Interindividual Variation in Carcinogen Metabolism and Bladder Cancer Risk

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Epidemiological studies indicate that subjects of the genetically based slow acetylator phenotype may be at higher risk for bladder cancer than fast acetylators, particularly when they are exposed to carcinogenic arylamines: *N*-acetylation is a detoxification step in the metabolism of some arylamines. We describe two collaborative studies on tobacco smoking, in which markers of internal dose (arylamine–hemoglobin adducts) and markers of genetically based metabolic polymorphism have been coupled. In the first investigation, we found that hemoglobin adducts formed by mononuclear arylamines have high reciprocal correlation coefficients, as do adducts from binuclear arylamines. This tendency of adducts with structurally similar arylamines to correlate reciprocally explains a large proportion of the residual variance seen after controlling for smoking habits (number and type of cigarettes). In the second study, the concentration of 4-aminobiphenyl–hemoglobin adducts varied according to three independent determinants: number of cigarettes, type of tobacco (air or flue cured), and acetylator phenotype (slow and fast). The dose–response relationship between the amount of tobacco smoked and level of 4-aminobiphenyl–hemoglobin adducts in the slow acetylators (with an immediate steep increase of the adducts) was different from that in the fast acetylators (with a more regular increase). These findings from "molecular epidemiology" may contribute to an understanding of the role of metabolic polymorphism in human carcinogenesis.

Introduction

Interindividual variability in the metabolism of arylamines has been suggested in several epidemiological investigations to be relevant to human bladder cancer. The purpose of this paper is to review such evidence briefly and to report the relevant findings of two studies on the "molecular epidemiology" of bladder cancer.

Epidemiological Evidence on *N*-Acetyltransferase Polymorphism and Bladder Cancer Risk

N-Acetyltransferase is involved in the detoxication of arylamines, a class of chemicals that includes some well-known bladder carcinogens. The activity of the enzyme shows genetically based polymorphism in human populations, with a clear-cut distinction between extensive metabolizers, who compose about 30–50% of the population, and poor metabolizers (PM), who represent 50–70% of the general population in western countries. Since some arylamines are potent bladder carcinogens and *N*-acetylation is involved in their detoxication, one would expect PM to be at higher risk for bladder cancer than extensive metabolizers.

In their seminal report, Cartwright et al. (*1*) described a case–control study of 111 bladder cancer cases and 207 controls. The *N*-acetylation phenotype was assessed by measuring the monoacetyldapsone: dapsone ratio, and 0.3 was used as the cut-off point between the phenotypes. The proportion of PM was 57% among the controls and 67% among the cases; however, this proportion was considerably increased in a group of 23 occupationally exposed cases (with possible exposure to benzidine and 2-naphthylamine). In fact, 22 out of 23 ($p = 0.005$) were PM; the only fast metabolizer had an adenocarcinoma, i.e., a histologic type different from that of the other 22, which were transitional-cell carcinomas.

The very high proportion of poor acetylators among the occupationally exposed bladder cancer cases described by Cartwright et al. has not been reported by others. A number of case–control studies have shown, however, a slight excess of PM among bladder cancer patients in comparison with controls. Table 1 gives the results of these studies. The numbers are generally small, and the confidence intervals of the odds ratios are large; nevertheless, the qualitative consistency of the findings is intriguing (see also reference *4* for a review). Overall, these data are compatible with an increased risk for bladder cancer of around 30% among PM, a figure much lower than that reported by Cartwright et al. Possible explanations for this series of observations and for their inconsistency with those of Cartwright et al. are the following: *a*) There may

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Table 1. Proportion (%) of slow acetylators among bladder cancer cases and controls in different studies.[a]

Location	Cases		Controls	
	%	No.	%	No.
Denmark	65	71	51	74
Huddersfield, UK	67	111	57	95
Rural Sweden	70	115	67	118
Wisconsin, USA	59	34	49	41
New York State, USA	46	26	69	26
Newcastle, UK	70	30	59	27
Liverpool, UK	66	66	60	510
Rural Denmark	64	228	54	100
Spain (*3*)	64	130	57	157
Spain (exposed to arylamines)	73	11	–	–

[a]From Mommsen et al. (*2*) and Ladero et al. (*3*).

