Comparison of DNA adduct levels in nasal mucosa, lymphocytes and bronchial mucosa of cigarette smokers and interaction with metabolic gene polymorphisms

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The recent introduction of biomarkers in population studies of lung cancer has improved the traditional epidemiological approach, especially in the detection of high risk groups. Many inhalable carcinogens form DNA adducts, an initial event in lung carcinogenesis, and therefore the identification of easily accessible sources of DNA for population studies is considered a leading priority in the field. In this study we compared the frequency of DNA adducts in samples from nasal brushing, bronchial biopsy and peripheral blood lymphocytes (PBL) in a group of 55 subjects, both smokers and non-smokers, undergoing bronchoscopy for diagnostic purposes. Polymorphisms in the CYP1A1, GSTM1 and GSTTI genes were also evaluated. The level of DNA adducts measured by 32P-labelling assay in nasal mucosa (10^8 relative adduct level, mean ± SD 1.10 ± 0.66) was higher than in bronchial mucosa (0.82 ± 0.36) and in PBL (0.54 ± 0.39, P < 0.01). DNA adducts measured in nasal mucosa and in PBL were correlated with those in nasal mucosa (0.82 ± 0.36) and in PBL (0.54 ± 0.39, P < 0.01). DNA adducts in smokers were significantly increased in both nasal mucosa and PBL, with a significant dose–response linear trend (P < 0.05). No significant effect on DNA adduction of the genetic polymorphisms investigated was found. Nasal mucosa brushing proved to be a suitable procedure for the 32P-labelling assay and its use in population studies should be further explored.

Abbreviations: CYP1A1, cytochrome P450 1A1; DRZ, diagonal radioactive zone; FR, frequency ratio; GST, glutathione S-transferase; PAs, polycyclic aromatic hydrocarbons; PBL, peripheral blood lymphocytes; RAL, relative adduct level; XPD, xeroderma pigmentosum group D; XRCC, X-ray repair cross-complementing group.

Introduction

Lung cancer is the most common malignancy in Western countries and one of the most lethal (1). The impact of therapy on survival is limited and, therefore, prevention of this disease is still considered a leading priority in public health. The traditional epidemiological approach has been supplemented in recent years by the use of biomarkers, which have provided new tools for exposure assessment, early effects recognition and, especially, the detection of high risk population groups.

The major role of tobacco smoking and other inhalable carcinogens in the aetiology of lung cancer has been well known for decades (2). Many compounds among those present in airborne carcinogens form DNA adducts and the measurement of this biomarker is an excellent index of exposure to DNA-damaging carcinogens in humans (3). The formation of carcinogen–DNA adducts is an initial event in carcinogenesis (4) and could be efficiently used to assess oncogenic risk in exposed individuals. The metabolic pathways leading to the detoxification or activation of these xenobiotics include enzymes that are polymorphic in humans. The evaluation of these enzymes is of great importance, since different genetic polymorphic combinations may result in different levels of DNA adduction and cancer risk (5).

A number of studies measuring DNA adducts in lung tissue have been published, and most of them found much higher levels of DNA adducts in smokers when compared with non-smokers (reviewed in 6). However, in these studies the specimens always came from cancer patients, since cells from the lung are difficult to sample in healthy subjects. Alternative sources of DNA have been used, of which peripheral blood lymphocytes (PBL), despite a certain lack of sensitivity, have been the most popular. Some studies in human populations have compared adduct levels in the lung and in PBL, reporting conflicting results (7–12).

Among tissues investigated as alternative sources of DNA, nasal mucosa is one of the most promising. Evidence that the level of carcinogen–DNA adducts measured in nasal mucosa is a sensitive and reliable estimate of exposure to tobacco smoke, correlated with similar damage occurring in the lung, emerged from animal studies (13). Recently, studies measuring DNA adducts in human nasal mucosa were published with encouraging results (14–16). The procedures used to obtain biological samples has been described as simple to perform and well tolerated in all these studies, although different techniques were employed. A correlation between the number of cigarettes smoked per day and the level of DNA adducts was observed by Peluso et al. (16). However, no study has been conducted so far in humans to simultaneously compare DNA adducts in various surrogate tissues and in the target organ.

In this study we compared the extent of DNA adduction in surrogate (nasal mucosa and PBL) and target (bronchial mucosa) tissues of smokers and non-smokers with the aim of validating nasal mucosa as a reliable surrogate tissue for...
monitoring exposure to inhalable carcinogens. We also considered the role of inter-individual variability in the metabolism of carcinogens, measuring the impact of relevant genetic polymorphisms on the levels of DNA adducts.

