DNA adducts in normal bladder tissue and bladder cancer risk

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Cigarette smoking is an established cause of bladder cancer. The direct relationship between smoking-induced DNA adducts in bladder cells and cancer risk at that site has, however, been poorly assessed. We therefore investigated the relationship between bladder cancer risk and levels of DNA adducts measured in normal bladder biopsies by 32P-post-labeling in a hospital-based case-control study of 59 bladder cancer patients and 45 controls submitted to surgery for prostatic hyperplasia or urinary incontinence. An approximately 2-fold risk for bladder cancer was found in individuals with an adduct level >14.8 (median among controls) compared with those with an adduct level <14.8 (OR = 1.9, 95% CI 0.8-4.3, P = 0.13). A dose-response relationship was also suggested (trend test, \( P = 0.13 \)): compared with adduct levels below 13.5, the OR for bladder cancer was 1.7 (95% CI 0.6-4.6) for adduct levels between 13.5 and 18.5 and 2.2 (95% CI 0.8-6.1) for adduct levels >18.5. These findings provide some evidence that DNA adducts in bladder tissue might predict smoking-induced bladder cancer. Larger studies are still warranted to confirm these results.

Introduction

Bladder cancer is the fourth most frequent cancer in men in the European Union, with approximately 52 000 new cases and 22 000 deaths occurring each year (Black et al., 1997). Cigarette smoking is an established cause of this malignancy, accounting for about two-thirds of the bladder cancer cases in men in industrialized countries (Brennan et al., 2000).

Tobacco smoke contains a highly complex mixture of compounds, including amines such as 2-naphthylamine and 4-aminobiphenyl. The carcinogenic potency of these substances has been clearly demonstrated and a role in the etiology of bladder cancer has been recognized (IARC, 1987). Many tobacco carcinogens can bind to DNA to form adducts, either directly or after cytochrome P-450 catalyzed modification. Unless repaired, such DNA lesions may lead to mutations and ultimately to cancer. The level of smoking-induced DNA adducts may therefore be a risk factor for the development of bladder cancer.

Several techniques have been developed for DNA adduct analysis, of which the 32P-post-labeling assay, involving adduct enhancement strategies that increase sensitivity for a wide variety of aromatic DNA adducts, has been widely used (Gupta, 1993). Because many target tissues for tobacco smoke-induced carcinogenesis are not readily obtainable at a population level, white blood cells (WBC) have usually been used as a surrogate for analysis of DNA damage.

WBC DNA adducts were found to be associated with the risk of developing bladder cancer (Peluso et al., 2000) and lung cancer (Tang et al., 1995; Li et al., 1996; Vulimiri et al., 2000; Tang et al., 2001). These findings suggest that WBC DNA adducts might predict smoking-related cancers, however, no study has been reported so far on the direct relationship between DNA adducts in bladder cells and cancer risk at that site, except a small investigation based on 20 cancer patients and 36 controls (Talaska et al., 1994).

Although the relationship between adduct concentrations in blood and target tissues has not been conclusively established, a good correlation was found between DNA adducts in blood cells and lung tissue (Tang et al., 1995; Wiencke et al., 1995) or larynx tissue (Szyfter et al., 1994).

We report here on the risk for bladder cancer associated with DNA adducts measured in normal bladder biopsies by the 32P-post-labeling method. We also investigated the correlation between adducts in WBC and bladder tissue from the same individuals.

Materials and methods

Study population

The case-control study was carried out in France between 1997 and 2001 in the Urology Department of three General Hospitals located in Paris.

Only newly diagnosed bladder cancer cases with histologically confirmed primary transitional cell carcinoma were included. Each time a new case was included in the study, we sought one control submitted to surgery for prostatic hyperplasia or urinary incontinence. Controls were matched with cases on age (±3 years), sex and hospital. All subjects were required to be Caucasians without previous malignant disease. In-person structured interviews were conducted by the same trained physician throughout data collection. Detailed information on demographic characteristics, medical history, lifetime use of tobacco products and occupational history was collected. Subjects who had smoked at least one cigarette, one cigar or one pipe a day for 6 months or longer were classified as ever-smokers. An average daily consumption of tobacco was calculated by dividing the cumulative lifetime tobacco consumption by the overall duration of smoking. Former smokers were defined as people who had stopped smoking at least 1 year prior to the diagnosis.

Samples of normal bladder tissue (20 mg) were resected at the time of surgery for both cases and controls. The tissue was immediately frozen and stored in liquid nitrogen until DNA extraction. Peripheral blood (5 ml) was collected at the time of recruitment and centrifuged within 24 h; buffy coat, red blood cells and plasma were separated and stored at -80°C.