be a common bias in the studies. In fact, most of the investigations reported in Table 1 included both incident and prevalent cases; if the PM phenotype is associated mainly with survival, then the excess of PM among bladder cancer patients would be due entirely to the prevalent cases. This explanation, however, does not hold for the study of Cartwright et al. Other sources of bias are unlikely, since methods for phenotyping are reliable and *N*-acetyltransferase is not inducible. Chance is also very unlikely to explain the observations. *b*) If the PM phenotype is relevant only to arylamine-induced bladder cancer, the different proportions of PM among the cases in the different investigations might be because different proportions of subjects were exposed to arylamines. In fact, a higher proportion of PM was present among the cases in the Spanish study when only workers with potential exposure to arylamines were selected. *c*) The populations that have been studied had qualitatively different exposures to arylamines. For example, the British workers studied by Cartwright et al. were probably exposed to high levels of benzidine and 2-naphthylamine; the nature of the occupational exposure in the study in Spain is uncertain. Tobacco smoke contains varying amounts of arylamines, including 4-aminobiphenyl (4-ABP) and 2-naphthylamine, and the composition of tobacco smoke is different in different countries, according to the type of tobacco used (*5*).

Hemoglobin Adducts Formed by Arylamines: Interindividual Variability According to Chemical Structure

In a molecular epidemiology investigation in which we were involved (*6*), we reported that the quantities of hemoglobin adducts with 3-aminobiphenyl (3-ABP) and 4-ABP were related to the quantity of cigarettes smoked and, in the case of 4-ABP, also to the kind of tobacco smoked (air or flue cured). Air-cured tobacco is known to contain larger amounts of arylamines than flue-cured product and has also been found to be associated with higher risks of bladder cancer in three epidemiological investigations (*7–9*).

In the molecular epidemiology investigation, the quantity and type of tobacco smoked were statistically significantly associated to the quantity of 4-ABP–hemoglobin adducts but could not explain a considerable proportion of the interindividual variation in the amounts of adducts. We reanalyzed the same set of data for smokers in order to ascertain whether the residual, unexplained variation was related to individual differences in susceptibility, possibly due to metabolic characteristics. If individual metabolic differences were responsible for part of the unexplained interindividual variation in the levels of hemoglobin adducts and if chemically similar substances have, at least in part, common metabolic pathways, then the concentrations of adducts with similar amines should be correlated in the same individual, allowing for the amount and type of tobacco smoked. In other words, subjects with high levels of 4-ABP adducts should also have high levels of 3-ABP adducts, allowing for tobacco consumption, and subjects with low levels of 4-ABP should have low levels of 3-ABP.

We considered the data for 25 nonsmokers, 18 smokers of air-cured tobacco, and 40 smokers of flue-cured tobacco, all living in the city of Torino (Turin). Blood samples were analyzed at the Massachusetts Institute of Technology (Cambridge) for hemoglobin adducts formed with 14 aromatic amines, using gas chromatography–mass spectrometry. The distribution of subjects by number of cigarettes smoked the day before collection is shown in Table 2.

For all quantities, except for 3, 8, 12, 14, 16, 17, and 18 cigarettes, at least two subjects reported exactly the same amount smoked. Only these 54 subjects were included in the statistical analyses as at least two smokers of the same amount were necessary to study interindividual variability not explained by the amount smoked. A model was fitted by the least-squares method for each aromatic amine, including the kind and amount of tobacco smoked; statistical residuals from this model were computed. Residuals are the differences between the observed levels of adducts and those expected on the basis of the model. This procedure is equivalent to computing the difference between the amount of adducts in each subject and the mean for all subjects who smoked exactly the same number of cigarettes, after adjusting for kind of tobacco. Since,

Table 2. First study on arylamine–hemoglobin adducts: distribution of subjects by number of cigarettes smoked.

No. of cigarettes	No. of subjects
0	25
2	3
3	1
6	2
7	2
8	1
10	3
12	1
13	2
14	1
15	11
16	1
17	1
18	1
20	20
25	2
30	6
40	3

for all amines, the residuals showed a log-normal distribution, the logarithm of adduct concentration was also used.

A correlation matrix of the residuals for the 14 aromatic amines was computed, and a principal component analysis was performed. The purpose of principal component analysis is to determine factors (i.e., principal components) in such a way as to explain as much of the total variation in the data as possible. The results of this analysis are reported in Table 3. Within each principal component ("factor"), the correlation coefficient of each arylamine with the factor is reported. The first principal component explains 49% of total variance and is related to all substances except 3-ABP, 4-ABP, and 2-naphthylamine; the second principal component explains a further 16% of the total variance and includes 3-ABP, 4-ABP, and 2-naphthylamine, whereas the coefficients for all other amines (except *p*-toluidine) are negative or close to zero. Comparable results are obtained if the log (adducts) are analyzed instead of residuals. Further details have been published elsewhere (*10*).

