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# Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis[1]

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**Abstract**

Differences in human urinary bladder cancer susceptibility have often been attributed to genetic polymorphisms in carcinogen-metabolizing enzymes, especially those involved in the biotransformation of aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs). Metabolic activation generally involves an initial cytochrome P450-dependent oxidation to form *N*-hydroxy, phenol, or dihydrodiol intermediates that undergo further conjugation or oxidation to form DNA adducts. The acetyltransferases, NAT1 and NAT2, can participate in these pathways by catalyzing detoxification (by AA *N*-acetylation) or further activation (by N-OH-AA *O*-acetylation) reactions. NAT2 polymorphisms, which are due to point mutations in the structural gene, have long been associated with higher risk for bladder cancer. In collaborative studies, we now have found that NAT1 is also expressed polymorphically in human bladder due to mutations in the NAT1 polyadenylation signal, which has recently been associated with increased bladder cancer risk. Moreover, we have found that the bladder NAT1*10 genotype and phenotype are correlated with significantly higher levels of putative AA-DNA adducts in human bladder as measured by $^{32}$P-postlabelling. Preliminary data have also suggested that putative PAH-DNA adducts in human bladder are correlated with a polymorphism in the total metabolism of benzo[*a*]pyrene (BP) by bladder microsomes and especially with the formation of BP-7,8-diol. Since each of these correlations was observed without adjusting for carcinogen intake, it would appear that, with ubiquitous human exposure to AAs and PAHs, the expression of carcinogen-metabolizing enzymes may be a more critical determinant of carcinogen-DNA adduct formation and of individual cancer susceptibility.

*Keywords:* Aromatic amines; Polycyclic aromatic hydrocarbons; Urinary bladder; Acetyltransferases; Polymorphisms; DNA adducts

## 1. Enzyme polymorphisms, DNA adducts, and cancer susceptibility

The metabolic activation and detoxification pathways associated with the carcinogenic aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs) provide a useful model of enzyme polymorphisms that can modulate human carcinogenesis. Such interindividual variations in AA- and PAH-metabolizing enzymes can be due to genetic factors or to sustained interaction with environmental factors, either of which can be shown to be a determinant of

* Corresponding author. The genotyping portion of these studies were carried out in collaboration with Drs. Douglas A. Bell and Ari Hirvonen at the National Institute for Environmental Health Sciences, Research Triangle Park, NC and are described in full in Refs. [17–19].

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cancer risk in epidemiological studies [1]. Our efforts have been focused on the risk factors associated with human urinary bladder carcinogenesis and the enzymatic systems that are known to play a major role in the biotransformation of carcinogenic AAs and PAHs such as the *N*-acetyltransferases (NATs) and the cytochromes P450 (CYPs). Metabolic polymorphisms in these enzymatic systems would thus be expected to affect the levels of DNA adducts in the carcinogen-target tissues and thus modulate bladder cancer risk [2].

For AA-induced urinary bladder carcinogenesis (Fig. 1), current hypotheses [3–5] indicate that most AAs are initially metabolized in the liver through either *N*-hydroxylation by CYP1A2 or *N*-acetylation by NAT2. The *N*-hydroxy metabolite can be further metabolized in the liver by sulfotransferases and glucuronyltransferases to form phenolic sulfates and *N*-glucuronides that are major excretion products; or it can enter the circulation where it can be oxidized to a nitrosoarene that forms covalent adducts with hemoglobin. The remaining *N*-hydroxy metabolite then undergoes renal filtration into the urinary bladder lumen where it can be reabsorbed into the bladder mucosa. Although *N*-hydroxy arylamines can react with DNA at acidic urinary pH, further activation by NATs in the bladder has been suggested as a final activation step leading to DNA adducts, mutations and neoplasia. Enzyme polymorphisms that have been previously associated with these pathways include CYP1A2 and NAT2 [6]. Accordingly, the slow NAT2 phenotype, which arises as a consequence of point mutations in an intron-less gene, has long been associated with increased bladder cancer risk [7]; and more recently, cigarette smokers who are slow NAT2 and rapid CYP1A2 were found to possess the highest level of AA-hemoglobin adducts [8].

