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The impact of interindividual variation in NAT2 activity on benzidine urinary metabolites and urothelial DNA adducts in exposed workers

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ABSTRACT **Several epidemiologic studies indicate that NAT2-related slow *N*-acetylation increases bladder cancer risk among workers exposed to aromatic amines, presumably because *N*-acetylation is important for the detoxification of these compounds. Previously, we showed that NAT2 polymorphisms did not influence bladder cancer risk among Chinese workers exposed exclusively to benzidine (BZ), suggesting that NAT2 *N*-acetylation is not a critical detoxifying pathway for this aromatic amine. To evaluate the biologic plausibility of this finding, we carried out a cross-sectional study of 33 workers exposed to BZ and 15 unexposed controls in Ahmedabad, India, to evaluate the presence of BZ-related DNA adducts in exfoliated urothelial cells, the excretion pattern of BZ metabolites, and the impact of NAT2 activity on these outcomes. Four DNA adducts were significantly elevated in exposed workers compared to controls; of these, the predominant adduct cochromatographed with a synthetic *N*-(3′-phosphodeoxyguanosin-8-yl)-*N*′-acetylbenzidine standard and was the only adduct that was significantly associated with total BZ urinary metabolites ($r = 0.68$, $P < 0.0001$). To our knowledge this is the first report to show that BZ forms DNA adducts in exfoliated urothelial cells of exposed humans and that the predominant adduct formed is *N*-acetylated, supporting the concept that monofunctional acetylation is an activation, rather than a detoxification, step for BZ. However, because almost all BZ-related metabolites measured in the urine of exposed workers were acetylated among slow, as well as rapid, acetylators (mean ± SD 95 ± 1.9% vs. 97 ± 1.6%, respectively) and NAT2 activity did not affect the levels of any DNA adduct measured, it is unlikely that interindividual variation in NAT2 function is relevant for BZ-associated bladder carcinogenesis.**

Aromatic amines must be metabolized within the host in order to exert mutagenic or carcinogenic activity (1). For many aromatic monoamines, including those found in tobacco smoke such as 4-aminobiphenyl (4-ABP) and 2-naphthylamine, *N*-acetylation appears to be a detoxification pathway, with the acetylated metabolite being excreted into the urine before it can be *N*-oxidized to a reactive form (2). In contrast, acetylation may be an activation pathway for benzidine (BZ), an aromatic diamine, in that *N*-acetylation of one amine group has been shown to facilitate *N*-oxidation to a reactive species by cytochrome P-450 (3, 4). Alternatively, BZ may be directly activated by prostaglandin-H synthetase in the bladder to benzidinediimine (the two-electron oxidation product), which can bind to DNA (5, 6). Thus, for BZ, the impact of acetylation on the production of the critical activated metabolite(s) is less certain and may be more complicated than for aromatic monoamines.

The capacity to *N*-acetylate is polymorphic in humans (2); slow acetylators are homozygotic for a mutated *N*-acetyltransferase gene (*NAT2*) that is generally responsible for decreased activity (7, 8). In 1979, Lower *et al.* (9) proposed that individuals with the slow NAT2 phenotype might be at higher risk for aromatic amine-associated bladder cancer; support for this followed from a series of epidemiologic studies that, overall, showed that individuals with the slow NAT2 phenotype (10) or genotype (11) are at greater risk of developing bladder cancer. Higher risks have generally been reported for individuals with occupational aromatic amine exposure (10), with the most striking examples being an English study showing a 17-fold risk (12) and a Polish study showing an 8-fold risk (13) for slow acetylators. Although BZ was reportedly present in these workplaces (12, 13), at least some subjects were also exposed to 2-naphthylamine (ref. 12; J. Hanke, personal communication). Since both compounds have been causally linked to bladder cancer in humans (14, 15), it is uncertain to what extent each compound contributed to the bladder cancer excess in these populations and influenced the impact of NAT2 phenotype on this process.

