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Carcinogen–DNA Adducts in Exfoliated Urothelial Cells: Techniques for Noninvasive Human Monitoring

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Detection of carcinogen–DNA adducts in DNA from exfoliated urothelial cells from animals and humans exposed to potential environmental carcinogens is described. In an animal model, 4-aminobiphenyl–DNA adducts were detected, and the shape of the dose–response curve was related to the levels of 4-aminobiphenyl–hemoglobin adducts. In a human study, five distinct adducts were two to nine times higher in smokers than in nonsmokers. The association of four adduct measures with smoking was corroborated by significant correlations with levels of 4-aminobiphenyl–hemoglobin adducts, type and number of cigarettes smoked, and/or urinary mutagenicity. One adduct seemed chromatographically similar to *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl. This adduct showed the strongest correlation with 4-aminobiphenyl–hemoglobin adduct levels. These data suggest that noninvasive techniques can be applied to the study of carcinogen–DNA adducts in the target organ of humans at risk for urinary bladder cancer.

Introduction

Because many carcinogens are organotropic and because surrogate tissues may not necessarily reflect the events occurring in the target organ, the development of noninvasive techniques to monitor carcinogen–DNA adducts in internal organs is a priority (*1–5*). This report describes the development and application of noninvasive methods to monitor carcinogen–DNA adducts in the human urinary bladder, a target organ for tobacco-induced cancers.

Methods

Materials, animal handling and treatment, the human study population, sample collection, isolation of cells and DNA from exfoliated urothelial cells, ^{32}P-postlabeling, 4-aminobiphenyl–hemoglobin adduct analysis, and determination of urinary mutagenicity have been described in detail in earlier reports (*6–10*). Briefly, exfoliated urothelial cells were collected from 24-hr urine samples by either centrifugation (dog studies) or filtration (human studies). DNA was isolated by a solvent extraction technique and quantitated during the ^{32}P-postlabeling analysis (*6*). Hydrolyzed DNA samples are ^{32}P-postlabeled under a condition of [^{32}P]ATP excess so that all nucleotides are labeled quantitatively. 4-Aminobiphenyl–hemoglobin adducts (ABP–Hb) were analyzed in blood samples by GC–MS of the parent compound following hydrolysis of the globin (*8*). Urinary mutagenicity was determined using *Salmonella typhimurium* strain TA98 and Aroclor-induced S-9 (*10*).

Results

Animal Studies

Figure 1 shows the time course of ABP–DNA and hemoglobin adduct accumulation during the five times per week for 2 weeks exposure period. Hemoglobin adduct levels rose rapidly to reach an apparent steady state after five treatments. However, the likelihood is low that a true steady state is achieved because there should be at least 1000 times more hemoglobin cysteinyl groups than *N*-hydroxy-4-aminobiphenyl at these doses. At the same time, there have been reports that similar levels of phenylhydroxylamine have significantly reduced erythrocyte life span, indicating that the apparent steady state may be induced by overt toxicity (*11*). After the initial increase, the slope of the ABP–DNA adduct curve is modest until the second week of treatment, when the slope increases. In addition, the ABP–DNA adduct levels in the exfoliated urothelium of one dog treated for 13 weeks

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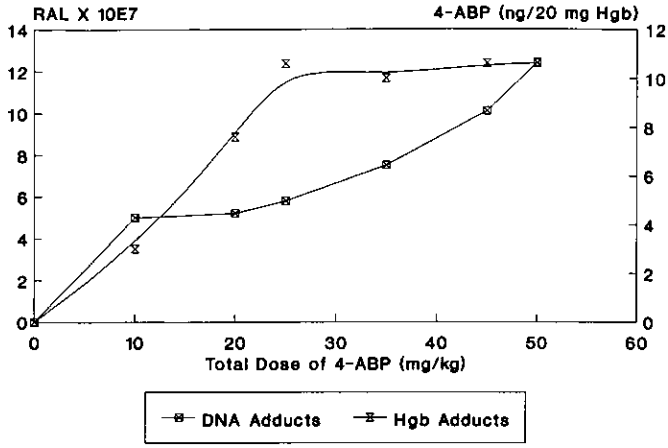

