Ovarian Aryl Hydrocarbon Hydroxylase Activity and Primordial Oocyte Toxicity of Polycyclic Aromatic Hydrocarbons in Mice

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ABSTRACT

Mouse ovarian aryl hydrocarbon hydroxylase (AHH, EC 1.14.14.2) activity was measured in control mice and in DBA/2N (hereafter called D2) and C57BL/6N (hereafter called B6) mice treated with 3-methylcholanthrene (MC). Basal ovarian AHH activity was similar in both strains (3 pmol/mg/min). Ovarian AHH was induced 2- to 3-fold in B6 mice after MC treatment, while no change was observed in similarly treated D2 mice. Primordial oocytes of both D2 and B6 mice were destroyed by the carcinogenic polycyclic aromatic hydrocarbons (PAH), MC, benzo(a)pyrene (BP), and 7,12-dimethylbenz(a)anthracene (DMBA), but not by the noncarcinogens, pyrene, a-naphthoflavone, and α-naphthoflavone. The rate of primordial oocyte destruction after PAH administration was faster in responsive B6 mice than in nonresponsive D2 mice. After a single i.p. injection of PAH (80 mg/kg), 50% of the oocytes were destroyed by the following times: DMBA, 1 day for B6, 2 days for D2; MC, between 2 and 3 days for B6, 6 days for D2; BP, between 2 and 3 days for B6, 12 days for D2. Dose-response curves of DMBA, MC, and BP also indicated greater primordial oocyte toxicity in responsive B6 mice than in nonresponsive D2 mice. The threshold dose for oocyte destruction 5 days after PAH injection was: DMBA, <1 mg/kg for B6, <2.5 mg/kg for D2; MC, <5 mg/kg for B6, ~80 mg/kg for D2; BP, <5 mg/kg for B6, ~80 mg/kg for D2. In MC-treated D2B6F1 × D2 backcross mice, PAH-inducible ovarian AHH activity and rapid primordial oocyte toxicity cosegregated with inducible hepatic AHH activity. Primordial oocyte toxicity was blocked by simultaneous treatment with α-naphthoflavone. The relative toxicity of the carcinogens to primordial oocytes in both D2 and B6 mice was DMBA > MC > BP.

INTRODUCTION

Spontaneous ovarian tumors are uncommon in most inbred (4) and wild (1) mice. However, malignant ovarian granulosa cell tumors can be initiated in mice with certain PAH2 (7). Shortly after treatment of mice with the carcinogenic PAH, MC, BP, or DMBA, primordial oocytes are destroyed, leading to early ovarian failure and subsequent tumor development. Destruction of the primordial oocytes is thought to be necessary for granulosa cell tumor formation, inasmuch as none develops until the oocytes are completely destroyed (9, 10).

PAH carcinogenesis and toxicity require metabolic activation of the parent compound by microsomal cytochrome P-450-dependent monoxygenases (6). The reactive epoxides formed may bind covalently to tissue macromolecules, spontaneously hydrolyze to dihydrodiols, or serve as substrates for glutathione transferase, UDP-gluconuronyltransferase, or epoxide hydrase (15). The dihydrodiols may be recycled through the monoxygenases forming the reactive diol-epoxides presently thought to be the proximate carcinogens (17, 21).

In earlier studies with the C57BL/6N (hereafter called B6) and DBA/2N (hereafter called D2) inbred strains of mice, we have demonstrated an association between inducible ovarian AHH activity and primordial oocyte toxicity (11, 12). It is well established that the inducibility of AHH by PAH exhibits genetic variation in the liver and other organs of inbred strains of mice (14, 19). It is probable that the genetic regulation of such induction involves several alleles at more than one genetic locus; however, the difference between the 2 phenotype strains, B6 being responsive and D2 being nonresponsive to AHH induction by PAH, is explained almost completely by a single gene difference in accordance with classical Mendelian segregation, the responsive allele being dominant (14, 19). The presence of PAH responsiveness in the mouse correlates highly with MC tumorigenesis (8), DMBA toxicity,3 and acetaminophen hepatotoxicity (18). [For reviews, see the papers of Nebert and Felton (14) and Thorgeirsson and Nebert (21)]. In this paper, we extend our observations on the strain difference in PAH induction of ovarian AHH activity and primordial oocyte toxicity of various carcinogenic and noncarcinogenic PAH in D2, B6, and D2B6F1 × D2 backcross mice.

MATERIALS AND METHODS

Animals. Inbred D2 and B6 mice were obtained from the Veterinary Resources Branch, NIH. D2B6F1 × D2 backcross mice were provided by Dr. Daniel Nebert, National Institute of Child Health and Human Development. For each experiment, the mice were age matched within 1 week; unless otherwise noted, animals used were 4 to 6 weeks old. Care of the animals has been described previously (12).

Chemicals. BP, MC, DMBA, ANF, and BNF were purchased from Sigma Chemical Co., St. Louis, Mo. Pyrene was a gift from Dr. Melvin Newman, Ohio State University. The PAH were dissolved in corn oil and administered i.p. to the mice.

