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DIFFERENCES IN BENZO(A)PYRENE METABOLIC PROFILE IN RAT AND MOUSE OVARY

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The ultimate effect of the treatment of animals with polycyclic aromatic hydrocarbons (PAH) depends on the relative efficiencies of the detoxifying and toxic pathways in the animal, organ or tissue studied. PAH are metabolized to toxins, mutagens and carcinogens by the combined actions of the microsomal cytochrome P-450-dependent mono-oxygenases and microsomal epoxide hydrases [1]. The proximate, reactive metabolite of the parent PAH is thought to be a diol-epoxide requiring sequential microsomal oxygenation, hydration and oxygenation [2]. As multiple forms of the mono-oxygenase exist with different substrate specificities as well as regioselectivities for oxygenation [3], measurement of aryl hydrocarbon hydroxylase (AHH) activity alone may not accurately reflect the relative proportion of substrate metabolized by toxic and nontoxic pathways.

Both mouse and rat ovaries have microsomal P-450-dependent mono-oxygenase [4] and epoxide hydrase [5] necessary for metabolic activation of PAH. Treatment of mice with the PAH, 3-methylcholanthrene (3-MC), benzo(a)pyrene (BP) or 7,12-dimethylbenz(a)anthracene (DMBA) will produce primordial oocyte destruction [6]. The rate of PAH ovotoxicity in the mouse is dependent on dose, ovarian AHH activity and intrinsic carcinogenicity of the PAH (D.R. Mattison and S.S. Thorgeirsson, unpublished data). In addition to oocyte destruction, the PAH will also initiate ovarian granulosa cell tumors in mice [7]. In fact, in mice, there is a striking interrelationship between oocyte destruction and ovarian granulosa cell tumor formation; no tumors are found in ovaries with oocytes present [8], and the ovotoxicity of a PAH is proportional to its ovarian carcinogenicity (DMBA > 3-MC > BP) [7].

Treatment of SD rats or B6 mice with 3-MC induces ovarian AHH activity to about the same extent [4]. The rat ovary, however, is strikingly resistant to PAH oocyte destruction [4], as well as PAH ovarian carcinogenicity [9]. Successful PAH ovarian tumor production in the rat requires initiation with the potent carcinogen DMBA followed by prolonged promotion with gonadotropins [9].

Table 1. Ovarian AHH activity and oocyte destruction*

	Ovarian AHH activity		Primordial oocytes remaining	
Species	Control	3-MC	Control	3-MC
B6 mice	7.0	19.6	2125 ± 800	60 ± 77
SD rats	3.1	10.6	7150 ± 783	6940 ± 1161

*Ovarian AHH activity (pmoles mg^{-1} min^{-1}) and primordial oocyte counts were determined in weanling SD rats and B6 mice obtained from the NIH Veterinary Resources Branch. Animals were maintained as described previously [4]. Control animals were used as obtained. Ovarian AHH activity was determined in control and 3-MC-treated (100 mg kg^{-1}, i.p. 3 days prior to death) animals. Animals were killed by cervical dislocation and decapitation and the ovaries were removed, washed in ice-cold Dulbecos phosphate buffered saline, homogenized with a Brinkman polytron (Westbury, NY) in a medium containing 0.02 M Tris (pH 7.4), 0.15 M KCl and 1 mM EDTA, and centrifuged at 9000 g for 20 min. Aliquots of the 9000 g supernatant were used for protein, AHH and BP metabolic profile determination. AHH assay was performed as described previously [4]. Protein was determined by the method of Lowry et al. [10]. Animals used in primordial oocyte determination were treated with 3-MC (B6 mice, 80 mg kg^{-1}, SD rats, 320 mg kg^{-1}) and killed 7 days later.

Table 2. Effect of 3-MC treatment on rat and mouse ovarian benzo(a)pyrene metabolism*

	Benzo(a)pyrene metabolism (fmoles/min/mg tissue)			
	SD rat		B6 mouse	
Benzo(a)pyrene metabolites	Control	3-MC	Control	3-MC
9,10-Diol	2.41 ± 0.5+(10)‡	4.80 ± 3.2 (4)	2.44 ± 0.3 (3)	5.98 ± 1.5 (2)
4,5-Diol	0.55 ± 0.2 (2)	1.11 ± 0.4 (1)	0.27 ± 0.1 (1)	Not detectable
7,8-Diol	3.56 ± 1.3 (15)	8.59 ± 2.1 (7)	11.67 ± 1.5 (16)	44.16 ± 6.8 (15)
9-OH	4.58 ± 0.9 (20)	27.22 ± 3.9 (22)	19.52 ± 2.6 (26)	72.22 ± 10.8 (24)
3-OH	11.08 ± 2.5 (47)	67.26 ± 2.4 (54)	37.77 ± 6.0 (50)	156.03 ± 23.7 (52)
Benzo(a)pyrene-1,6-dione	0.29 ± 0.1 (1)	5.35 ± 0.7 (4)	1.18 ± 0.9 (2)	6.10 ± 1.0 (2)
Benzo(a)pyrene-3,6 + 6,12-dione	0.92 ± 1.1 (4)	9.64 ± 2.1 (8)	2.09 ± 0.3 (3)	13.37 ± 0.3 (4)
Total benzo(a)pyrene metabolism	23.39 ± 6.6	123.97 ± 14.8	74.94 ± 12.1	297.86 ± 44.1

