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2-Aminofluorene–DNA adducts in mouse urinary bladder: effect of age, sex and acetylator phenotype

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Formation of urinary bladder DNA–2-aminofluorene adducts in inbred and acetylator congenic mice was measured 3 h after a 60 mg/kg dose of the arylamine carcinogen. The sensitivity of ^{32}P-postlabeling with HPLC analysis permitted quantitation of adducts in individual mouse bladders. Acetylator phenotype was a significant determinant of DNA damage in female mice as slow acetylators had higher levels of bladder DNA adducts than rapids. This correlation is the reverse of that seen with hepatic DNA. Age was also a significant determinant of DNA damage as older mice (20–23 weeks) formed more bladder DNA adducts than young (7 week) mice. The age-related increase in bladder adduct formation was seen in both sexes of all mouse lines. Male B6 mice exposed to 2-aminofluorene at 20–23 weeks of age showed a 26-fold higher level of bladder DNA adducts than males exposed at 7 weeks. In addition to the large increase in total adduct level, the older male B6 mice produced significant amounts of an unidentified, early-eluting adduct peak that had chromatographic properties similar to an aminofluorene–DNA adduct produced through peroxidative activation. These results indicate that age, sex and acetylator phenotype are all important determinants of aromatic amine–bladder DNA adduct formation in mice.

Introduction

In order to initiate carcinogenesis, aromatic amines require activation to metabolites capable of combining covalently with cellular DNA. The metabolic pathways for activation or detoxification of arylamines may involve C- or N-oxidation by cytochrome P450s, acetyl transfer reactions, and sulfo- and glucuronyl transfer reactions. There is also considerable evidence that peroxidation such as by prostaglandin H synthase (PHS*) is an activation pathway for arylamines and aryl hydroxamic acids, especially in extrahepatic tissues (1–4). The exact degree of utilization of the various metabolic pathways for arylamine carcinogens varies with the specific arylamine, the animal (or plant) species involved, the particular tissue studied, and the genetic makeup of the individual. Other factors such as sex, age and nutritional or health status may also be significant.

We have previously examined the effect of the genetically determined acetylator status of inbred and congenic mice on 2-aminofluorene (2-AF)–hepatic DNA adduct formation and found a correlation between hepatic acetyl coenzyme A:arylamine *N*-acetyltransferase (NAT) activity toward *para*-aminobenzoic acid (PABA) and 2-AF and hepatic 2-AF–DNA adduct formation (5). As both liver and bladder are target organs for arylamine carcinogenesis in mice, we have now investigated the role of the acetylator polymorphism in the formation of urinary bladder DNA–2-AF adducts in mice of defined acetylator genotype. The results of studies of bladder DNA adduct formation may shed light on the mechanisms responsible for the statistical correlation between slow acetylation and bladder cancer observed in humans occupationally exposed to arylamines (reviewed in 6).

In order to study the formation of adducts in individual mouse bladders, we used the sensitive ^{32}P-postlabeling procedure with HPLC analysis of adducted nucleotides (7) as we did in previous work on hepatic DNA adducts in acetylator (5) and *Ah*-responsive (8) congenic lines. We now report the formation of urinary bladder DNA–2-AF adducts in acetylator congenic mice of both sexes and at young (7 weeks) and older (20–23 week) ages. Like most studies of adduct formation, tumorigenesis studies will be necessary to verify the applicability of our findings to risk assessment.

*Abbreviations: PHS, prostaglandin H synthase; 2-AF, 2-aminofluorene; NAT, acetyl coenzyme A:arylamine *N*-acetyltransferase; PABA, *para*-aminobenzoic acid; B6, C57BL/6J mice; A, A/J mice; B6.A, B6.A-*Nat*^{s} mice; A.B6, A.B6-*Nat*^{r} mice; 2-AAF, *N*-acetyl-2-aminofluorene; dG-C8-AAF, *N*-acetyl-*N*-(deoxyguanosin-8-yl)-2-aminofluorene-3',5'-diphosphate; dG-C8-AF, *N*-(deoxyguanosine-8-yl)-2-aminofluorene-3',5'-diphosphate.