Ovarian AHH Activity. Mice were treated with the test compound or vehicle 40 hr prior to sacrifice. Animals were sacrificed by cervical dislocation, and ovaries were removed. The

1 To whom requests for reprints should be addressed, at Reproductive Toxicology Unit, Pregnancy Research Branch, National Institute of Child Health and Human Development, NIH, Building 10, Room 13N266, Bethesda, Md. 20014.
2 The abbreviations used are: PAH, polycyclic aromatic hydrocarbons; MC, 3-methylcholanthrene; BP, benzo(a)pyrene; DMBA, 7,12-dimethylbenz(a)anthracene; AHH, aryl hydrocarbon hydroxylase; ANF, α-naphthoflavone; BNF, β-naphthoflavone.
3 D. R. Mattison and S. S. Thorgeirsson, unpublished data.
9000 x g supernatant was prepared and assayed as described previously (12).

**Primordial Oocyte Number.** Animals were given i.p. injections of the compounds and sacrificed at the times noted in the legends. Ovaries were then fixed in Bouin’s medium and processed as described previously (12).

**RESULTS**

Basal ovarian AHH activity was similar for both strains of mice (3 pmol/mg/min; Table 1). Treatment with MC resulted in a 2- to 3-fold induction of B6 ovarian AHH activity, while no change was observed in D2 ovarian AHH activity. Backcross mice treated with MC and phenotyped by hepatic AHH activity differed in ovarian AHH activity and oocyte number (Table 2). The responsive phenotype had ovarian AHH activity close to that observed in MC-treated responsive B6 mouse ovary (9.0 pmol/mg/min), while the nonresponsive phenotype had ovarian AHH activity similar to that observed in MC-treated D2 mouse ovary (3.2 pmol/mg/min). The primordial oocyte number in the MC-treated responsive phenotype was significantly below that in the nonresponsive phenotype.

Oocyte number in D2 and B6 mice treated with corn oil, BNF, and pyrene did not differ significantly from that in control animals (Table 3). The mice treated with the carcinogens BP, MC, or DMBA, however, had significantly fewer primordial oocytes (with the exception of BP-treated D2 mice). In addition, the toxicity of PAH to primordial oocytes appeared graded with DMBA, causing more complete destruction than did MC, which was in turn more toxic than was BP.

The time course of primordial oocyte destruction after a single injection of the PAH varied with both mouse strain and carcinogen (Chart 1). B6 mice lost 50% of their primordial oocytes by 2 days after BP treatment, between 2 and 3 days after MC treatment, and within 1 day after DMBA treatment. The rate of oocyte destruction in nonresponsive D2 mice was much slower, with 50% destruction occurring at 10 days for BP, 6 days for MC, and 2 days for DMBA. Primordial oocytes were completely destroyed in responsive B6 mice by all 3 carcinogens but with different end points (BP, 7 days; MC, 6 days for MC, and 2 days for DMBA). Primordial oocytes were completely destroyed in responsive B6 mice by all 3 carcinogens but with different end points (BP, 7 days; MC, 6 days; and DMBA, 2 days) than in D2 mice. Complete destruction of primordial oocytes in nonresponsive D2 mice occurred at 6 days with DMBA and 15 days with MC. Fourteen days after treatment of D2 mice with BP only 65% of the primordial oocytes had been destroyed, and destruction appeared to have stopped.

The dose-response studies also reflected similar strain and carcinogen differences (Chart 2). B6 mice treated with BP showed destruction of primordial oocytes at 5 mg/kg (the lowest dose tested), while D2 mice did not show loss of primordial oocytes below 40 mg/kg. Treatment with MC at 5 mg/kg also produced significant oocyte destruction in B6 mice, while none was seen in D2 mice treated with less than 40 mg/kg. The most potent of the carcinogens tested, DMBA, destroyed approximately 60% of the primordial oocytes in B6 mice at 1 mg/kg and destroyed about 30% of the primordial oocytes in D2 mice at 2.5 mg/kg. Once the threshold for oocyte destruction was exceeded, the rate of dose-dependent loss seemed the same for all 3 carcinogens and in both strains of mice. A plateau of destruction appeared when the primordial oocyte number fell below 90% of control, while unprotected animals (with the exception of BP-treated D2) had less than 50% remaining at 5 days.

A comparison of the rate of oocyte destruction in weaning (5-week) and middle-aged (8-month) D2 and B6 mice treated with DMBA or MC suggested a decreased rate of oocyte loss in B6 mice with age, while in D2 mice the rate of primordial oocyte loss appeared to increase with age (Table 4).

**DISCUSSION**

The ovaries of D2 and B6 mice have similar levels of AHH activity.
Ovarian AHH and Oocyte Toxicity

activity (3 pmol/mg/min). Treatment of both strains with MC results in induction of ovarian AHH activity in responsive B6 mice (up to 9 pmol/mg/min), while no change occurs in ovarian AHH activity in nonresponsive D2 mice. Induction of ovarian AHH activity by MC in D2B6F1 x D2 mice corresponds with induction of hepatic AHH activity. The responsive phenotype, based on induction of hepatic AHH activity, has MC-inducible ovarian AHH activity while the nonresponsive phenotype does not. These data are consistent with previous observations on the induction of AHH activity by PAH in liver and extrahepatic tissues of these 2 inbred strains and their genetic crosses (19).