To evaluate the risk of the NAT2 slow phenotype for bladder cancer among workers exposed exclusively to BZ, we identified a cohort of workers in China who had been employed in BZ production and use facilities. There was an overall 25-fold increased risk of bladder cancer among exposed workers, increasing to 158-fold for the most heavily exposed subjects (16). A case-control study of surviving bladder cancer cases from the cohort (17) showed that neither the slow NAT2 phenotype nor genotype was associated with an increased risk of bladder cancer (odds ratio for slow phenotype = 0.3; 95% confidence interval = 0.1–1.3). In addition, all *NAT2* alleles identified in this population had been previously detected in Caucasians and similarly correlated with NAT2 phenotype (18). These results suggest that *N*-acetylation by NAT2 is not a key detoxifying pathway for BZ.

The precise role of *N*-acetylation in the metabolism of BZ in humans cannot easily be studied experimentally since there is no animal model that exactly reflects both human metabolism and BZ-associated cancer risk. Specifically, humans *N*-acetylate (2) and are at excess risk for only bladder cancer from BZ exposure (19–22). In contrast, rats *N*-acetylate, form acetylated BZ DNA adducts in liver cells (3, 4, 23–25), and develop only liver cancer from BZ exposure (14, 26), whereas

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Abbreviations: BZ, benzidine; ABZ, *N*-acetylbenzidine; DBZ, *N,N*′-diacetylbenzidine; NAT2, *N*-acetyltransferase; C8dG, *N*-(3′-phosphodeoxyguanosin-8-yl); 4-ABP, 4-aminobiphenyl.

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dogs do not *N*-acetylate BZ (14, 25) and develop only bladder cancer from this exposure (14).

Since DNA adducts have not been characterized previously in humans exposed to BZ, the critical activating metabolic pathway(s) for this compound is uncertain. To provide insight into this process, and to evaluate the biologic plausibility that the slow NAT2 phenotype is not a risk factor for BZ-associated bladder cancer in humans, we carried out a cross-sectional study of 33 male workers exposed to BZ and 15 unexposed male controls in Ahmedabad, India. Here, we report the pattern of BZ metabolites found in the urine of exposed workers, the presence and type of DNA adducts detected in their exfoliated urothelial cells, and the impact of NAT2 activity on these outcomes.

MATERIALS AND METHODS

Subject Enrollment and Biologic Sample Collection. The field phase of the study took place in 1993 in Ahmedabad, India. Consenting subjects were enrolled into the study using Institutional Review Board-approved procedures. Eligibility criteria for BZ-exposed subjects were being in good general health, having worked during the previous 6 mo in a factory that manufactured BZ-related compounds, and having no history of cancer. Eligibility criteria for controls were as above, apart from never having worked in a job with potential for exposure to aromatic amines. Fifteen subjects were enrolled from four factories that manufactured BZ dihydrochloride (BZ workers), and 18 subjects were enrolled from three factories that manufactured BZ-based dyes (primarily Direct Black 38), using BZ dihydrochloride as a starting product (BZ dye workers). The factories were generally dusty, and workers had extensive dermal and respiratory contact with these compounds. Fifteen subjects from a building construction company (controls) were frequency-matched to the exposed workers on age (5-yr intervals) and current smoking status.

Subjects were administered a questionnaire by trained interviewers, collecting information on occupational and medical history, current and lifelong tobacco use, and usual alcohol intake. Subjects provided a first morning void urine sample for 2 successive days and a post-workshift urine sample. For determination of NAT2 phenotype, subjects consumed caffeinated coffee and provided urine samples as described (27). In addition, a 14-ml peripheral blood sample was collected from each subject.

First morning urines were made 20% glycerol to prevent cell lysis during freezing. Urine samples for caffeine metabolite analysis were acidified with ascorbic acid at 20 mg/ml. Peripheral blood samples were fractionated into plasma, leukocytes, and red blood cells. All samples were stored at −20°C for up to 3 weeks, shipped on dry ice to a National Cancer Institute biorepository, and stored at −70°C. Laboratories receiving biologic samples for analysis were "blinded" with respect to the exposure status of study subjects.

BZ Metabolite Analysis. Post-workshift urine samples were analyzed at the Veterans Affairs Medical Center in St. Louis for BZ, *N*-acetylbenzidine (ABZ), and *N,N'*-diacetylbenzidine (DBZ) by capillary gas chromatography/negative chemical ion mass spectrometry as described (28).