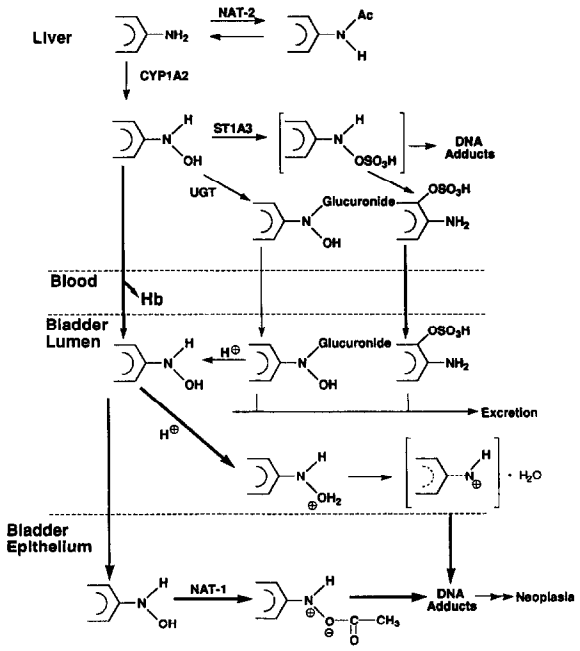


Fig. 1. Proposed metabolic activation and detoxification pathways for AA-induced human urinary bladder carcinogenesis.

In comparison to the AAs, the activation and detoxification pathways for PAHs in relation to urinary bladder carcinogenesis are less clear. However, benzo[*a*]pyrene (BP) is metabolized by cultured human bladder systems [9] and its metabolism in other human tissues such as lung and larnyx is consistent with the initial formation of BP-7,8-diol and 9-hydroxy-BP and their subsequent conversion to DNA-reactive bay-region diol-epoxides and K-region oxides (Fig. 2). CYPs 1A1, 2C9, and 3A4 have each been shown to catalyze these oxidative reactions in human tissues (reviewed in [10]); while glutathione *S*-transferases (GSTs) serve an important role in the detoxification of the arene oxides. In this regard, individuals lacking the GST M1 gene have been associated with a significantly increased risk to bladder cancer [11].

Carcinogen-DNA adducts have been detected in human urinary bladder using $^{32}$P-postlabelling methods (butanol extraction and nuclease P1 enhancement) that are selective for AA- and PAH-DNA adducts, respectively [12]. Current cigarette smokers had 2–3-fold higher adduct levels; and nearly half of the adducts detected were similar to those derived from PAHs, while the remainder exhibited properties consistent with their identity as AAs. Of these, the C8-dG adduct of 4-aminobiphenyl (ABP), which accounted for about 10% of the smoking-related adducts, was specifically identified and its pres-

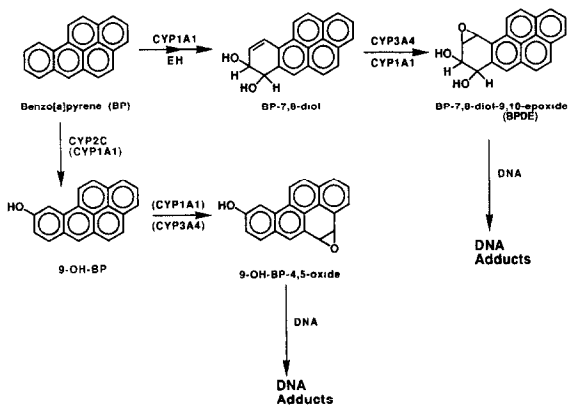


Fig. 2. Potential pathways for the metabolic activation of BP and other PAHs in human tissues.

ence in human bladder DNA was subsequently confirmed by GC/MS [13].

## 2. Metabolism of AAs and PAHs by human urinary bladder tissues and its role in carcinogen-DNA formation

To assess further the role of AAs and PAHs in human urinary bladder carcinogenesis, we have recently examined the relationship between metabolism and DNA adduct levels in 26 human bladder tissue samples obtained through the US Cooperative Tissue Network. Patient history and exposure information was not available for these samples, although historically some 80–90% of such samples originated from current cigarette smokers. The metabolic *N*-acetylation of *p*-aminobenzoic acid (PABA) to *N*-acetyl-PABA (NAT1 activity) and of sulfamethazine (SMZ) to *N*-acetyl-SMZ (NAT2 activity), and the *O*-acetylation of *N*-hydroxy-ABP (OAT activity; catalyzed by NAT1 and NAT2) was measured in bladder cytosols; and the capacity of bladder microsomes to catalyze oxidative metabolism of BP was determined. DNA was also isolated for determination of *NAT1* and *NAT2* genotype and for analyses of carcinogen-DNA adducts using the butanol extraction and nuclease P1 enhancement methods.