Materials and methods

Chemicals, animals and treatments

PHS was obtained from Oxford Biomedical Research, Oxford, MI, indomethacin and arachidonic acid from Sigma, St Louis, MO, and 2-amino[ring-G-^{3}H]fluorene (1.6 Ci/mmol) from Chemsyn Science Laboratories, Lenexa, KS. Other chemicals and enzymes were of purities and from sources reported previously (5). Mice of C57BL/6J (B6) and A/J (A) lines were from stock maintained at the University of Michigan. Acetylator congenic mice (B6.A and A.B6) were from lines produced as described (9). For *in vivo* adduct formation studies, 2-AF in DMSO (16 mg/ml) was injected i.p. at a dose of 60 mg/kg, while control mice received DMSO alone. Animals were killed 3 h after injection and bladders were removed, cleaned, quick frozen and stored at −20°C until DNA was isolated and prepared for analysis. For studies of the time course of adduct formation and disappearance 7 week old male B6 and A mice were dosed as above but were killed 3, 6, 12 and 24 h after injection.

Isolation of DNA and ^{32}P-postlabeling

Urinary bladder DNA modified *in vivo* was prepared from individual mice by homogenization of the minced tissue in a 2 ml Tenbroeck glass homogenizer in 200 μl of 50 mM Tris, 25 mM KCl, 5 mM $MgCl_2$, 250 mM sucrose, pH 7.5. The homogenate was then added to 200 μl of 1 M NaCl, 0.1 M EDTA, 2% SDS, pH 8 and incubated at 60°C for 1 h. Following isolation, the DNA was purified by protease K and RNase digestion, phenol and chloroform extraction, and precipitation with ethanol as described (7). The DNA samples were dissolved in 100 μl of 20 mM succinate/10 mM $CaCl_2$, pH 6.0. Typical yields of DNA, as determined by absorbance at 260 nm, were 12–20 μg/bladder. Hydrolysis of DNA (1–4 μg) by micrococcal nuclease and spleen phosphodiesterase II, enrichment of adducts by butanol extraction in the presence of tetrabutylammonium chloride, and ^{32}P-labeling of extracted nucleotides with [γ-^{32}P]ATP and polynucleotide kinase were as described previously (5,7).

In vitro activation of 2-AF by PHS and formation of DNA adducts

2-AF was activated by reaction with arachidonic acid and purified PHS by a modification of the method of Flammang *et al.* (10). 2-Amino-[ring-G-^{3}H]fluorene (5 μCi) at 1.6 Ci/mmol was added to a 2 ml centrifuge tube and evaporated to dryness. One milliliter of 2 mg/ml calf thymus DNA in 50 mM potassium phosphate pH 7.4 buffer was added followed by 5 μl (200 U) PHS. After a 1 min incubation at 37°C, 10 μl of 0.1 mM hematin was added and incubation continued for 1 min. The reaction was started by the addition of 10 μl of 30 mM arachidonic acid in ethanol and incubated at 37°C for 5 min. Additional incubations were carried out using PHS heated to 70°C for 5 min, omitting arachidonic acid, or containing 100 μM indomethacin. The reactions were

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terminated and DNA precipitated by adding the reaction contents to 5 ml of 95% ethanol/1% phenol. The DNA was recovered by centrifugation and purified by repeated cycles of solution in 50 mM potassium phosphate pH 7.4 buffer and ethanol precipitation until no change in specific radioactivity was detectable.

Samples of DNA modified by PHS activation of 2-AF were digested to 3'-nucleotides with micrococcal nuclease and spleen phosphodiesterase. Some samples were extracted with butanol and tetrabutylammonium chloride as described for DNA modified *in vivo*. Nucleotides derived from PHS-activated 2-AF−DNA were phosphorylated with polynucleotide kinase and non-radioactive ATP to give nucleotide-3',5'-diphosphates, which were analyzed by HPLC as for ^{32}P-labeled samples.

