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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

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Abstract

Fetal mice are more sensitive to chemical carcinogens than are adults. Previous studies from our laboratory demonstrated differences in the mutational spectrum induced in the Ki-*ras* gene from lung tumors isolated from [D2 × B6D2F1]F2 mice and Balb/c mice treated in utero with 3-methylcholanthrene (MC). We thus determined if differences in metabolism, adduct formation, or adduct repair influence strain-specific responses to transplacental MC exposure in C57BL/6 (B6), Balb/c (BC), and reciprocal F1 crosses between these two strains of mice. The induction of *Cyp1a1* and *Cyp1b1* in fetal lung and liver tissue was determined by quantitative fluorescent real-time PCR. MC treatment caused maximal induction of *Cyp1a1* and *Cyp1b1* RNA 2–8 h after injection in both organs. RNA levels for both genes then declined in both fetal organs, but a small biphasic, secondary increase in *Cyp1a1* was observed specifically in the fetal lung 24–48 h after MC exposure in all four strains. *Cyp1a1* induction by MC at 4 h was 2–5 times greater in fetal liver (7000- to 16,000-fold) than fetal lung (2000- to 6000-fold). *Cyp1b1* induction in both fetal lung and liver was similar and much lower than that observed for *Cyp1a1*, with induction ratios of 8- to 18-fold in fetal lung and 10- to 20-fold in fetal liver. The overall kinetics and patterns of induction were thus very similar across the four strains of mice. The only significant strain-specific effect appeared to be the relatively poor induction of *Cyp1b1* in the parental strain of B6 mice, especially in fetal lung tissue. We also measured the levels of MC adducts and their disappearance from lung tissue by the P^{32} post-labeling assay on gestation days 18 and 19 and postnatal days 1, 4, 11, and 18. Few differences were seen between the different strains of mice; the parental strain of B6 mice had nominally higher levels of DNA adducts 2 (gestation day 19) and 4 (postnatal day 1) days after injection, although this was not statistically significant. These results indicate that differences in Phase I metabolism of MC and formation of MC-DNA adducts are unlikely to account for the marked differences observed in the Ki-*ras* mutational spectrum seen in previous studies. Further, the results suggest that other genetic factors may interact with chemical carcinogens in determining individual susceptibility to these agents during development.
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Keywords: Fetus; *Cyp1a1*; *Cyp1b1*; 3-methylcholanthrene; Lung tumors; Transplacental carcinogenesis; DNA adducts

Abbreviations: AHH, aryl hydrocarbon hydroxylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; RAL, relative adduct labeling; *Pas*, pulmonary adenoma susceptibility; RT-PCR, reverse transcription polymerase chain reaction.

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Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States in both men and women (Jemal et al., 2005). The association between cigarette smoking (Loeb et al., 1984; Thun et al., 2002), air pollution (Dockery et al., 1993), and lung cancer incidence suggest that gene-environmental interactions strongly influence individual susceptibility for the risk of lung cancer (Idle, 1991; Minna et al., 1988; Perera et al., 1996). While the association between exposures to environmental toxicants and lung cancer induction in adults has been well documented, the effects of in utero exposures to these agents are still uncertain (Anderson et al., 2000).

Following exposure to PAHs, drug metabolic enzymes are induced to metabolically detoxify and eliminate the parent PAHs. During the course of metabolism, PAHs are also converted to reactive electrophiles capable of binding to and damaging DNA. Both murine and human tissues utilize the same metabolic pathways to produce the same types of adduct profiles following exposure to PAHs and metabolism by CYP1A1 (Gonzalez, 1988; Jaiswal et al., 1985; Randerath et al., 1986; Wheeler et al., 1990). Murine lung adenocarcinomas resemble human lung adenocarcinomas in terms of histology, etiology, and some of the genetic lesions that are prevalent in the tumors of both species (Dragani et al., 1995; Malkinson, 1989; Witschi, 1991), including mutations in Ki-*ras* and alterations in expression of members of the *Ink4a*/*Rb*/cyclin D1 pathway (Malkinson, 1992, 1998; Miller, 2004). Like their human counterparts, murine lung adenocarcinomas arise from cells of peripheral origin, and several lines of evidence suggest that they may be derived from alveolar type II and/or Clara cells (Malkinson, 1992; Malkinson et al., 1997). Thus, similar mechanisms of tumor pathogenesis are utilized in the two species, making the mouse an excellent model in which to study the interaction between environmental and genetic factors in lung tumorigenesis (Dragani et al., 1995; Malkinson, 1989, 1992; Miller, 1994; Witschi, 1991).