*[^{3}H]BP (generally labeled) and [7,10-^{14}C]BP were purchased from Amersham/Searle (Arlington Heights, Il.) and purified prior to use by high pressure liquid chromatography (HPLC) [11]. HPLC determination of BP metabolism was according to the method of Yang et al. [11], with the following modifications. The reaction mixture, in a total volume of 1.0 ml, contained: [7,10-^{14}C]BP (sp. act. 60.7 nCi/nmole in 1.5% methanol) or [^{3}H]BP (sp. act. 2554 mCi/mmole in 1.5% methanol), 60 mM Tris buffer (pH 7.4), 10 mM EDTA and 3 mM NADPH. The amount of tissue added to the reaction mixture was 80-120 mg. The incubation mixture was preincubated at 37° for 2 min, and the reaction was started by the addition of 50 µmoles BP. The mixture was incubated at 37° for 60 min. The reaction was stopped with 1.0 ml acetone. One to two µmoles vitamin E was then added. The mixture was then further extracted with 2.0 ml ethyl acetate and the organic phase was transferred to tubes containing 5-6 µmoles vitamin E. An aliquot (1.0 ml) of the extract was evaporated under nitrogen at 41° and reconstituted in 100 µl methanol containing unlabeled metabolites of BP. The metabolites of BP were separated by HPLC on a Whatman Partisil column with a methanol solvent system adjusted on a linear gradient from 60 to 100%. Flow rate was 0.80 ml/min, delay time was 1.0 min, and gradient time was 20 min. When tritiated BP was used as the radioactive substrate, the BP metabolites were first separated from BP on a silica gel column and then applied to the HPCL. The radioactivity of each metabolite was measured quantitatively by liquid scintillation spectrometry. The enzymatic activities are expressed as the amount of BP metabolites formed/min/mg of tissue. The amount of BP metabolized varied between 1 and 5 percent.

+Values are the mean of three determinations ± S.D.

‡Numbers in parentheses represent the percent of the total metabolites formed.