BZ–DNA Adduct Analysis. First morning void urine samples were analyzed at the University of Cincinnati for DNA adducts. Cell isolation and ^{32}P-postlabeling were done essentially as described (29, 30), with the exceptions noted below. Samples were rapidly thawed and then vacuum filtered using 4–12 10-μm nylon filters. Cells collected were stored in 10% glycerol until DNA isolation and ^{32}P-postlabeling were done. Samples were postlabeled using 250 μCi [^{32}P]ATP (1 Ci = 37 GBq) per sample as reported earlier (29, 31). Analysis of unadducted nucleotides indicated that there was an excess of radiolabeled ATP in each sample. Chromatographic conditions detected *N*-(3'-phosphodeoxyguanosin-8-yl) (C8dG)-ABZ (25), C8dG-BZ (32), and the major smoking-associated DNA adducts—e.g., C8dG-4-ABP (29, 31, 33). Adducts were quantified as described (29, 30) and expressed as relative adduct labeling (calculated as $cpm_{adducts}/cpm_{unadducted\ nucleotides}) \times 10^9$. The value reported for each individual is the mean of at least two and as many as six independent replications (samples were repeated if there were discrepancies between replicates within a run).

Table 1. Demographic characteristics and BZ metabolite levels by exposure group, in a study of workers exposed to BZ in Ahmedabad, India, 1993

Characteristic	Exposure group		
	Controls (n = 15)	BZ dye (n = 18)	BZ (n = 15)
Age, yr	26.5 ± 5.1	23.8 ± 3.7	23.3 ± 4.1
Bidi use, no./day	2.2 ± 3.7	3.2 ± 4.4	2.2 ± 4.1
Current job duration, mo.	68.3 ± 40.3	42.0 ± 19.3	38.9 ± 15.9
Urinary BZ metabolites,* ng/μmol	0.0	2.7 ± 5.6†	46.5 ± 30.5‡

Levels are given as mean ± SD.
*BZ + ABZ + DBZ (ng/μmol of creatinine) in post-workshift urine samples.
†Range, 0.2–24.0 ng/μmol of creatinine.
‡Range, 10.2–110.6 ng/μmol of creatinine.

NAT2 Phenotype Analysis. Urine samples collected for evaluation of NAT2 phenotype were analyzed at the National Institute for Occupational Safety and Health for the caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil and 1-methylxanthine by HPLC as reported (27). Subjects with urinary 5-acetylamino-6-formylamino-3-methyluracil/1-methylxanthine/molar ratios <0.6 were considered slow acetylators and those with values ≥0.6 were designated rapid acetylators.

***NAT2* and *NAT1* Genotype Analysis.** DNA was extracted from peripheral leukocytes by high-salt precipitation and resuspended in TE buffer (10 mM Tris/1 mM EDTA). *NAT2* genotype was determined at the National Institute of Environmental Health Sciences by PCR-restriction fragment length polymorphism (34) and allele-specific PCR (35) for the five most common functional and low-activity *NAT2* alleles (WT, M1, M2, M3, and M4 corresponding to *NAT2*4, NAT2*5B, NAT2*6A, NAT2*7A*, and *NAT2*14A*, respectively). The method of Bell *et al.* (36) was used to test for the four common sequence variants in the 3' region of *NAT1* near the polyadenylylation signal (37) including the *NAT1*10* allele, which has been associated with elevated *N*-acetylation activity in human bladder samples (38), and *NAT1*3, NAT1*4*, and *NAT1*11*, which are thought to have substantial but lower activity.

Statistical Analysis. Summary demographic and BZ-related data are presented as mean ± SD or median (range). The Kruskall–Wallis test was used to assess group differences in adduct levels and the Wilcoxon sign rank test was used to determine if a given adduct was significantly higher compared to other adducts within each study population. Samples with nondetectable values were assigned half the value of the sample with the lowest value in the same population.

Linear regression was used (*i*) to test for a trend in adduct levels (using the mean value of total BZ metabolites for each group), (*ii*) to evaluate the association between BZ urinary metabolites and DNA adducts, (*iii*) to determine the impact of interindividual variation in *N*-acetylation on the ratio of acetylated to total BZ metabolites and DNA adduct levels, and (*iv*) to assess potential confounding by age, tobacco, and alcohol use. All models used DNA adduct and total BZ metabolite values normalized by a natural logarithmic transformation. Two-sided *P* values were calculated throughout; *P* values <0.05 were considered significant.

