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PRELIMINARY COMMUNICATION

GENETIC DIFFERENCES IN MOUSE OVARIAN METABOLISM OF BENZO[a]PYRENE AND OOCYTE TOXICITY

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The polycyclic aromatic hydrocarbons (PAH) [benzo[a]pyrene (BP), 3-methylcholanthrene (3-MC) and 7,12-dimethylbenz[a]anthracene (DMBA)] are carcinogens capable of initiating ovarian granulosa cell tumors in mice.[1] The mechanism of tumor initiation by PAH is assumed to be dependent on metabolic activation of the PAH in the ovary because treatment of the ovary with PAH in organ culture and transplantation into castrated, PAH-free hosts result in ovarian granulosa cell tumor formation.[2] The subsequent development of PAH-initiated ovarian tumors appears to require depletion of primordial follicles[3] as well as gonadotropin promotion.[4] However, the interrelationship of ovarian PAH metabolism and oocyte destruction has not been previously demonstrated. We have investigated ovarian metabolism of BP to 3-hydroxybenzo[a]pyrene by aryl hydrocarbon hydroxylase (AHH, EC 1.14.14.2, EN 1972) and changes in oocyte number after treatment with 3-MC in C57Bl/6N (B6) and DBA/2N (D2) mice. These two inbred strains of mice were chosen because they differ in expression of induction of AHH after PAH treatment. The B6 strain, homozygous for the Ah^b locus, exhibits a several-fold increase in AHH activity after treatment with PAH in all tissues investigated.[5] The D2 mice, homozygous for the Ah^d locus, however, do not exhibit induction of AHH after treatment with PAH. The locus for the induction of aryl hydrocarbon hydroxylase activity by aromatic hydrocarbons is designated Ah for aromatic hydrocarbon responsiveness. The Ah^b allele represents the autosomal dominant responsive trait as observed in the prototype B6 mice. The Ah^d allele represents the recessive nonresponsive trait as typified by D2 mice.[5] Associated with the Ah^b locus is the formation of the microsomal cytochrome P_1-450, increases in several mono-oxygenase activities[5] as well as 3-MC-initiated subcutaneous fibrosarcomas.[6]

Ovarian AHH activity was increased 3-fold in the B6 ovary after treatment with 3-MC but no increase was found in the D2 ovary after 3-MC treatment (Table 1). This strain difference in ovarian response to 3-MC is the same as that observed in the liver, lung, kidney, bowel, skin, lymph nodes and bone marrow of these strains.[5] Further experiments using genetic crosses between these two strains suggest that induction of ovarian AHH activity by PAH segregates as an autosomal dominant trait (D. R. Mattison, in preparation). The PAH induction of ovarian AHH in B6 mice as compared to D2 mice will result in different rates of ovarian metabolism of PAH as well as different metabolic profiles of PAH which could lead to differences in both the kind and the degree of observed toxicity. For example, it has been shown that death from hepatic necrosis in the first 24 hr after administration of large doses of acetaminophin occurs only in 3-MC-pretreated B6 mice, and not in 3-MC-pretreated D2 mice. In addition, the extent of hepatic necrosis in the survivors was greater in the 3-MC-treated B6 mice than in the 3-MC-treated D2 mice.[8]

Table 1. Mouse ovarian AHH activity*

Mouse strain	Untreated	3-Methylcholanthrene-treated
DBA/2N	1.57 ± 0.86 (3)	1.43 ± 0.43 (11)†
C57Bl/6N	0.93 ± 0.35 (3)	3.46 ± 0.98 (8)‡

*Mouse ovarian AHH activity (mean ± S.D.) is expressed in pmoles of fluorescent metabolite(s) equivalent to 3-hydroxybenzo[a]pyrene standard formed/mg of protein/min. The number of animals assayed is given in parentheses. Six-week-old B6 and D2 mice were obtained from the NIH Veterinary Resources Branch. Animals treated with 3-MC were given a single intraperitoneal dose of 80 mg/kg dissolved in corn oil 40 hr prior to sacrifice. Control animals were untreated after initial experiments demonstrated no effect of corn oil on AHH activity. Both ovaries were removed from an animal, dissected free of connective tissue and fat and homogenized with a Willems Polytron in 0.5 M Tris buffer, pH 7.8, containing 0.45 mM NADPH and 0.6 mM NADH. The homogenate was centrifuged at 9000 g for 20 min and the supernatant used in the assay. All preparative procedures were carried out at 0-4°. Duplicate aliquots from each animal were assayed and averaged. The AHH assay was performed at 37°. The reaction was started by adding benzo[a]pyrene (Sigma) dissolved in methanol to a final concentration of 0.1 mM in the incubation medium. Each assay flask contained approximately 1 mg protein.[7] The reaction was stopped after 45 min with ice-cold hexane-acetone (3.25:1). The phenolic metabolites were extracted from the organic phase into 1 N sodium hydroxide and the fluorescence was determined immediately with an Aminco fluorocolorimeter (American Instrument Co., Silver Spring, Md.).