Our hypothesis was that individual metabolic differences, represented by the correlations between adducts with different aromatic amines, could explain part of the interindividual variability that is not explained by the characteristics of smoking habits. The results we obtained are clearly in agreement with this hypothesis, since the correlation among one group of residuals explained 49% of the total variance of residuals, and the correlation among residuals of a second group explained a further 16% of the variance. Interestingly, the variables that contribute to the first principal component are all mononuclear aromatic amines, and those included in the second component are binuclear amines (3-ABP, 4-ABP, 2-naphthylamine). Residuals of compounds in one group (e.g., mononuclear) are correlated with residuals of other amines within the same group (i.e., subjects who have higher levels of one compound than expected have higher levels of the other compounds too) but are independent of residuals of compounds in other groups.

This finding seems to suggest that two different metabolic pathways are involved—one for mononuclear and the other for binuclear aromatic amines—and that interindividual variability exists for these pathways. Such variability would explain a considerable proportion of the differences in levels of hemoglobin adducts that are not explained by smoking habits.

N-Acetyltransferase Polymorphism and 4-ABP–Hemoglobin Adducts

Several sources of information indicate that 4-ABP is related to bladder carcinogenesis in smokers: two studies on hemoglobin adducts, including that already mentioned (*6,11*); one study on DNA adducts in exfoliated bladder cells (*12*); one study of bladder biopsy samples from humans (*13*); and studies on bladder biopsy samples from dogs. Talaska et al. (*14*) showed that administration of 4-ABP to dogs resulted in formation of a main DNA adduct in bladder cells, *N*-(deoxyguanosin-8-yl)-4-ABP. Subsequently, they studied DNA adducts in biopsy samples from 42 subjects with bladder cancer and again found that *N*-(deoxyguanosin-8-yl)-4-ABP was one of the main adducts in smokers (*13*). Finally, a comprehensive collaborative investigation has been conducted among 97 volunteers in Torino to measure the levels of 4-ABP adducts with hemoglobin (*11*) and with DNA in exfoliated bladder cells (*12*). The levels of hemoglobin adducts clearly correlate with the number of cigarettes smoked, with urinary cotinine and nicotine, and with the type of tobacco used (air or flue cured). In addition, the levels of 2 out of 12 DNA adducts found among smokers were clearly correlated with both the number of cigarettes smoked and the concentration of 4-ABP–hemoglobin adducts; one of the two DNA adducts was quite similar to *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (*12*). There are good reasons to believe, therefore, that 4-ABP, and perhaps other arylamines, play an important role in smoking-induced bladder carcinogenesis.

In the study mentioned above on 97 volunteers, the concentration of 4-ABP–hemoglobin adducts was also measured according to *N*-acetylation phenotype (defined on the basis of the ratio of two metabolites of caffeine, 5-acetylamino-6-formylamino-3-methyluracil, and 17-methylxanthine; measurements made at the National Cen-

Table 3. Principal component analysis of residuals for 14 aromatic amines[a] (weights of residuals for each substance in the first 7 factors).

Substance	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
4-ABP	0.07	0.32	0.56	0.11	0.47	−0.44	−0.11
3-ABP	0.07	0.52	0.18	−0.13	0.25	0.62	0.33
2-Naphthylamine	0.01	0.52	0.06	0.21	−0.51	−0.37	−0.32
o-Toluidine	0.22	0.05	−0.03	0.73	0.01	0.26	−0.35
m-Toluidine	0.32	0.13	0.04	−0.01	−0.08	−0.05	−0.54
p-Toluidine	0.23	0.38	−0.25	−0.15	−0.30	0.19	−0.22
2,4-Dimethylamine	0.27	0.01	−0.40	0.13	0.42	0.01	0.13
2,6-Dimethylamine	0.22	−0.13	0.49	−0.35	−0.11	0.23	−0.24
2,3-Dimethylamine	0.33	−0.18	0.16	−0.07	−0.30	−0.04	0.09
3,5-Dimethylamine	0.34	−0.01	−0.15	−0.23	0.21	−0.23	0.04
3,4-Dimethylamine	0.34	0.05	−0.21	−0.16	0.09	−0.25	0.00
2,5-Dimethylamine	0.31	−0.28	0.19	0.13	−0.16	0.07	0.28
3-Ethylamine	0.36	0.07	−0.13	−0.16	−0.01	−0.01	0.17
2-Ethylamine	0.30	−0.23	0.20	0.30	0.02	0.05	0.35
Proportion of total variance	49%	16%	10%	7%	5%	4%	3%