Several studies utilizing in utero exposure protocols have shown that the developing fetus displays a higher sensitivity toward certain chemical and physical carcinogens than do adult animals, suggesting that exposure of the pregnant mother to environmental toxicants may place the fetus at high risk for the development of cancer (Anderson et al., 2000; Miller, 2004; Rice, 1979). Studies by several laboratories have shown that the developing fetus exhibits limited drug metabolic activity (Anderson et al., 1989; Hines and McCarver, 2002; McCarver and Hines, 2002; Miller, 1994; Miller et al., 1996). In general, the fetus lacks expression of many forms of CYP found in adults and, in response to exposure to PAHs, demonstrate a marked induction of *Cyp1a1* in the absence of a similar induction of detoxifying Phase II enzymes. It has thus been proposed that this differential induction of activating Phase I enzymes may be one of the factors that accounts for the enhanced sensitivity of the fetus to chemical carcinogens (Miller, 1994; Miller et al., 1996).

We and others have shown that treatment of pregnant mice with MC results in a high incidence of lung tumors in the offspring 9 to 13 months following in utero exposure to a single dose of the carcinogen (Anderson et al., 1985; Miller et al., 1998; Wessner et al., 1996). In this animal model, the C57BL/6 (B6) and DBA/2 (D2) strains of mice were used because of their differences in inducibility for *Cyp1a1* (Gielen et al., 1972; Thomas et al., 1972). The B6 strain contain the dominant Ah^b allele, which codes for the wild type *Ah* receptor mediating PAH-responsive induction of *Cyp1a1*. D2 mice, on the other hand, contain the defective, nonresponsive Ah^d allele and fail to up-regulate expression of *Cyp1* family members following exposure to PAHs (Chang et al., 1993; Okey et al., 1989). A backcross between inducible F1 mice (B6D2F1) containing the hybrid genotype (Ah^bAh^d) and the noninducible D2 strain yields a second generation litter in which half of the offspring are responsive and half are nonresponsive to *Cyp1a1* inducing agents. Utilizing this genetic cross, we have shown that inducibility for *Cyp1a1* influenced the incidence of lung tumors, as fetuses exhibiting an inducible phenotype exhibited a 2- to 2.5-fold increase in tumor incidence (Miller et al., 1989, 1990b, 1998; Wessner et al., 1996). When the backcrossed offspring exhibited the responsive phenotype, >80% of the transplacentally exposed mice developed lung tumors 1 year after birth (Wessner et al., 1996).

Utilizing similar treatment protocols, we examined the effects of in utero exposure to MC in the more susceptible Balb/c strain (Gressani et al., 1999). Balb/c mice demonstrated a reduced latency for lung tumor formation as the mice exhibited a 100% tumor incidence 6 months after a 45 mg/kg dose of MC compared to an 84% tumor incidence at 12 months in inducible [D2 × B6D2F1]F2 backcrossed mice treated with 30 mg/kg of MC. The two strains also exhibited a markedly different mutational spectrum in the Ki-*ras* gene. Whereas G→T transversions were found in 84% of lung lesions from [D2 × B6D2F1]F2 backcrossed mice with mutations, 62% of the Ki-*ras* mutations in lesions from Balb/c mice were G→C transversions (Gressani et al., 1999; Leone-Kabler et al., 1997; Miller et al., 2000). In addition, we also noted that 100% of the liver tumors isolated from [D2 × B6D2F1]F2 backcrossed mice contained the mutant Ki-*ras* G13R allele; thus, the same mice harboring G→T mutations in their lung tumors contained G→C mutations in their liver tumors (Gressani et al., 1998). While the mechanism mediating this difference in the *ras* mutational spectrum is unknown, possibilities include differences in the metabolism of MC resulting in either increased activation of MC to reactive electrophiles as a result of induction of *Cyp1* genes or decreased detoxification due to differences in Phase II enzymes and/or differences in adduct formation and/or repair of adducts. Alternatively, it is possible that these strain-specific effects may be the result of contributions from other genetic