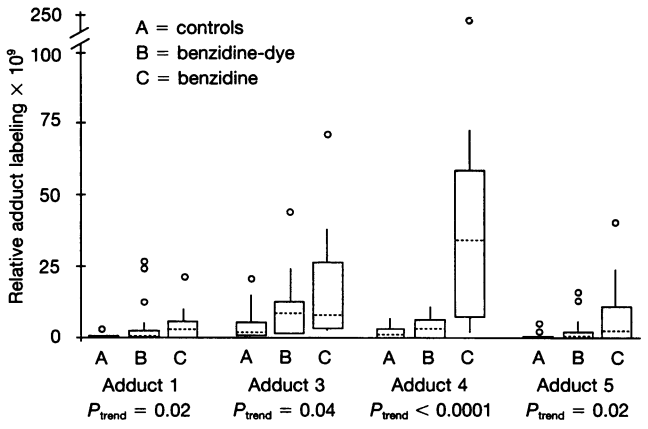

FIG. 1. Box and whisker plots for ^{32}P-postlabeled DNA adducts in exfoliated urothelial cells from controls, workers who manufactured BZ-based dyes (benzidine-dye), and workers who manufactured BZ dihydrochloride (benzidine). Whiskers represent upper and lower 25th percentile of data, respectively. - - -, Median; ○, outlier (defined as >1.5 × height of box) (test for trend by linear regression).

RESULTS

The age and bidi (predominant form of tobacco) use of subjects in the control, BZ dye, and BZ groups were comparable (Table 1). Workers in the latter two groups had been employed in their factories for similar periods of time (Table 1) and rotated through all jobs.

Current BZ exposure was assessed by measuring urinary BZ metabolites in post-workshift urine samples, because these integrate exposure received by both inhalation and percutaneous routes (the latter being a major route of absorption in humans) (14, 39) (Table 1). BZ-related compounds (sum of BZ, ABZ, and DBZ) were nondetectable in control subjects, present at relatively low levels in BZ dye workers, and ≈17 times higher, on average, in BZ workers.

A total of 11 putative DNA adducts were identified in the exfoliated urothelial cells of the 48 subjects, but not all were present in samples from any one subject. Four of the 11 adducts were significantly different (or approached significance) between the three groups; adduct 1 (P = 0.02), adduct 3 (P = 0.06), adduct 4 (P = 0.0001), and adduct 5 (P = 0.05). Adduct 4 showed the strongest dose–response relationship across controls, BZ dye workers, and BZ workers (Fig. 1), which changed minimally after adjustment for age, alcohol use, and bidi use. Further, adduct 4 was the predominant adduct present in the BZ workers (median = 34.7 relative adduct labeling × 10^9) and was significantly higher (P < 0.01) than levels of adducts 1, 3, or 5 (median = 3.8, 5.7, and 2.6 relative adduct labeling × 10^9, respectively). Adducts 2 and 6–11 were present at levels substantially below adduct 4 and were similarly distributed across the three study populations (data not shown). Autoradiograms from a control subject and two BZ workers are shown in Fig. 2 *A–C*, respectively. Fig. 2*D* is an autoradiogram of synthetic C8dG-ABZ, the acetylated BZ–DNA adduct standard, which chromatographed to the same position as adduct 4 shown in Fig. 2 *B* and *C*.

To confirm that adduct 4 was C8dG-ABZ, we performed cochromatography experiments using the C8dG-ABZ, C8dG-BZ, and C8dG-4-ABP standards. Fig. 3*A* is an autoradiogram of a mixture of the three DNA adduct standards. Fig. 3 *B* and *C* are autoradiograms of urothelial cell DNA from a BZ worker alone and combined with the adduct standard mixture, respectively, demonstrating that C8dG-ABZ cochromatographed with adduct 4. Further, cochromatography studies of DNA from two BZ workers showed that radioactivity in the adduct 4 position was increased by 92 ± 10% of the amount expected by the addition of the C8dG-ABZ standard. These studies also indicated that C8dG-BZ, the nonacetylated BZ–DNA adduct, was not present in the study subjects because the standard chromatographed to a different position than any adduct found in samples from the subjects. The possibility that adduct 3 represents C8dG-4-ABP cannot be completely excluded because the standard chromatographed close to its position (Fig. 3*C*).