Substantial levels of both PABA and OAT activities were found in all of the bladder cytosols (Table 1). However, SMZ activities were below the assay limits of detection. This observation confirmed the findings that NAT2 activity is poorly expressed in human bladder cytosols in relation to NAT1 activity [14]. In addition, nearly all of the bladder microsomes metabolized BP at low but readily detectable levels (Table 1).

$^{32}$P-Postlabelling analysis for putative car-

Table 1
Metabolism and DNA adduct levels (RAL) in human urinary bladder

| Measurement (units; number of individuals) | | Mean ± S.D. |
|---|---|---|
| (A) | PABA *N*-acetylation (nmol/min/mg protein; $n = 26$) | 2.9 ± 2.3 |
| | SMZ *N*-acetylation (pmol/min/mg protein; $n = 26$) | <10 |
| | *N*-Hydroxy-ABP OAT (pmol bound/mg DNA; $n = 26$) | 43 ± 21 |
| | Total BP metabolism (pmol/min/mg protein; $n = 22$) | 1.95 ± 0.62 |
| | RAL (AA-DNA adducts/$10^8$ dNp; $n = 22$) | 2.4 ± 2.0 |
| | RAL (PAH-DNA adducts/$10^8$ dNp; $n = 22$) | 2.5 ± 2.1 |
| (B) | RAL (AA adducts/$10^8$ dNp); rapid NAT1 phenotype ($n = 13$) | 1.7 ± 1.7* |
| | RAL (AA adducts/$10^8$ dNp); slow NAT1 phenotype ($n = 13$) | 3.2 ± 2.2* |
| | RAL (PAH adducts/$10^8$ dNp); rapid BP metabolizer ($n = 11$) | 1.6 ± 1.1** |
| | RAL (PAH adducts/$10^8$ dNp); slow BP metabolizer ($n = 11$) | 3.6 ± 2.2** |
| (C) | PABA *N*-acetylation (nmol/min/mg protein); *NAT1*4/NAT1*4* genotype ($n = 17$) | 2.3 ± 2.6*** |
| | PABA *N*-acetylation (nmol/min/mg protein); *NAT1*4/NAT1*10* genotype ($n = 8$) | 4.6 ± 1.6*** |
| | RAL (AA adducts/$10^8$ dNp); *NAT1*4/NAT1*4* genotype ($n = 17$) | 1.8 ± 1.9**** |
| | RAL (AA adducts/$10^8$ dNp); *NAT1*4/NAT1*10* genotype ($n = 8$) | 3.5 ± 2.1**** |

* $P = 0.05$; ** $P = 0.02$; *** $P = 0.03$; **** $P = 0.05$, using the Mann-Whitney rank sum test.

cinogen-DNA adducts gave distinct chromatographic profiles that were indicative of both AA- and PAH-DNA adducts and at similar levels (Table 1A). Furthermore, the AA-DNA adduct levels in these tissues correlated with their NAT1-dependent PABA activities; and the PAH-DNA adducts correlated with both the total BP-metabolizing capacity as well as with the formation of BP-7,8-diol, a known proximate carcinogenic metabolite (Table 2).

Statistical analyses and probit and NTV plots indicated that both NAT1 activity and total BP metabolism in the human bladder tissue samples were not normally distributed and appeared bimodal. For NAT1 and BP activities, these observations allowed arbitrary designation of slow and rapid (2-fold higher) acetylation phenotypes, with cutpoints near their median values. Within each of these subgroups, NAT1 correlated well with OAT, which provided additional support for the existence of 2 NAT1 phenotypes since this correlation was unobtainable when all NAT1 and OAT data points were examined together. Moreover, the apparent polymorphisms both in NAT1 and in BP metabolism were associated with significant differences in the levels of the corresponding DNA adducts in the bladder tissues examined (Table 1B). Specifically, the rapid NAT1 and rapid BP metabolizer phenotypes each exhibited a 2–3-fold higher level of putative AA- and PAH-DNA adducts, respectively. Thus, even in the absence of exposure information in these individuals, DNA adduct levels in the human urinary bladder appear to be influenced predominantly by individual differences in bladder NAT1- or BP-metabolizing activity.