HPLC and quantitation of DNA adducts

HPLC of ^{32}P-postlabeled nucleotides from DNA modified *in vivo* was performed using a reverse-phase C_{18} ion-pairing procedure as described previously (7). Samples were eluted with 30 mM potassium phosphate pH 6.0/CH_3CN (90:10) for 10 min followed by a linear gradient of 90% 30 mM potassium phosphate, pH 6.0, 5 mM tetrabutylammonium phosphate/10% CH_3CN increasing to 50% at 50 min. The flow rate was 1.5 ml/min. The HPLC column was washed with 20% potassium phosphate buffer/80% CH_3CN and re-equilibrated to the starting conditions between each sample. Samples contained an internal UV standard of *N*-acetyl-*N*-(deoxyguanosin-8-yl)-2-aminofluorene-3',5'-diphosphate (dG-C8-AAF) (7).

Radioactivity in ^{32}P-labeled samples was quantitated by Cerenkov counting of collected fractions and the extent of adduct formation was determined from the specific activity of the [^{32}P]ATP. For ^{3}H-labeled nucleotides, fractions were collected and radioactivity determined using Ecolite scintillation fluid. The amount of adduct formation was calculated from the specific activity of the [^{3}H]2-AF used in the incubation.

Statistical analysis

Results from the different groups of mice were compared for statistical significance using the *t*-test with unknown variances.

Results

Results of 24 h time course

Urinary bladder DNA−2-AF adducts were measured in individual 7 week old male B6 and A mice killed at 3, 6, 12 and 24 h after a 60 mg/kg i.p. dose of 2-AF. The 3 h time point gave the highest adduct level with gradually declining levels of adduct at later time points. B6 mice had higher adduct levels than A mice for each point examined (Figure 1). Since the 3 h point gave the highest adduct values in bladder, as was also the case in liver (5), this time was selected for the comparison of bladder adduct formation between mouse lines, sexes and ages.

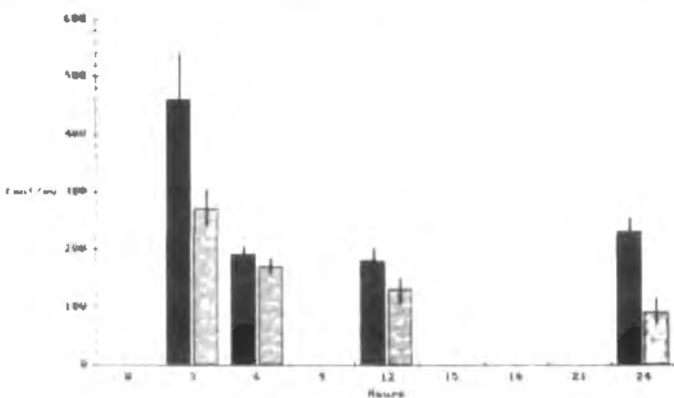

Fig. 1. Urinary bladder DNA−2-AF adducts over 24 h after 60 mg/kg 2-AF. The level of adducts found in bladder DNA (10^{-15} mol/mg DNA) was determined in 7 week old male mice at 3 h (n = 8 mice), 6 h (n = 3), 12 h (n = 3) and 24 h (n = 3). ■ B6 mice. ▨ A mice

Urinary bladder DNA adducts

Our initial results indicated a wide variation of urinary bladder DNA adduct formation in male B6 mice. It was determined that most of the variation could be eliminated by using mice of a single age. The first mice examined were 7 week old males and females of the parental inbred lines B6 and A and the two acetylator congenic lines B6.A and A.B6. The degree of adduct formation in these young mice is shown in Table I. Among the females, slow acetylators had ~2.3 times the bladder adduct levels found in the corresponding rapid acetylators (B6.A verus B6, A versus A.B6). Only the difference between A and B6 was statistically significant ($P < 0.01$). This result differs from our examination of hepatic 2-AF−DNA adducts where rapid acetylators formed higher levels of adducts than slow acetylators (5). For the young male mice, the acetylator phenotype had less effect on bladder adduct formation than for females. Male rapid acetylators had slightly higher (25−45%) adduct formation than the corresponding slow acetylators with none of the differences being statistically significant.