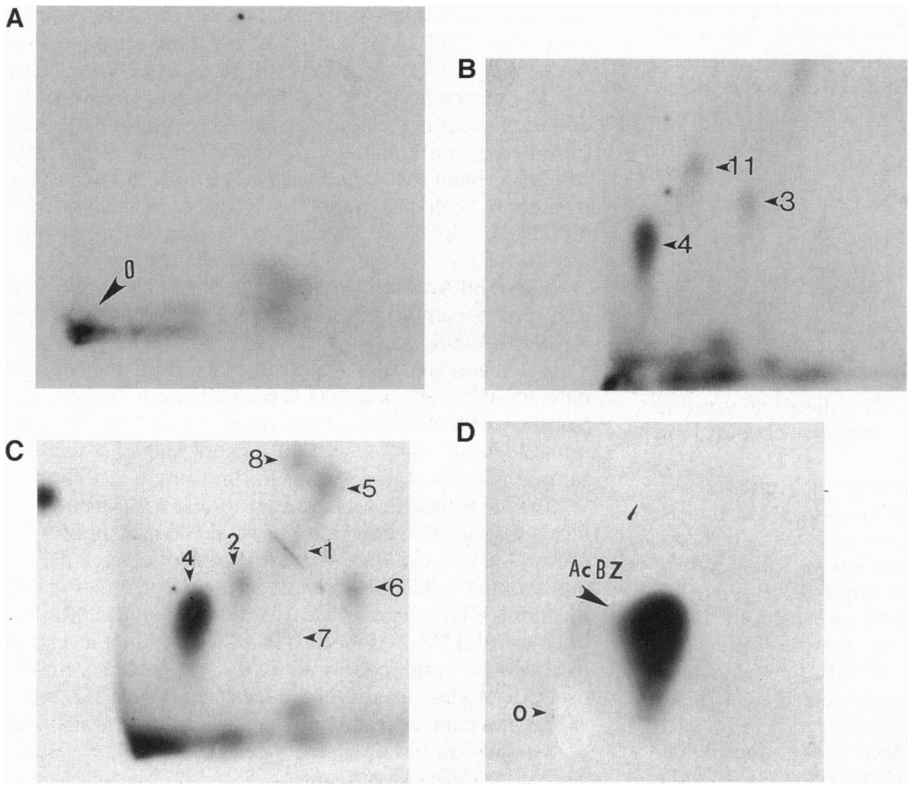

FIG. 2. Autoradiograms of ^{32}P-postlabeled human exfoliated urothelial cell DNA obtained from a subject control, BZ workers (*B* and *C*), and a synthetic C8dG-ABZ (AcBZ) standard (*D*). The origin is located in the lower left of all autoradiograms.