Materials and methods

Study subjects

Subjects included in the study were patients undergoing bronchoscopy for diagnostic purposes in two clinical departments of the San Martino Regional Hospital and in the Pneumology Unit of the National Cancer Research Institute, Genoa. Patients with infectious diseases or with a diagnosis of cancer at the moment of examination were excluded. Sample collection started after approval by all three ethical committees involved. Written consent to participate in the study was requested from all subjects fulfilling inclusion criteria, after receiving a detailed description of the sampling procedures and the aims of the study. Fifty-five (58.5%) out of 94 eligible subjects agreed to participate in the study. The subjects who refused (mainly due to anxiety) were not significantly different from the participants in terms of gender, age, or purpose of the bronchoscopy. A questionnaire concerning current and past smoking habit and possible exposures to carcinogens was administered to all participants at the start of the study by trained interviewers.

The mean age of the study group was 61.6 years (SD = 14). Males represented 76.4% of the sample (42 subjects). All the subjects were followed-up to ascertain their pathology at the end of the diagnostic process and 19 were finally diagnosed as having a respiratory tract cancer (16 lung cancers). Fourteen per cent of the males and half of the females were classified as never smokers (P < 0.02). Smoking status of many subjects was different if considered in the last month before bronchoscopy (38.2% claimed to be former smokers and 25.5% reported smoking >10 cigarettes/day) or in the last week before examination (47.3 and 10.9%, respectively). Given the dramatic change in smoking habits over the last month before examination, former and current smokers were classified together as ever smokers.

Professional or leisure activities were considered as another possible source of carcinogen exposure. Sixteen subjects declared themselves to have recently been exposed to known or suspected carcinogenic agents, i.e., solvents, varnish, ammonia, detergents, pesticides, wood dust and diesel exhaust.

Sampling process

Each subject who signed the consent form was interviewed and underwent a blood draw (10 ml). A trained otorhinolaryngologist performed nasal mucosa brushing with a PAP test cytobrush after washing the nasal cavity with saline solution (0.9% NaCl) and cleaning with a cotton swab. At the end of this procedure subjects received the scheduled bronchoscopy, which included a biopsy of bronchial mucosa. All specimens were blind coded.

Collection was incomplete for 10 subjects; samples most frequently missing were from bronchial mucosa, mainly for clinical reasons (such as bleeding).

32P-post-labelling analysis of DNA adducts

Nasal cells were gently rinsed from the cytobrush into a tube containing 6 ml of saline solution (0.9% NaCl) and 10% acetylcysteine (200 mg/ml) and incubated for 30 min at room temperature (100 cycles/min shaking frequency). After centrifugation, the cell pellets were kept at −80°C until DNA extraction. Bronchial biopsies (2 × 2 mm average size) were frozen shortly after removal and stored at −80°C until DNA isolation. Whole blood (10 ml) collected in EDTA vacutainer tubes was applied to Ficoll density gradients to separate lymphocytes, which were subsequently stored at −80°C.

Frozen bronchial mucosa tissues were powdered in liquid N2 using steel mortars and pestles and homogenized in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4). DNA was isolated and purified using a method that requires enzymatic digestion of RNA and proteins followed by phenol chloroform extractions according to Reddy and Randerath (17).

DNA was gently dissolved in distilled water and its concentration and purity were determined using a spectrophotometer. DNA was stored at −80°C until laboratory analysis.

The 32P-post-labelling assay was used to determine the presence of DNA adducts (16). Coded DNA samples (5–10 µg) were digested using micrococcal nuclease (0.46 U) and spleen phosphodiesterase (0.0174 U) for 4.5 h at 37°C. The DNA digest was treated with nuclease P1 (5 µg) for 20 min at 37°C. The modified DNA was labelled with [32P]ATP (30–40 Ci/mmol) by incubation with T4 polynucleotide kinase (10 U) for 30 min at 37°C (18). Resolution of 32P-labelled DNA adducts treated with nuclease P1 was performed on polyethyleneimine-cellulose thin layer chromatography plates using the contact transfer technique (19). Resolution of aromatic DNA adducts was carried out using a solvent system known to be efficient for the analysis of DNA adducts induced by polycyclic aromatic hydrocarbons (PAHs), such as those contained in cigarette smoking (16): D1, 1 M NaH2PO4, pH 6.8; D3, 7.5 M urea, 4 M lithium formate, pH 3.5; D4, 0.65 M lithium chloride, 0.45 M Tris–HCl, 7.7 M urea, pH 8.0; D5, 1.7 M NaH2PO4, pH 5.0. The presence of DNA adducts in the chromatogram was then visualized by autoradiography for 48–72 h at −80°C. The level of DNA adducts was determined by excising areas of chromatograms and measuring the levels of radioactivity present by Cerenkov counting. Quantification of normal nucleotides was carried out as described above (20). Results are expressed as relative adduct labelling (RAL) × 106 nucleotides, calculated as RAL = (cpm in adducts/cpm in total nucleotides) × (1/dilution factor) (20). Benzo[a]pyrene-modified DNA was routinely processed in the analysis. The reproducibility of the 32P-post-labelling assay was verified by analysing ∼30% of the DNA samples in a second independent experiment and the results of the two analyses were in agreement (r = 0.63, Spearman’s correlation coefficient).