In a second series of experiments, older (20−23 week) mice were exposed to 2-AF for 3 h. Table I shows the formation of bladder DNA−2-AF adducts in these older mice. In addition, a single 10 week old male B6 was examined and found to have an adduct level (2.2 pmol/mg) intermediate between that of the 7 week and 20 week age groups. Similarly, two 11 week old male A mice had bladder DNA adduct levels of 0.56 pmol/mg, also intermediate between the 7 week and 20 week groups. For all lines and for both sexes, 20−23 week old mice produced more bladder DNA adducts in response to 2-AF than did 7 week old mice. The degree of increased response varied with sex and strain. Females showed the least amount of increase with age, ranging from just above unity to >2-fold. In the males the range was greater: 5-fold in B6.A and > 26-fold in B6. The degrees of increase are presented in Table II.

In addition to the quantitative changes in adduct formation found, there was a qualitative difference in the HPLC elution profile of adducts from male mice. In the young mice the elution of ^{32}P from the HPLC column was very similar to that reported previously for mouse liver DNA after 2-AF exposure (5). Two major adduct peaks were found, presumably corresponding to dG-C8-AAF and dG-C8-AF. The older male mice differed from this pattern in that an additional unidentified ^{32}P peak was found in their bladder DNA. This new, early-eluting peak was most prominent in the B6 males. Figure 2 illustrates the elution of ^{32}P for 7 and 20 week old male B6 bladder DNA. The early-eluting peak was not observed in females of any line and was much less prominent in males of lines other than B6. The increase in adduct

Table I. 2-AF urinary bladder DNA adducts in 7 and 20−23 week old mice

Mouse line	Acetylator genotype	7 weeks (pmol adduct/mg DNA)		20−23 weeks (pmol adduct/mg DNA)	
		Male	Female	Male	Female
B6	RR	0.51 ± 0.11 (5)	0.24 ± 0.05 (6)	13.4 ± 5.61 (4)	0.54 ± 0.22 (3)
B6.A	rr	0.41 ± 0.27 (5)	0.56 ± 0.32 (4)	2.06 ± 0.70 (4)	0.61 ± 0.18 (4)
A	rr	0.27 ± 0.03 (4)	0.92 ± 0.17 (5)	2.83 ± 0.79 (4)	1.12 ± 0.31 (4)
A.B6	RR	0.39 ± 0.09 (5)	0.40 ± 0.13 (4)	2.63 ± 0.66 (4)	0.77 ± 0.24 (4)

Values are mean ± standard error. Number of mice used shown in parenthesis.

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formation in these other lines of mice was primarily due to an increase in the more familiar adduct peaks. We had not observed an age-related change in adduct formation in our earlier study of hepatic DNA following 2-AF exposure.

To determine if the early eluting adduct observed in the older male B6 mice could be formed by peroxidative activation of 2-AF, PHS-activated 2-AF–DNA adducts were prepared *in vitro* (3,10) and their chromatographic profile compared. PHS and arachidonic acid caused the activation of 2-AF to a DNA-binding metabolite. As shown in Table III both active PHS enzyme and arachidonic acid are required for the activation to be efficient. Furthermore, addition of indomethacin, an inhibitor of the cyclooxygenase activity of PHS, greatly diminished the binding of 2-AF to DNA. HPLC analysis of nucleotides from PHS-activated 2-AF–DNA showed an elution pattern very similar to that seen for 2-AF–DNA from bladders of older male B6 mice. The early-eluting peak noted in urinary bladder DNA from these mice also occurs in the *in vitro* activated DNA. HPLC