## 3. A genetic polymorphism in *NAT1* and its relation to NAT1 phenotype and AA-DNA adduct levels in human urinary bladder

*NAT1*, which codes for the acetyltransferase activity originally thought to be monomorphic, has recently been shown to exhibit polymorphisms in non-coding regions [15]. The predominant alleles, designated NAT1*4 and *NAT1*10*, differ by a single base at nt 1088, which represents an alteration in the consensus polyadenylation signal. Examination of DNA sequence polymorphisms in the *NAT1* gene by PCR have demonstrated that the *NAT1* polyadenylation polymorphism is associated with significant differences in bladder NAT1 enzyme activity (Table 1C). Accordingly, NAT1 activity in the bladder of individuals with the heterozygous *NAT1*10* allele was 2-fold higher than in subjects with the homozygous *NAT1*4* allele. Furthermore, putative AA-DNA adduct levels in the urinary bladder were similarly found to be 2-fold higher in individuals with the heterozygous *NAT1*10* allele, as compared to those with *NAT1*4* allele. Thus, these data provide strong support for the hypothesis that NAT1 activity in the urinary bladder mucosa represents a major

Table 2
Correlation coefficients ($r$) and $P$ values

| Comparisons | $r$ ($P$) |
|---|---|
| NAT1 activity vs. RAL (AA-DNA adducts) | 0.52 (0.01) |
| OAT activity vs. RAL (AA-DNA Adducts) | ns[a] |
| Total BP metabolism vs. RAL (PAH-DNA adducts) | 0.52 (0.02) |
| BP-7,8-diol formation vs. RAL (PAH-DNA adducts) | 0.71 (0.02) |
| 9-Hydroxy-BP formation vs. RAL (PAH-DNA adducts) | 0.44 (0.06) |
| NAT1 activity vs. OAT | ns[a] |
| Slow NAT1 phenotype vs. OAT | 0.66 (0.01) |
| Rapid NAT1 phenotype vs. OAT | 0.71 (0.01) |

Correlation coefficients and $P$ values were calculated using the Spearman rank correlation test.
[a] ns, not significant.

bioactivation step that converts urinary *N*-hydroxy arylamines to reactive *N*-acetoxy esters that form covalent DNA adducts (Fig. 1).

Since previous studies have indicated that hepatic NAT2 activity is an important detoxification step for bladder carcinogenesis and individuals with slow *NAT2* genotype were found to be at higher risk for bladder cancer [16], one would predict that individuals who possess a slow *NAT2* and the rapid *NAT1*10* genotypes would be at highest risk of developing this type of neoplasm. Indeed, this combined genotype (slow *NAT2/NAT1*10*) indeed exhibited the highest carcinogen-DNA adduct level in our study. Moreover, slow NAT2 phenotype and rapid CYP1A2 have been implicated in the activation (*N*-oxidation) and detoxification (*N*-acetylation) of AAs for human bladder carcinogenesis.

In conclusion, these data provide the first evidence for phenotypic and genotypic polymorphisms in both NAT1 and NAT2 and a phenotypic polymorphism in the metabolic activation of BP that are predictive of DNA adduct levels in the human urinary bladder. Moreover, the enzyme which has long been thought to be responsible for monomorphic NAT1 activity, is shown to be regulated by a polymorphic gene, *NAT1*, and it is bimodally distributed in the human urinary bladder in a manner that is significantly correlated with the DNA adduct levels found in this tissue. Together with the known polymorphisms for NAT2, CYP1A1, CYP1A2, GSTM1 and sulfotransferase, it is evident that metabolic phenotypes/genotypes can significantly influence DNA adduct formation in urothelial cells and could ultimately lead to a wide variation in urinary bladder cancer risk. Therefore, future epidemiological studies will need to include molecular biomarkers of individual susceptibility, especially those involved in carcinogen activation and detoxification.

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