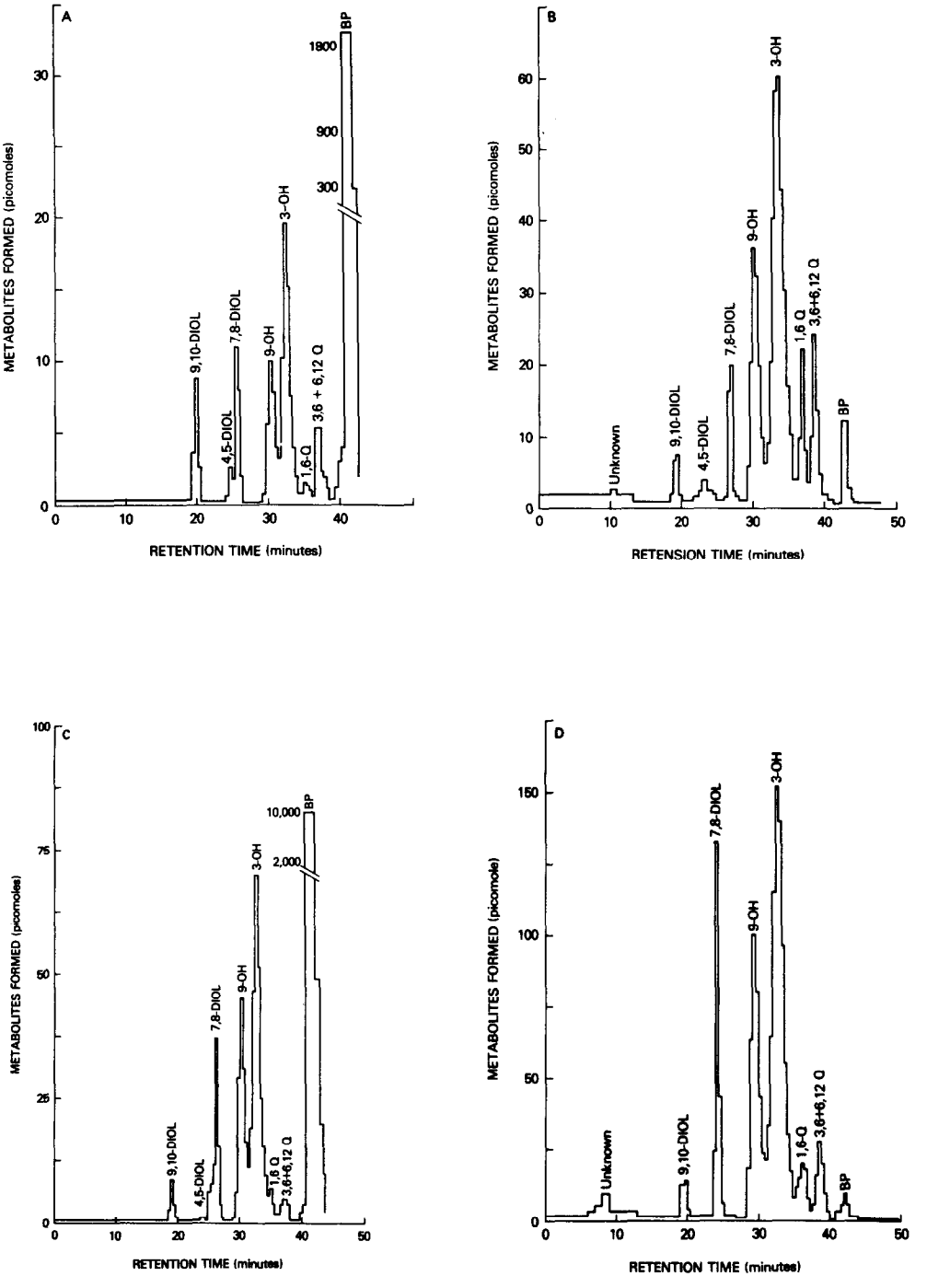

Figure 1. BP metabolites produced by SD rat and B6 mouse ovarian 9,000 g supernatant. (A) Control SD rat. (B) 3-MC-treated SD rat. (C) Control B6 mouse. (D) 3-MC-treated B6 mouse.

These differences in PAH ovotoxicity and ovarian carcinogenicity suggest differences in toxic and nontoxic pathways of PAH metabolism in the rat and mouse ovary. As these differences may reside in oxygenases, hydrases or conjugases singly or in combination, it is necessary to investigate each in a step-wise fashion. In this paper we report differences in the metabolic profile of BP in rat and mouse ovary.

Ovarian AHH activity, as determined by the fluorescent assay, was approximately 2-fold greater in B6 mice than in SD rats for both control and 3-MC treated animals (Table 1). Although the fluorescent assay for AHH activity only measures the phenolic fluorescent metabolites of BP (predominantly 9-OH-BP and 3-OH-BP), it does seem to correspond to the total BP metabolism. However, it cannot differentiate between toxic and nontoxic pathways for BP metabolism. This is reflected in differences in primordial oocyte toxicity after 3-MC treatment of B6 mice and SD rats (Table 1). No primordial oocytes were destroyed in SD rats treated with 3-MC, while 87 percent were destroyed by similar treatment of mice.

The HPLC metabolic profiles of BP for control and 3-MC-induced rat and mouse ovarian mono-oxygenase are presented in Fig. 1 and quantitated in Table 2. Determination of BP metabolism as well as 3- and 9-hydroxy-BP formation by HPLC agree with the fluorescent assay for AHH activity. K-region metabolism, as reflected by 4,5-dihydrodiol-BP formation, was found to be greater in the SD rat than in the B6 mouse for both control and 3-MC-induced oxygenases. In fact, the 4,5-dihydrodiol-BP could not be detected in 3-MC-treated B6 mice. Early evidence suggested that the K-region oxides were the proximate carcinogens of BP metabolism [12]. If the 4,5-oxides were important in ovotoxicity and ovarian carcinogenesis, the rat might have been expected to be more sensitive to PAH oocyte destruction and ovarian carcinogenesis than the mouse but, in fact, the opposite is observed. This is consistent with more recent experiments which suggest that the K-region oxides are not the proximate carcinogens [2].

In control and 3-MC-treated SD and B6 ovary, 9,10-dihydrodiol-BP production is similar. Control B6 mouse ovary produces four times more 7,8-dihydrodiol-BP and 9-hydroxy-BP than the SD ovary. After 3-MC treatment the B6 ovary produces five times more 7,8-dihydrodiol-BP and three times more 9-hydroxy-BP than the SD ovary. 7,8-Dihydrodiol-BP formation in the control rat represents 15 percent of the total BP metabolism, while after 3-MC treatment 7,8-dihydrodiol-BP formation accounts for only 7 percent of the BP metabolized by rat ovarian mono-oxygenases. At the present time, the proximate carcinogen from BP metabolism is thought to be the 7,8-diol-9,10-oxide [2]. Production of this reactive metabolite requires formation of the 7,8-oxide, diol formation via epoxide hydrases, and reoxidation at the 9,10 positions by the mono-oxygenase. As multiple forms of the mono-oxygenase and epoxide hydrase are known to exist with different substrate specificities, in addition to positional selectivity for the mono-oxygenase, it is not surprising that the gonads of two different species differ in BP metabolic profiles. The lower rate of production of 7,8-diol by the rat ovarian mono-oxygenase is consistent with the greater resistance of the rat ovary to PAH ovotoxicity and ovarian carcinogenicity.

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