Table II. Ratio of 2-AF urinary bladder DNA adducts in old to young mice

Mouse line	Ratio	
	Male	Female
B6	26.3	2.3
B6.A	5.0	1.1
A	10.5	1.2
A.B6	6.7	1.9

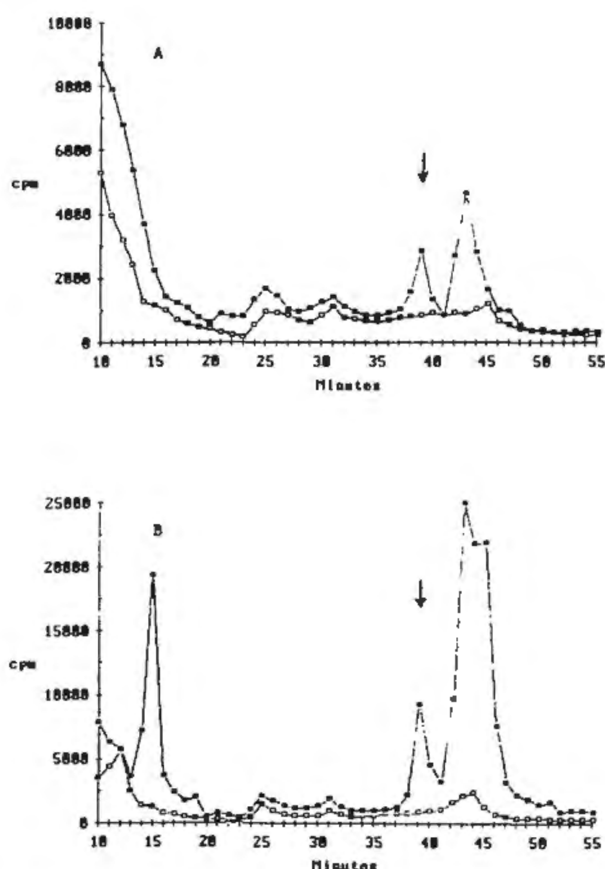

Fig. 2. HPLC elution profile of ^{32}P-postlabeled nucleotide adducts from 2-AF-treated and control male B6 urinary bladders. (A) Mouse treated at 7 weeks of age. (B) Mouse treated at 20 weeks of age. □——□ control (DMSO only) ■——■ 2-AF treated. Arrow indicates elution of a standard of dG-C8-AAF.

analysis of DNA from the incubation containing indomethacin did not show the early-eluting peak (Figure 3). Other peaks eluting later in the chromatogram also showed sensitivity to indomethacin. The identity of the material in these later peaks is unknown.

Our previous results with hepatic DNA–carcinogen adducts (5) showed greater adduct formation in females than males at the 3 h time point. Our present results with bladder DNA in the young mice show a slightly greater formation of adducts in males than females for the rapid acetylator B6 strain (2-fold) and a somewhat greater adduct formation in females than males for the slow acetylator A strain (3-fold). However, in the older mice, adduct levels found in male bladder DNA were consistently higher than in females. The ratio of adduct levels in males to females was highest in the case of B6, where males had nearly 25 times the adduct level found in females.

Discussion

The formation of urinary bladder DNA–carcinogen adducts in the mice studied here is the result of metabolism of the carcinogen by different tissus. Combinations of activation and deactivation pathways in liver, blood, kidney, bladder, and perhaps other organs all contribute to the results obtained by measuring adducts in bladder DNA. The balance of activation and deactivation is determined by many factors, including age, sex and specific enzyme polymorphisms. We have attempted to study the effect of the acetylator polymorphism on the formation of DNA–carcinogen adducts in urinary bladder. Previously we found that rapid acetylators had higher levels of hepatic DNA–2-AF adducts than slow acetylators (5). Formation of hepatic DNA–carcinogen adducts is thought to be relatively independent of metabolism of carcinogen by extrahepatic tissues as the bulk of metabolism by arylamine carcinogens is thought to occur in the liver, and metabolites from tissues such as kidney and bladder are not transported to the liver to a significant extent. The situation in bladder differs greatly in that metabolites from most other organs, after passing via blood through the kidney, accumulate in the urine stored in bladder. Cells of the bladder are thus exposed to carcinogens and their metabolites both from the urine and from the blood supply to the bladder. Adduct formation in bladder cells could conceivably occur via simple chemical changes such as acid-catalyzed hydrolysis of arylhydroxylamine conjugates in the urine as well as via metabolic activation of carcinogens arriving in the blood or in the urine.

