A) or 1A2-luc mice (C) were treated with CO or MC, and luciferase expression was analyzed by bioluminescent imaging in real time at 1, 8, 15, or 22 days after MC or CO withdrawal. Quantitation of bioluminescent imaging data of CYP1A1-luc (B) or 1A2-luc (D) mice was conducted using IVIS imaging software. Values represent mean $\pm$ S.E. (n = 5). *, Statistically significant differences between MC- and CO-treated mice at P < 0.05, as determined by two-way ANOVA.

Figure 2.

Effect of MC on endogenous hepatic EROD (CYP1A1) (A), MROD (B), and pulmonary EROD activities (C). Adult male WT (CD-1), CYP1A1-luc or CYP1A2-luc mice were treated with CO or MC, and hepatic EROD (A), MROD (B), and pulmonary EROD activities (C) were determined in the liver microsomes at 1, 8, 15, or 22 days after MC withdrawal. Data represent mean $\pm$ SE of fold induction versus controls from at least 4 individual animals.

Figure 3.

Representative Western blot showing effect of MC on endogenous hepatic CYP1A1 and 1A2 (A) and pulmonary CYP1A1 protein (B) expression in mice. Adult male WT (CD-1), CYP1A1-luc or CYP1A2-luc mice were treated with CO or MC, and CYP1A1/1A2 apoprotein expression was analyzed in the microsomes (20 μ g) at the indicated time points by Western blotting.

Figure 4.

Effect of MC on endogenous hepatic CYP1A1 (A), CYP1A2 (B), and pulmonary mRNA (C) in mice. Adult male WT (CD-1), CYP1A1-luc or CYP1A2-luc mice were treated with CO or MC, and hepatic CYP1A1 (A), CYP1A2 (B), and pulmonary CYP1A1 (C) mRNA levels were determined by real time RT-PCR at 1, 8, 15, or 22 days after MC withdrawal. Data represents mean $\pm$ S.E. of fold induction versus controls from at least four individual animals. *, Statistically significant differences between MC- and DMSO-treated cells at $P < 0.05$, as determined by two-way ANOVA.