FIGURE 1. Mean levels of ^{32}P-postlabeled 4-aminobiphenyl (ABP)–DNA adducts in exfoliated urothelial cells and ABP–hemoglobin adducts from eight dogs treated over 2 weeks with 5 mg/kg 4-aminobiphenyl orally five times per week. The lines in the figure were drawn using the lines and curves formats for DNA and hemoglobin adducts of Harvard Graphics computer software.

increased until the sixth week of treatment to relative adduct levels (RAL) × 10^7 of about 20–25, while the hemoglobin adducts remained at the levels shown in Figure 1. After 6 weeks both markers appeared to be at steady state. These data suggest that at these high doses toxicity may hamper the protective function of hemoglobin. This might be expected because *N*-OH-ABP is thought to be the key metabolite involved in both DNA and hemoglobin adduct formation (*8*).

Human Studies

Complete 24-hr urine samples were obtained from 73 volunteers. Exfoliated urothelial cells were isolated and their DNA was ^{32}P-postlabeled. Sufficient DNA for ^{32}P-postlabeling analysis was obtained from 40 of these 73 urine samples. The 40 samples included 21 from nonsmokers, 18 from current smokers, and one from a pipe smoker, which was excluded from further analysis.

The mean levels of the 8 of 12 individual carcinogen–DNA adducts measured increased in smokers (Table 1). Several of the adducts that did not increase displayed chromatographic behavior similar to indigenous adducts reported by ourselves and others (*1,2,11*). As can be seen from Table 1, these adducts were 1.6, 7.0, 1.7, 3.9, 1.2, 1.1, 3.7, 27, and 1.3 times higher in smokers compared to nonsmokers. However, these differences were not significant at the 0.05 level using the Mann-Whitney statistic.

The data were then analyzed to determine if there were correlations between reported cigarette intake, levels of ABP–Hb, and urinary mutagenicity. As shown in Table 2, there were statistically significant Spearman correlation coefficients for the adducts 2, 4, 10, and total diagonal-zone adducts with the number of cigarettes smoked, 4-ABP–Hb adduct levels, and/or the levels of mutagens excreted in the urine by smokers.

Adduct 4 appeared qualitatively very similar to butanol adduct 4 (B4) in a urinary bladder biopsy study, which was characterized as *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (*2*). The average level of adduct 4 were somewhat higher in smokers of black tobacco than in smokers of blond tobacco (RAL values of 8.8 and 7.7, respectively), but these levels were not different statistically than those of the nonsmokers (RAL = 4.8). Many samples from smokers and nonsmokers were negative for particular adducts. Adduct 4 was reported positive (nonzero) in 13/21 nonsmokers and in 10/18 smokers. It is interesting that the proportion of positive samples in smokers of blond and black tobacco were 5/11 (45.5%) and 5/7 (71.4%), respectively. In addition, when smokers were segregated into three tobacco consumption groups of approximately equal size: light 1–14 cigarettes/day; medium, 15–29 cigarettes/day; or heavy, > 30 cigarettes/day, the proportion of positive samples increased from 17 to 80% and 71%, respectively, and a dose–response relationship was observed with the respective average RAL values increasing from 0.9 to 9.5 and 13.3. Adduct 4 was better correlated than any other adduct in the exfoliated urothelium with individual 4-ABP–hemoglobin adducts. Figure 2 plots the data for the two biomarkers in tobacco smokers. Interestingly, the regression lines are parallel, indicating that at this level of exposure there may be a proportionality between the two markers, in contrast to the data presented above for the dog.

Table 1. Relative adduct levels × 10^9 for adducts higher in the exfoliated urothelium of smokers.

	Adduct number							
Group	1	2	4	6	7	9	10	Total[a]
Smokers	12.6	9.9	8.1	3.2	5.5	3.7	5.4	24.5
Nonsmokers	8.1	1.4	4.8	2.6	5.2	1.0	0.2	19.3

[a]Total diagonal-zone adducts.

Table 2. Spearman correlation coefficients and *p*-values for smoking-related adducts in smokers.