The effect of the slow acetylator phenotype on 2-AF bladder DNA adduct formation is seen in the female mice. Both at 7 weeks and at 20–23 weeks slow acetylator females formed greater levels of adducts than the corresponding rapid acetylators. The difference is most pronounced on the A background (A versus A.B6) but is also observed on the B6 background (B6.A versus B6) and is larger in young mice than in the older group, possibly due to maturation of additional, acetylator-independent, pathways of arylamine activation (as will be more fully discussed later). The wider difference in adduct levels between rapid and slow acetylators on the A background compared to the B6 background is similar to that reported for liver DNA adducts (5). Comparing adduct formation in hepatic and bladder DNA in female mice shows a reciprocal relation in terms of acetylator phenotype. In hepatic DNA, rapid acetylators have more DNA adducts than slow, whereas in bladder, slow acetylators have more DNA adducts than rapids. A possible explanation of this difference in the relationship between DNA adduct formation and acetylator status is that the rapid acetylators activate greater

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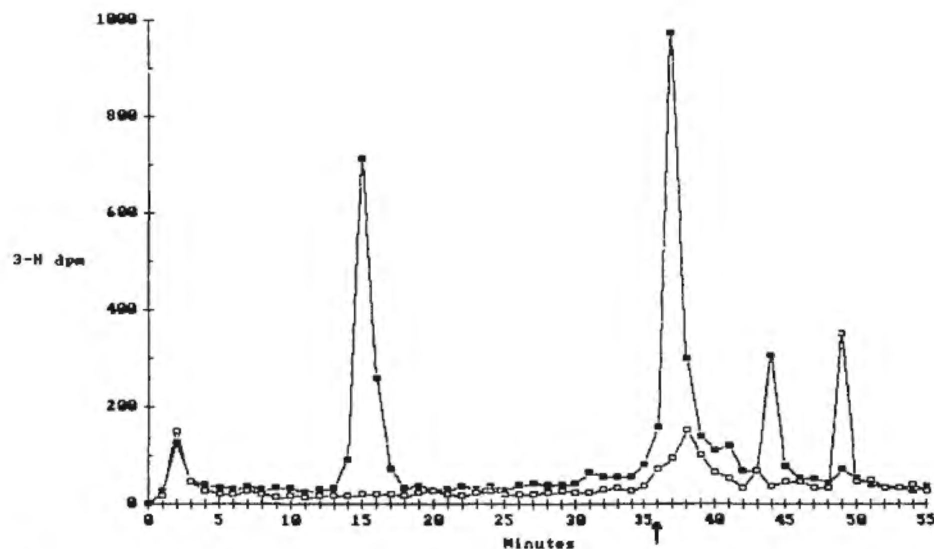

Fig. 3. HPLC elution profile of nucleotide adducts from DNA treated with 2-amino-[^{3}H]fluorene activated with PHS and arachidonic acid. Nucleotides were phosphorylated and resolved by HPLC. ■——■ DNA treated with 2-AF, PHS and arachidonic acid. □——□ DNA treated as above but with 100 μM indomethacin added. Arrow indicates elution of a standard of C8-dG-AAF.

amounts of arylamines in the liver to form hepatic DNA adducts, while slow acetylators pass more unmetabolized carcinogen to the extrahepatic tissues such as kidney and bladder where activation and adduct formation can occur. In mice the acetylator phenotype as determined in liver is reflected in blood, kidney and bladder (11,12). Thus, the rapid acetylation of 2-AF in one tissue would also occur in the other tissues if sufficient concentrations of the carcinogen were available. The actual concentration of carcinogen available for acetylation is determined in part by the activity of tissues upstream of the organ in question. Consideration must also be given to the fact that in liver and blood, at least, the K_M values of mouse NAT for 2-AF are lower in slow acetylators than in rapids (12).