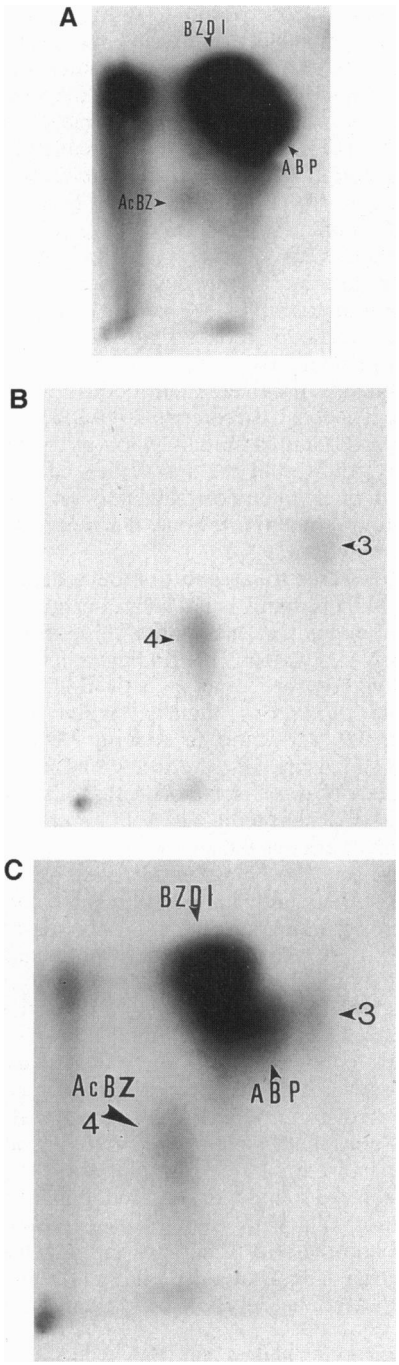

FIG. 3. Cochromatography studies of ^{32}P-postlabeled adduct 4 with aromatic amine DNA standards. (*A*) Synthetic C8dG-ABZ (AcBZ), C8dG-BZ (BZDI, benzidine diimine), and C8dG-4-ABP (ABP) standards. (*B*) Exfoliated urothelial cell DNA from a BZ worker. (*C*) Cochromatography of the mixed adduct standards added to the sample from the BZ worker.

The association between total urinary BZ metabolites and adducts 1, 3, 4, and 5 for exposed workers is presented in Fig. 4. There was a strong and highly significant positive correlation only for adduct 4 (Pearson $r = 0.68$, $P < 0.0001$). The number of months subjects worked in their factories was not associated with any DNA adduct (data not shown).

NAT2 phenotype was assessed for the 48 subjects. Of these, 33 (68.7%) were slow acetylators and 15 (31.3%) were rapid acetylators, consistent with a previous report on the distribution of acetylation phenotype in India (2). There was a concordance between the phenotype predicted by *NAT2* genotype and the phenotype measured by caffeine metabolites for 45 of 48 (93.8%) subjects. Three subjects were phenotypically slow acetylators but had only one mutant *NAT2* allele detected.

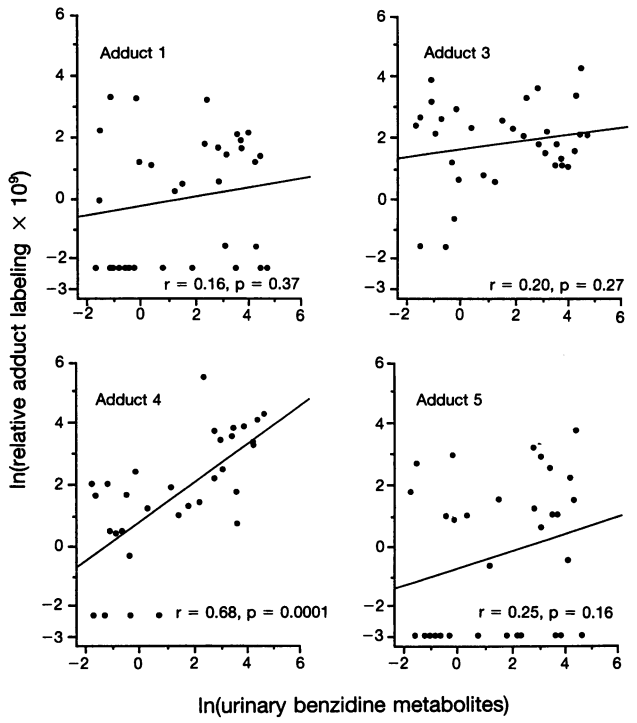

FIG. 4. Correlation between total BZ urinary metabolites (BZ + ABZ + DBZ, ng/μmol of creatinine) and ^{32}P-postlabeled DNA adducts in exfoliated urothelial cells from 33 workers exposed to BZ (r = Pearson correlation coefficient).

There was only a marginal difference in the ratio of acetylated to total measured urinary BZ metabolites—i.e., (ABZ + DBZ)/(ABZ + DBZ + BZ)—for subjects with slow ($n = 23$) versus rapid ($n = 7$) phenotypes; 95.0 ± 1.9% of BZ metabolites were acetylated among slow acetylators, whereas 97.0 ± 1.6% of BZ metabolites were acetylated among rapid acetylators (Fig. 5). Although this difference was significant ($P = 0.01$, Wilcoxon rank sum test), the magnitude of the difference was quite small (2%). Similar results were obtained using subjects categorized by *NAT2* genotype into individuals with two mutations ($n = 20$, 95.0 ± 2.0% of BZ metabolites acetylated) versus individuals wild type or with only one mutation ($n = 10$, 96.5 ± 1.9% of BZ metabolites acetylated; $P = 0.06$ for difference).