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The study protocol was approved by the local Ethical Committee (Kremlin-Bicêtre Hospital, France). Written informed consent was obtained from all subjects.

Analysis of DNA adducts using the $^{32}\text{P}$-post-labeling assay

Bladder biopsy samples were obtained from 120 bladder cancer cases and 71 controls. Of these, 68 cases and 51 controls had adequate amounts of bladder tissue for adduct analysis. After treatment with collagenase, DNA was extracted and purified using the SEVAG phenol procedure. DNA adducts were analyzed using the butanol extraction procedure of the $^{32}\text{P}$-post-labeling assay (Gupta, 1985), reported to be more suitable for the detection of aromatic amine adducts than the nuclease P1 digestion method (Gupta and Early, 1988).

Each DNA specimen was analyzed at least twice in duplicate. There was a sufficient amount of DNA (4 × 5 μg) for these four analyses for 59 cases and 45 controls. Radioactivity was counted throughout the entire rectangular area of chromatograms which were cut into 42 small squares (Figure 1). The background radioactivity was defined as the smallest of these 42 values; the total adduct level for each chromatogram was equal to the sum of these 42 values minus 42 × the background value. This level was calculated based on the labeling efficiency of 10 pmol of dAp during the same session. The adduct level for each individual was the mean of the results of at least three chromatograms, excluding outliers. It was expressed as relative adduct level (RAL) per $10^8$ nucleotides.

Blood samples from 38 individuals (20 cases and 18 controls) included in the present study were used to evaluate the correlation between DNA adducts in WBC and bladder tissue. WBC DNA extraction was performed using standard protocols and adducts were measured by the $^{32}\text{P}$-post-labeling technique as described above.

Results

The main characteristics of the study population are displayed in Table I. Practically all subjects were men and the mean age at diagnosis was similar among cases and controls. All the controls, except one, were hospitalized for prostatic hyperplasia. The proportion of ever-smokers (former and current) was higher among cases (85%) than among controls (76%), and cases had a higher daily tobacco consumption than controls (19 versus 16 g/day, respectively); these differences were not significant, however.

Bladder DNA adduct levels according to the case/control and smoking status are presented in Table II. The median of bladder DNA adducts/$10^8$ nucleotides was higher, although not significantly, among cases than among controls, overall (17.6 versus 14.8, $P = 0.17$) and for current smokers (22.5 versus 14.7, $P = 0.18$). No difference was found between cases and controls within each of the two other smoking categories. Bladder cancer risks associated with DNA adducts, adjusted for age and smoking status, are shown in Table III. An approximately 2-fold risk for bladder cancer was found in individuals with an adduct level >14.8 adducts/$10^9$ nucleotides (median level in the control population) compared with those with an adduct level of ≤14.8 (OR = 1.9, 95% CI 0.8–4.3, $P =$...
0.13). Controlling for daily tobacco consumption did not materially alter the present observations of association between DNA adducts and bladder cancer (OR = 1.9, 95% CI 0.8–4.4, \( P = 0.16 \)). When DNA adducts were considered in tertiles, a dose–response relationship was suggested (trend test, \( P = 0.13 \)) compared with adduct levels below 13.5, the OR for bladder cancer was 1.7 (95% CI 0.6–4.6) for adduct levels between 13.5 and 18.5 and 2.2 (95% CI 0.8–6.1) for adduct levels >18.5.

A strong association was found between adduct levels and smoking categories among cases (\( P = 0.001 \)) but not among controls (\( P = 0.87 \)) (Table II): among cases, the median of adducts/10^8 nucleotides was clearly elevated in current smokers (22.5) compared with former (15.6) or never-smokers (13.7).

There was no significant correlation between DNA adducts in WBC and in normal bladder tissue (correlation coefficient = 0.0096, \( P = 0.95 \)).

### Discussion

The major strength of this study is the measure of DNA adducts in normal bladder tissue from both bladder cancer patients and controls and, to our knowledge, this is the largest study to evaluate the predictive value of smoking-induced DNA adducts at a target organ site on cancer risk at that site. Indeed, most target tissues are not readily obtainable as they require surgical procedures which are, for practical and ethical reasons, difficult to perform among control individuals.

Our findings suggest that DNA adducts in normal bladder tissue might influence the risk of bladder cancer; almost a 2-fold risk of bladder cancer was found among individuals with elevated DNA adducts. This relatively weak association may be explained by the fact that adducts express recent exposure, because of the rapid cell turnover in the bladder, and that only a fraction of the total DNA adducts could be carcinogenic to the bladder (e.g. arylamines). A trend of increased risk with increasing levels of adducts was also suggested. The higher levels of adducts among bladder cancer cases than among controls, after adjustment for smoking exposure, may express a higher susceptibility of those who develop cancer. However, our results were not statistically significant, likely because of the small number of tissue samples suitable for DNA adduct analysis. Our results may be biased due to the selection of a subset of cases and controls with usable DNA adduct levels, however, the main characteristics were not different between subjects with and without usable tissue samples. It is also unlikely that our results were biased by differential DNA adduct measurement errors among cases and controls since adducts were measured on coded samples.