The increase in bladder adduct formation in older mice may be explained, in part, by the maturation of NAT and P450 activities in kidney and tissues of the urinary system. Renal NAT activity shows an increase with age in B6 mice up to 6–8 weeks of age (T.Smolen, J.Brewer and W.W.Weber, in preparation) with male kidney activity increasing somewhat further to the 16–20 week stage. Similarly Brusick *et al.* (13) have shown that mouse kidney microsomes obtained from females are practically unable to activate dimethylnitrosamine to a mutagen for *Salmonella*. Kidney microsomes from male mice were unable to activate dimethylnitrosamine unless obtained from mice >6 weeks of age. Mutagenic activation ability in male kidney microsomes continued to increase with age. Interestingly, the ability of liver microsomes to carry out this activation was independent of sex and age (13).

The very large increase in bladder DNA adducts seen in older male B6 mice may be the combined effect of maturation of metabolic pathways for activation of 2-AF and of sex-linked differences in arylamine metabolism. B6 mouse kidney shows a sex-specific difference in 2-AF NAT activity, with males having more than twice the activity of females (11). The sensitivity of the NAT assay used for this determination was not great enough to detect any kidney 2-AF NAT activity in either male or female A mice, so it is not known if the sex-related difference in kidney 2-AF NAT activity is specific for B6 mice. However, the same sex-related difference in kidney NAT activity was observed in A mice using PABA as a substrate (T.Smolen *et al.*, in preparation). Male sex hormones have been shown to increase activation of arylamine carcinogens by kidney. Both androgenic hormones and *tfm* (testicular feminization mutant) mice have been used to demonstrate the role of sex hormones on renal metabolism and activation of carcinogens (13,14; T.Smolen *et al.*, in preparation). In addition to a direct effect on renal metabolism of carcinogens, it has been shown that androgens stimulate renal β-glucuronidase production (15). An increase in β-glucuronidase activity could increase hydrolysis of glucuronic acid conjugates of carcinogens which are formed in the liver and transported to the bladder. Increased β-glucuronidase activity could release additional quantities of activated carcinogen metabolites able to react with bladder DNA. A number of other gene products involved in the metabolism or elimination of foreign compounds have been found to be androgen inducible in mouse tissues (16).

The formation of an additional early-eluting adduct peak in bladder of older B6 males could indicate that an additional activation pathway for the arylamine is functioning in these mice.

Table III. DNA–2-AF adduct formation *in vitro* after activation by PHS

Incubation	pmol adduct/mg DNA
Complete system[a]	84.7
Heated PHS	13.2
– Arachidonic acid	9.7
+ Indomethacin (100 μM)[b]	24.0

[a]Incubation as described in Materials and methods.
[b]Indomethacin was added 2 min before arachidonic acid.

Table IV. Ratio of urinary bladder DNA–2-AF adducts to hepatic DNA–2-AF adducts

Mouse line	Males		Females	
	7 week	20–23 week	7 week	20–23 week
B6	2.9	98.0	0.5	1.2
B6.A	4.1	20.8	3.5	3.7
A	3.9	39.3	4.4	5.4
A.B6	0.5	3.6	0.5	1.0