The relationship between various measures of NAT2 function, the proportion of urinary BZ metabolites acetylated, and DNA adduct levels were evaluated by multiple linear regression. After adjusting for total BZ metabolites, NAT2 activity (as a continuous measure) and rapid NAT2 phenotype were significantly associated with a tendency to form acetylated BZ metabolites (Table 2). In contrast, there were no significant associations detected between any measure of NAT2 function and adducts 1, 3, 4, or 5 (Table 2). These relationships were minimally changed after adjustment for age, alcohol use, and bidi use.

Human recombinant NAT1 has been shown to *N*-acetylate BZ (40) *in vitro* about 50 times more efficiently—i.e., V_{max}/K_m—than NAT2 (D. Hein, personal communication). Further, NAT1 is expressed in human liver and urothelial cells (41–43), and the *NAT1*10* polymorphism has been associated with elevated *N*-acetylation activity (38) and DNA adduct levels (44) in human bladder samples, most of which were presumed to have come from current tobacco smokers (44). We there-

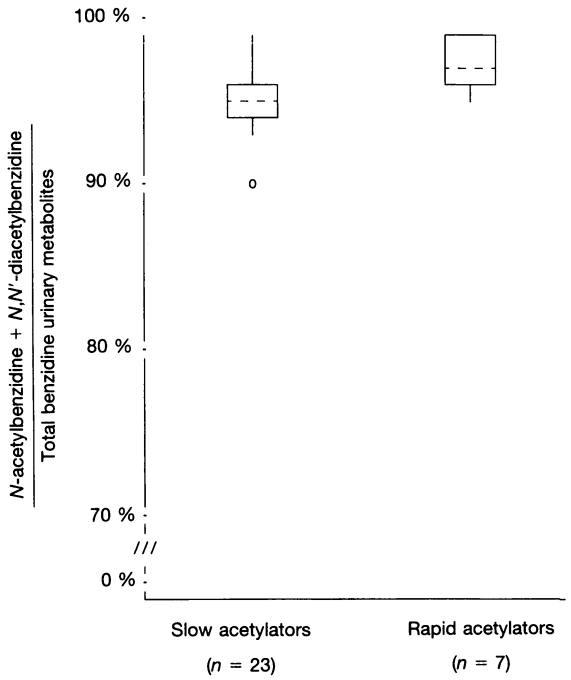

FIG. 5. Ratio of acetylated urinary BZ metabolites to all measured urinary BZ metabolites by NAT2 phenotype ($P = 0.01$ by Wilcoxon rank sum test). Data are presented for 30 BZ-exposed subjects who had detectable urinary BZ metabolites.

fore evaluated the impact of this allele on the proportion of BZ urinary metabolites acetylated and urothelial cell DNA adduct levels. Twenty of 30 workers with detectable urinary BZ metabolites carried the *NAT1*10* allele, but it was not significantly associated with any outcome tested (Table 2).

DISCUSSION

We studied the pattern of BZ metabolites, the presence and structure of DNA adducts in exfoliated urothelial cells, and the impact of NAT2 activity on these outcomes in a cross-sectional study of 33 workers currently exposed to BZ and 15 unexposed controls. More than 90% of measured urinary BZ compounds were present in acetylated form in every exposed subject who had detectable metabolites, consistent with a previous study of workers exposed to BZ-based dyes (45) and with *in vitro* studies of human S9 preparations (46) and fresh human liver slices (47). There was no evidence that *N*-acetylation of benzidine became rate-limiting as exposure levels increased to very high levels, even among subjects with the slow NAT2 phenotype. For example, 95% of urinary BZ metabolites were acetylated in the most highly exposed worker with the slow acetylation phenotype, whose urine contained BZ at a level comparable to that found in workers from a factory where bladder cancer risk had been exceptionally elevated—i.e., 0.06 mg of BZ/liter versus a mean of ≈0.04 mg of BZ/liter (48), respectively.

Four DNA adducts were elevated in exfoliated urothelial cells from exposed workers compared to controls. Of these, adduct 4 was present at the highest level among the BZ workers, cochromatographed with a synthetic acetylated standard, and was the only adduct significantly associated with post-workshift urinary BZ metabolites. Interestingly, this adduct has been presumptively identified as the predominant DNA adduct in liver cells of rodents exposed *in vivo* and *in vitro* to BZ (3, 4, 23–25) and in human lymphocytes incubated with BZ (49). The identities of the other three adducts are uncertain, but are unlikely to be nonacetylated BZ adducts because they chromatographed to locations that were distinctly different from the standard.