Adduct number	Cigarettes per day	Hemoglobin adduct levels	Urinary mutagenicity
2	0.499*	0.410	0.26
	(0.03)[a]	(0.09)	(0.30)
4	0.505*	0.601*	0.509*
	(0.03)	(0.01)	(0.03)
10	0.502*	0.491*	0.455
	(0.03)	(0.04)	(0.058)
Total[b]	0.337	0.510*	0.654*
	(0.17)	(0.03)	(0.003)

*Correlation coefficients significant at the 0.05 level.
[a] *p*-Values in parentheses.
[b] Total diagonal-zone adducts.

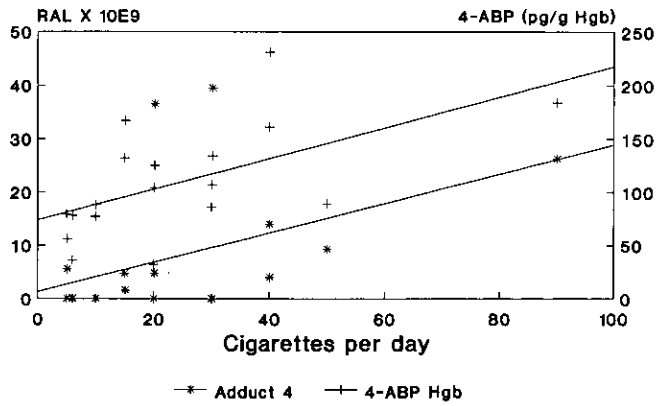

FIGURE 2. Individual values for adduct 4 in exfoliated urothelial cells and 4-aminobiphenyl–hemoglobin adducts for cigarette smokers.

There was an overall 7-fold increase in adduct 2 in smokers compared to nonsmokers (mean RAL values 9.9 and 1.4, respectively). Adduct 2 was correlated significantly with the number of cigarettes smoked (Table 1). Although there was no significant difference between the mean adduct 2 levels for smokers of blond tobacco (1.9) and nonsmokers (1.4), the average in the smokers of black tobacco (22.5) was significantly different from the nonsmokers ($p \leq 0.001$). In addition, the proportion of samples with adduct 2 levels greater than zero increased from 4/21 (19%) to 3/11 (27%) and 3/7 (43%) in nonsmokers, blond-, and black-tobacco smokers. Adduct 2 was also detected in 57% of the samples of the heaviest smokers (> 30 cigarettes/day), but only in about 20 of the samples of nonsmokers or those who smoked less than 14 cigarettes/day.

The average level of adduct 10 in smokers was 5.4, while the mean level in nonsmokers was 0.14. However, only 1 of 21 nonsmokers and 4 of 14 smokers were positive for this adduct, making further comparisons tenuous. As shown in Table 1, adduct 10 was correlated significantly with amount smoked and levels of 4-aminobiphenyl–Hb adducts.

The level of total diagonal-zone adducts in smokers was correlated significantly with 4-aminobiphenyl–Hb adducts and excretion of urinary mutagens. Total diagonal-zone adducts is the summation of adducts 3, 4, 9, and the activity above background associated with the diagonal radioactive zone not incorporated into discrete adducts.

Discussion

This paper describes the development and application of noninvasive methods for the detection of carcinogen–DNA adducts from an internal target organ by collection of exfoliated urothelial cells from the urine. Several carcinogen–DNA adducts were elevated in cigarette smokers; the increase in particular carcinogen–DNA adducts ranged from 1.6- to over 7-fold, which is approximately the range seen in an earlier study of human bladder biopsies. This is also the range of excess risk of bladder cancer in smokers as reported by various epidemiological studies (*2*). However, these increases were not statistically significant. Nevertheless, further analysis indicated that three adducts and a summary adduct measure were correlated significantly with quantity of tobacco smoked, 4-aminobiphenyl–Hb adducts, and/or urinary mutagenicity. Two of these carcinogen–DNA adducts (2 and 4) were qualitatively similar to adducts observed in bladder biopsy samples of cigarette smokers. Adduct 4, which was chromatographically similar to *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl, was correlated significantly with levels of 4-aminobiphenyl-Hb adducts. We have also found that the relationship between these adducts appears different at high doses in animal studies as compared with the low doses of human exposures. These techniques are relatively simple and should be useful for monitoring the consequences of exposure to urinary bladder carcinogens.

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