Consistent with the epidemiological evidence for an association between bladder cancer and smoking, we found that the levels of DNA adducts in bladder tissue were higher in smokers than in non-smokers among cases. These findings are in agreement with the relationship between DNA adduct levels and smoking status previously reported in different human tissues (reviewed in Phillips, 1996, 2002).

Previous case–control studies of the effects of DNA adducts on the risk for smoking-related cancers were conducted using blood cells as a surrogate for the target tissue. Consistent with our findings, an increase in risk of bladder cancer with increasing levels of WBC DNA adducts was recently demonstrated (Peluso et al., 2000). Higher levels of adducts in blood cells were also found in lung cancer patients than in control individuals in all studies so far reported (Tang et al., 1995; Li et al., 1996; Vulimiri et al., 2000; Tang et al., 2001), except one (Hou et al., 1999). Overall, our results, along with those from previous studies, support the hypothesis that DNA adducts could predict the onset of smoking-related cancers. These findings are biologically plausible since DNA adducts are at the origin of mutation and, ultimately, cancer development.

Table II. Bladder DNA adduct levels (RAL/10^8 nucleotides) according to case/control and smoking status

<table>
<thead>
<tr>
<th></th>
<th>Never smokers</th>
<th>Current smokers</th>
<th>Total</th>
<th>OR value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Median [range]</td>
<td>Median [range]</td>
<td>n</td>
<td>Median [range]</td>
</tr>
<tr>
<td>Bladder cancer patients</td>
<td>9 13.7 [9.4–27.0]</td>
<td>16 22.5 [10.2–62.9]</td>
<td>59 17.6 [7.9–62.9]</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>11 14.0 [11.3–24.0]</td>
<td>10 14.7 [11.0–46.3]</td>
<td>45 14.8 [8.8–46.3]</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*Kruskal–Wallis test (comparison between smoking categories).

Table III. ORs (95% CI) of bladder cancer associated with levels of DNA adducts in normal bladder tissue

<table>
<thead>
<tr>
<th>DNA adducts/10^8 nucleotides (above/below the median)</th>
<th>Cases (n = 59)</th>
<th>Controls (n = 45)</th>
<th>OR† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 14.8 )</td>
<td>19 (32%)</td>
<td>22 (49%)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>( &gt;14.8 )</td>
<td>40 (68%)</td>
<td>23 (51%)</td>
<td>1.9 (0.8–4.3)</td>
</tr>
<tr>
<td>Tertiles of DNA adducts/10^8 nucleotides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;13.5 )</td>
<td>12 (20%)</td>
<td>15 (33%)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>13.5–18.5</td>
<td>20 (34%)</td>
<td>15 (33%)</td>
<td>1.7 (0.6–4.6)</td>
</tr>
<tr>
<td>( \geq18.5 )</td>
<td>27 (46%)</td>
<td>15 (33%)</td>
<td>2.2 (0.8–6.1)</td>
</tr>
</tbody>
</table>

*Adjusted for age and smoking status (never, former and current smokers).

\( P \) value

\( P \) trend

\( 0.79 \) 0.74 0.18 0.17

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The choice of the butanol extraction procedure for DNA adduct analysis in our study was based on the increasing evidence that the excess bladder cancer observed in smokers could be attributable to arylamines rather than to other aromatic compounds such as polycyclic aromatic hydrocarbons produced by tobacco smoke (Vineis and Pirastu, 1997). In keeping with this, higher levels of adducts have been detected by the butanol extraction procedure than by the nuclease P1 digestion method of the 32P-post-labeling assay in bladder biopsies and exfoliated cells recovered from urine (Talaska et al., 1991a,b; Phillips and Hewer, 1993).

No correlation was found between DNA adducts in WBC and bladder biopsies in a recent study (Airoldi et al., 2002); this result may be explained by the different methodologies applied for adduct measurement in both tissues and differences in types of adducts measured. We also found that DNA adduct levels in WBC were not correlated with DNA adducts in bladder tissue using the same 32P-post-labeling assay; differences in the kinetics of DNA adduct formation and removal, as well as different values for cell lifetimes, in WBC and in bladder tissue might explain the lack of correlation.

To conclude, the present results provide some evidence that DNA adducts in bladder tissue may be a risk factor for smoking-induced bladder cancer. These findings are, however, based on relatively small numbers and need to be confirmed in larger studies.

Acknowledgements

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References


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