Abbreviations: 4-ABP, 4-aminobiphenyl; 3-ABP, 3-aminobiphenyl.
[a]From Ronco et al. (*10*).

ter for Toxicological Research, Jefferson, AR). The concentration of adducts is clearly higher in slow acetylators (Table 4). When smoking habits (dose, type of tobacco) and metabolic phenotype were included in a multivariate model, an independent, statistically significant contribution of the latter was found. This finding agrees with *a priori* expectations, since *N*-acetylation is expected to deactivate 4-ABP, thus preventing covalent binding to macromolecules.

Dose–Response Relationships

The shape of dose–response curves in cancer epidemiology has usually been interpreted to infer the number of stages in the carcinogenic process, within the frame of multistage carcinogenesis. An example is the quadratic dose–response relationship seen between the number of cigarettes smoked and the risk for lung cancer; the inference is that tobacco smoke acts at two stages of the carcinogenic process in the lung (*15*). The dose–response relationship can also be an expression of other phenomena; one of these is metabolic polymorphism within the study population, i.e., the presence of subgroups with different susceptibilities to the action of carcinogens.

In epidemiological studies of bladder cancer, a convex dose–response relationship has been observed repeatedly between the number of cigarettes smoked and the relative risk, rather than the linear or exponential curve seen in lung cancer (*16*). In other words, the relative risk increases quickly, then seems to reach a plateau. For example, in the largest case–control study reported, the relative risks by number of cigarettes smoked (with nonsmokers as the reference category) were: < 20, 1.8 (based on 658 cases); 20–39, 2.6 (1102 cases); and ≥ 40, 2.6 (392 cases) (*17*). In a Danish investigation, the following relative risks were found: for 1–14 cigarettes, 4.2; 15–29, 4.9; and ≥ 25, 4.3 (*18*). In other investigations, however, the plateau was less clear (*16*).

A convex dose–response curve was also observed for the correlation of the concentration of 4-ABP–hemoglobin adducts with markers of recent smoking (number of cigarettes, urinary cotinine and nicotine) (*6,11*). Such a relationship would be expected *a priori* if the population included two subgroups—one that metabolizes the relevant chemical rapidly and the other, slowly. In fact, when the data on hemoglobin adducts were subdivided according to metabolic phenotype (slow or fast acetylator), the picture shown in Table 5 emerged. Slow acetylators clearly have a high level of adducts at low levels of smoking, whereas the curve for fast acetylators increases more regularly. The association of the two curves leads to a less than linear relationship. The intercept of the curve for slow acetylators is 72 pg/g hemoglobin, which is significantly different from the intercept for fast acetylators ($p = 0.01$). Unfortunately, these observations are still based on small numbers, as expressed by the large standard errors in Table 5, and warrant further investigation.

It would be an oversimplification to conclude that the convex dose–response curve observed between the number of cigarettes smoked and the relative risk for bladder cancer indicates the same phenomenon as hypothesized for hemoglobin adducts. In fact, carcinogenesis is more complex than the simple binding of arylamines to macromolecules. Nevertheless, the similarity of the dose–response curves is suggestive.

Table 4. Means (and SEs) of concentrations of 4-aminobiphenyl–hemoglobin adducts in blood of smokers of black and blond tobacco and of nonsmokers (pg/g hemoglobin), by acetylation phenotype.[a]

Smoking status	Acetylation phenotype: Slow	Acetylation phenotype: Fast	Ratio slow:fast
Nonsmoker	31.7 (3.8)	19.4 (4.9)	1.6
Blond tobacco	111.8 (13.0)	86.4 (14.5)	1.3
Black tobacco	175.0 (11.0)	117.5 (13.7)	1.5

[a]From Bartsch et al. (*11*).

Table 5. Levels of 4-aminobiphenyl–hemoglobin adducts according to level of cotinine plus nicotine in urine, by metabolic phenotype, smokers only.

Cotinine plus nicotine, μmole/mmole	Adducts, pg/g (mean ± SE): Fast acetylators	Adducts, pg/g (mean ± SE): Slow acetylators
< 0.5	44[a]	153 ± 41
0.5–1.4	66 ± 12	114 ± 17
1.5–2.4	92 ± 36	134 ± 15
≥ 2.5	121 ± 16	148 ± 20

[a] One subject only.

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