To our knowledge, this is the first demonstration that BZ forms DNA adducts in urothelial cells of exposed humans and that the predominant adduct under these chromatographic conditions is *N*-acetylated. This finding supports the hypothesis that acetylation represents an activation step for at least one BZ-related adduct in humans, similar to the pathway proposed for BZ activation in rodents (3, 4, 23, 24, 50). However, no measure of interindividual variation in *N*-acetylation activity was associated with adduct 4, consistent with the small or absent impact of *NAT2* and *NAT1* alleles on the proportion of urinary BZ metabolites that were acetylated.

BZ was suspected to be a bladder carcinogen before 1940 (14) and was subsequently banned in many countries. Nevertheless, thousands of workers are still at increased cancer risk from past exposure. Similarly, though India banned the production and use of BZ and BZ-based dyes in 1994, workers who were exposed to these compounds will be at substantially increased risk of bladder cancer in the future. The results reported here, combined with our finding that *NAT2* polymorphisms were not associated with bladder cancer among workers exposed only to BZ (17), suggest that slow NAT2 activity does not identify a subgroup of BZ-exposed workers who are at increased risk of developing this disease.

In conclusion, we found that the predominant DNA adduct formed in urothelial cells of workers exposed to BZ was *N*-acetylated and that NAT2 activity had only a small impact on the formation of acetylated BZ metabolites in urine and no impact on DNA adduct levels. To the extent that BZ-related

Table 2. Influence of various measures of NAT2 function and *NAT1* genotype on BZ metabolite pattern and DNA adduct formation in a study of workers exposed to BZ in Ahmedabad, India, 1993

	NAT2 Activity†			NAT2 Phenotype‡			*NAT2* Genotype§			*NAT1* Genotype¶		
Outcome*	*b*	(SE)	*P* value	*b*	(SE)	*P* value	*b*	(SE)	*P* value	*b*	(SE)	*P* value
Acetylated metabolites/ total metabolites‖	0.03	(0.01)	0.03	0.02	(0.008)	0.03	0.01	(0.008)	0.09	0.002	(0.009)	0.80
Adduct 1	−1.1	(1.2)	0.35	−0.1	(0.9)	0.87	−1.1	(0.7)	0.13	−1.3	(0.8)	0.10
Adduct 3	−0.8	(0.8)	0.29	−1.0	(0.6)	0.09	−0.3	(0.5)	0.50	−0.4	(0.5)	0.45
Adduct 4	−0.6	(0.9)	0.49	−0.2	(0.7)	0.80	0.04	(0.6)	0.94	−0.08	(0.6)	0.90
Adduct 5	1.3	(1.4)	0.37	0.6	(1.1)	0.61	1.5	(0.9)	0.09	−0.05	(0.9)	0.96

The study included 30 BZ-exposed subjects with detectable metabolites. *b*, Regression coefficient.
*Linear regression on ln(outcome), adjusted for ln(total BZ metabolites).
†NAT2 activity as a continuous measure (range, 0.10–1.13).
‡NAT2 phenotype [coded as slow = 1 ($n = 23$) and rapid = 2 ($n = 7$)].
§*NAT2* genotype [coded as subjects with two copies of any combination of *NAT2*5B, NAT2*6A, NAT2*7A, NAT2*14A* = 1 ($n = 20$), associated with slow activity; subjects with no or one copy of any of these alleles = 2 ($n = 10$), associated with rapid activity].
¶*NAT1* genotype [coded as subjects with no copies of the *NAT*10* allele = 1 ($n = 10$); subjects with one or two copies of the *NAT1*10* allele = 2 ($n = 20$), associated with elevated activity.]
‖(ABZ + BZ)/(BZ + ABZ + DBZ) measured in post-workshift urine samples.

bladder carcinogenesis is mediated via DNA adduct formation, our findings indicate that *N*-acetylation is an activation step for BZ-induced bladder cancer in humans but that the known *NAT2* and *NAT1* polymorphisms are unlikely to be risk factors for this process. Finally, our studies suggest that gene–environment interactions can be highly exposure-specific, and they reinforce the need to accurately assess chemical exposures in investigations that seek to evaluate such relationships.

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