The presence of AHH and epoxide hydrase (15) in the ovary, a target organ for PAH toxicity and carcinogenicity, is not surprising. The ovary, therefore, has the capacity to metabolize PAH in vivo to diol-epoxides, potent mutagens, and carcinogens (6, 16, 21). This is in agreement with the experiments of Jull (7), in which ovaries incubated in medium containing PAH produced ovarian tumors when implanted in nonexposed syngeneic hosts. Baster and Rohrborn (2) have also demonstrated meiotic errors in oocytes from mice treated with BP. However, these data do not preclude the possibility that extragonadal metabolic activity of PAH may also play a role in gonadal toxicity, germ cell mutations, and gonadal carcinogenesis.

Murine oocytes appear to be selectively sensitive to carcinogenic PAH, since pyrene, a noncarcinogenic PAH, produces no oocyte destruction in responsive B6 mice, the strain most sensitive to oocyte toxicity by carcinogenic PAH. Oocyte destruction also does not occur in mice treated with ANF or BNF, nor does toxicity occur in follicular cells or ovarian stromal cells of mice treated with MC (5).

The mouse strain difference in PAH induction of the ovarian AHH activity is also reflected in PAH primordial oocyte toxicity. Responsive B6 mice have a significantly greater rate of primordial oocyte destruction after treatment with BP, MC, or DMBA than do nonresponsive D2 mice. Responsive D2B6F1 x D2 mice also have greater oocyte loss following treatment with MC than do similarly treated nonresponsive backcross mice (Table 2), indicating that primordial oocyte toxicity is greater in mice with PAH-inducible ovarian AHH activity. Increased toxicity of xenobiotics as a result of more rapid activation in the responsive B6 strain has been investigated using other systems and is consistent with the data reported here (14, 19). The variation in oocyte destruction with respect to time for both strains indicates that toxic levels of reactive metabolites are reached more quickly in the B6 mouse ovary but also indicates their production in the nonresponsive D2 mouse. Although the strains do differ in rate of metabolism, the eventual outcome of sufficient PAH exposure will be complete primordial oocyte destruction in either strain. This is consistent with the observed ovarian carcinogenicity of the PAH in nonresponsive mouse strains (7, 10). Of special interest, however, are the graded destruction of primordial oocytes by carcino-

Chart 1. Time course of primordial oocyte destruction by PAH. All mice were treated on Day 0 with PAH (90 mg/kg I.P.) and sacrificed on the day indicated on the ordinate. Abscissa, number of primordial oocytes per ovary, expressed as percentage of control. Each point represents the mean of 6 to 10 ovaries.  •, B6 mice; □, D2 mice.

Chart 2. Dose response of primordial oocyte destruction by PAH. All mice were treated on Day 0 with the PAH dose indicated on the ordinate and sacrificed on Day 5. Abscissa, percentage of primordial oocyte destruction compared with control. Each point represents the mean of 6 to 8 ovaries.  •, B6 mice; □, D2 mice.
PAH. Oocyte destruction occurs at much lower doses in the responsive B6 strain than in the nonresponsive D2 strain, reflecting higher levels of toxic metabolites in the responsive ovary. Carcinogenic PAH themselves have no intrinsic oocyte toxicity, as demonstrated by the ANF protection experiments. ANF is known to compete with the PAH for the enzyme active site in the monooxygenase (20), so that blocking the metabolism of PAH blocks the primordial oocyte toxicity of these compounds.

The carcinogens tested, BP, MC, and DMBA, are known to produce murine ovarian granulosa cell tumors through a mechanism which requires destruction of primordial oocytes (7, 9, 10). The graded response of oocyte destruction observed here with DMBA > MC > BP is the same as the relative ability of these compounds to produce ovarian tumors (7) as well as their relative carcinogenicity and DNA binding in a mouse skin tumor system (3). Oocyte destruction by the carcinogenic PAH tested appears dependent on both the carcinogenicity of the PAH and the metabolic activity of the target tissue, suggesting that the mouse primordial oocyte toxicity assay may provide a useful in vivo system for studying relative carcinogenicity of certain xenobiotic compounds activated by the microsomal monooxygenase system (13).

Human ovarian cancer is an environmentally associated disease (10). The recent demonstration of early menopause in women smokers underscores the sensitivity of the human ovary to PAH (6). The similarities among PAH exposure, oocyte destruction, and ovarian cancer in the mouse, PAH exposure (via cigarette smoke and other environmental pollutants) and early menopause, and rising PAH pollution and increasing incidence of ovarian cancer suggest that human exposure to PAH, ovarian metabolism to ovotoxic reactive intermediates, and subsequent ovarian tumorigenesis may be linked (14).

**REFERENCES**


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