† Not different from untreated values: $P > 0.2$ (t-test).

‡ Different from untreated value: $P < 0.005$ (t-test).

The event common to all experimentally induced, spontaneous or genetically predisposed mouse ovarian tumors is loss of the primordial oocytes.[3] Therefore, we were interested in determining if the difference in induction of ovarian AHH in response to 3-MC was accompanied by differences in the rate of primordial oocyte destruction. One possible explanation for the destruction of primordial oocytes after PAH exposure could be the formation of a toxic metabolite(s) from the parent PAH. To explore this possibility, 4-week-old animals

were treated with corn oil or 3-MC. The animals in the 3-MC-treated group were given a single intraperitoneal injection of 3-MC (80 mg/kg) dissolved in corn oil. The corn oil-treated animals were given the appropriate volume of corn oil on a weight basis. The animals were sacrificed 7 days later. The ovaries were removed, fixed in Bouins, serially sectioned at 6 μm and stained with hematoxylin and eosin. The result of a 1-week exposure to 3-MC was the destruction of 97 percent of the primordial oocytes in the responsive B6 strain, while only 50 percent of the primordial oocytes in the nonresponsive D2 strain were destroyed (Table 2).

Table 2. Strain difference in 3-methylcholanthrene primordial oocyte toxicity*

Mouse strain	Corn oil-treated	3-Methylcholanthrene-treated
DBA/2N	2394 ± 565 (7)	1299 ± 369 (8)†
C57Bl/6N	1980 ± 733 (8)	60 ± 77 (8)†

*Primordial oocytes per ovary (mean ± S.D.) in corn oil-treated and 3-MC-treated (80 mg/kg) D2 and B6 mice. Both ovaries from each mouse were used for oocyte counts. The number of ovaries counted is given in parentheses. Ovaries were prepared as described in the text; every tenth section was counted and the total determined by multiplication. Primordial oocytes were counted without knowledge of the mouse strain or treatment.
†Different from corn oil-treated: $P < 0.005$ (*t*-test).

The almost complete destruction of primordial oocytes in the B6 mice in comparison to the extent of ovotoxicity in D2 mice parallels the previously noted effects of 3-MC in induction of fibrosarcomas[6] and acetaminophin hepatotoxicity[8] suggesting metabolic activation of PAH as a cause of ovotoxicity in the PAH-treated mice. The sensitivity of primordial oocytes to PAH and radiation treatment has been previously noted, but the mechanism remains obscure. The striking concordance of increased ovarian AHH activity and oocyte destruction in the B6 mouse compared with that in the D2 mouse suggests that oocyte destruction is mediated by formation of one or more toxic metabolites, which are formed more rapidly in the B6 than in the D2 mouse. Because the D2 mouse ovary does indeed have constitutive AHH activity, we would expect to find oocyte destruction occurring at a slower rate. Preliminary results from experiments now in progress show that D2 mice sacrificed at periods greater than 1 week after PAH treatment demonstrate more extensive primordial oocyte loss.

At the present time we do not know if there is a difference in AHH activity between the several cell types represented in the ovary. The destruction of the primordial oocyte with no apparent histological change in the granulosa, theca or stroma may suggest that the AHH activity resides in the oocyte itself. Alternatively, the stroma, theca or granulosa cells may possess the AHH activity with the primordial oocyte being a sensitive target for the pre-

sumed toxic metabolite(s). Modern primary culture techniques give us a means of separating these cell types and studying their capacity to metabolize PAH and may provide the information needed to answer these questions. The ovary may indeed provide a model for studies, both in vivo and in vitro, of carcinogenicity and cytotoxicity of PAH.

Existing data suggest that PAH are potent ovarian carcinogens in nonresponsive as well as responsive mouse strains.[1] The doses of PAH used, however, were generally higher and exposure time considerably longer than those used in our experiments. Dose and time response curves will allow us to characterize the differential rate of primordial oocyte destruction in these different mouse strains. The striking regional variation in human ovarian cancer with high incidence in industrialized countries,[9] regions where PAH pollution is common, as well as a 3-fold increase in incidence in the past 40 years, underscores the need for further study of ovarian PAH metabolism as well as the effects of metabolites on ovarian components.

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