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A strong possibility is that in examining bladder DNA, we are seeing an adduct formed in extrahepatic tissue by the peroxidative pathway of arylamine activation as catalyzed by PHS. Krauss and Eling (2) described an early-eluting 2-AF–DNA adduct formed *in vitro* with either ram seminal vesicle microsomes and arachidonic acid or with horseradish peroxidase and hydrogen peroxide. The adduct formed in these incubations was extractable into n-butanol at low pH only in the presence of tetrabutylammonium chloride, the very conditions we used for adduct enrichment. The occurrence of this unique early-eluting 2-AF adduct has been used by Krauss *et al.* (3) as a probe for *in vivo* peroxidative activation of arylamines. They used two male beagle dogs dosed with 2-AF and determined the DNA adduct profiles after 24 h. In both dogs N-hydroxylation was the only activation pathway for arylamines in liver, but in kidney and urothelium one of the dogs (dog A) had significant peroxidative activation of 2-AF. For dog A the renal cortex showed mainly the usual dG-C8-AF adduct, but the renal medulla produced the peroxidative adduct in excess of dG-C8-AF. The pattern of adduct formation parallels the relative cytochrome P450 and PHS content of cortex and medulla (4). Furthermore, dog A had only the peroxidative adduct in the urothelium (3). We have shown that *in vitro* activation of 2-AF with PHS and arachidonic acid in the presence of DNA yields an adduct with the chromatographic properties of the additional bladder DNA adduct formed *in vivo* by older male B6 mice. It would be interesting to determine if the level of PHS activity changes with age in these mice and if such changes correlate with changes in the pattern of DNA adducts produced after exposure to arylamine carcinogens.

If the additional adduct found in mouse bladder DNA from older male B6 mice (Figure 2, fraction 15) is indeed due to peroxidative activation, its formation may still be influenced by the acetylation polymorphism. Although aromatic amines are substrates for PHS, aromatic amides are not. However, aryl hydroxamic acids are oxidized by PHS (1). Thus 2-AF would be oxidized, but if it were acetylated to 2-AAF it would not be activated by PHS. If, however, cytochrome P450 N-oxidative of the amide formed N-OH-AAF, peroxidation could occur. The influence of the acetylator phenotype, sex and age on these complex and intertwined metabolic pathways cannot be sorted out by the experiments reported here.

Table IV compares the levels of 2-AF adducts formed in bladder as reported here with 2-AF hepatic DNA adducts reported previously (5). It is apparent that in 7 week old female mice acetylation is an important determinant of adduct formation: slow acetylators (A and B6.A) show 3–4 times more adduct in bladder than liver, while rapid acetylators (B6 and A.B6) have about half as much. For the young males the correlation with acetylator phenotype is not found. This would suggest that some additional factor in the male mice leads to increased urinary bladder DNA adduct formation after exposure of 2-AF. Although we have discussed possibilities as to the nature of this factor, we cannot determine its identity from the present information.

In the older females, hepatic and bladder DNA is adducted to almost equal levels in the rapid acetylators while the slow acetylators continue to show 3–5 times higher adduct levels in bladder than liver. For older male mice the large increase in bladder DNA adduct formation is reflected in the very large increase in the bladder to liver adduct ratio. In the older males the ratio of DNA–2-AF adducts in bladder to those in liver would indicate that bladder DNA is far more susceptible to arylamine-induced damage than liver DNA, except possibly in the A.B6 line. The ratios in Table IV indicate that the difference between bladder and liver DNA susceptibility to damage from acute exposure to 2-AF is less for the older females of both acetylator phenotypes than for the older males.

Adduct levels in DNA after exposure to 2-AF suggest that rapid acetylator female mice are at elevated risk of hepatic carcinogenesis, while male mice are at greatest risk for bladder tumors. Tumorigenesis studies have shown the prevalence of arylamine-induced liver tumors in female mice, in contrast to formation of bladder tumors in males (17,18). However, neither a slow acetylator mouse strain nor an acetylator congenic line has been studied for arylamine-induced carcinogenesis. Thus, additional tumorigenesis studies are required to interpret the importance of the different levels of adducts found in the sexes, at different ages, in different tissues, and in the different acetylator phenotypes. High levels of adducts in a tissue may not be indicative of higher risk of tumor in that tissue. Among the reasons for this are differing repair capabilities, different rates of cell proliferation (19), cell death without DNA replication or mutation in cells with high levels of DNA adducts, and whether or not the adduct present at high concentration is the adduct responsible for carcinogenesis. For these reasons we have begun tumor studies in both sexes of mice of differing acetylator phenotypes.

Acknowledgements

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