Genotype determination

The GSTM1 and GSTT1 genotypes were examined together. The method used both glutathione S-transferase (GST) primer sets (GSTM1, 5′-AATCTCCCT-GAAAAGCTAAAGG and 5′-GTGTTGGGCTTAAATACGTTGG; GSTT1, 5′-TCTTATGCTGGCTTACATCTC and 5′-TCACGGATGATGC-CAGCA) in the same PCR and included a third primer set for albumin as an internal control (5′-GCCCTCTGCTAAACAGTCTCTAC and 5′-CCCTAAAAA-GAAAAATGCAAATC). PCR consisted of 30 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min and extension at 72°C for 1 min, in the presence of trisphosphate nucleotides, ammonium sulphate and magnesium chloride buffer and Taq DNA polymerase. Reaction products were identified by agarose gel electrophoresis and stained with ethidium bromide.

Cytochrome P450 IA1 (CYP1A1) polymorphism analysis was performed by PCR followed by digestion with restriction enzymes (restriction fragment length polymorphism). An analysis procedure using HincII digestion was followed (21).

Statistical analysis

The estimate of proper sample size for the study was based on findings from correlation studies on carcinogen–DNA adducts in lung tissue and PBL (12) and on the expected difference in the mean level of DNA adducts between smokers and non-smokers (16). Parametric tests on transformed data were performed in the univariate analysis. Multivariate analysis was performed by means of the log-normal regression model. Age, gender, exposure to chemicals and tumour diagnosis were evaluated as confounding variables and included in the model when influencing the regression coefficients. The ratio of DNA adduct median frequency in exposed subjects to controls, i.e. the frequency ratio (FR), was used as a measure of effect.

Results

The numbers of DNA adducts in nasal mucosa cells, bronchial mucosa and PBL according to selected characteristics of the study subjects are reported in Table I. DNA adducts levels were different in the three tissues, with nasal mucosa showing a 34% higher frequency than bronchial mucosa, and more than double that of PBL (P = 0.002).

DNA adduct levels were slightly higher in all tissues in males than in females, probably reflecting different smoking patterns in the two genders, and in nasal brushings of subjects who received a diagnosis of cancer at the end of clinical assessment. No trends were evident by age class or by exposure to chemicals.

Selected polymorphisms of genes involved in detoxification metabolic pathways were determined in the study group. We examined one polymorphic gene involved in metabolic activation (phase I reactions), i.e. CYP1A1, and two genes involved in phase II detoxification reactions, i.e. GSTM1 and GSTT1 (Table II). The frequencies of the polymorphisms were as follows: GSTM1 null, 57%; GSTT1 null, 17%; CYP1A1 variant, 17% (8 heterozygote subjects and 1 homozygote). This is in agreement with the distribution of genotypes in Caucasians reported in the literature (22). No significant differences in DNA adduct frequencies appeared after stratifying by...
DNA adduct levels of cigarette smokers

Table I. DNA adducts levels expressed as $10^8$ RAL in nasal brushings, bronchial biopsies and circulating lymphocytes according to selected variables

<table>
<thead>
<tr>
<th></th>
<th>Nasal brushing</th>
<th></th>
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<th>Bronchial biopsy</th>
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<th>Lymphocytes</th>
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<tr>
<td></td>
<td>$n$ (%)</td>
<td>Mean (SD)</td>
<td>Median</td>
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<td>$n$ (%)</td>
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<td>$n$ (%)</td>
<td>Mean (SD)</td>
<td>Median</td>
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<tr>
<td>Males</td>
<td>42 (77.8)</td>
<td>1.14 (0.66)</td>
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<td>36 (80.0)</td>
<td>0.85 (0.57)</td>
<td>0.75</td>
<td></td>
<td>42 (79.2)</td>
<td>0.55 (0.43)</td>
<td>0.49</td>
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<tr>
<td>Females</td>
<td>12 (22.2)</td>
<td>0.96 (0.65)</td>
<td>0.81</td>
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<td>9 (20.0)</td>
<td>0.74 (0.54)</td>
<td>0.44</td>
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<td>11 (20.8)</td>
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<tr>
<td>&lt;60</td>
<td>19 (35.2)</td>
<td>1.17 (0.80)</td>
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<td>14 (31.1)</td>
<td>0.87 (0.63)</td>
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<td>18 (34.0)</td>
<td>0.48 (0.33)</td>
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<td>60–69</td>
<td>18 (33.3)</td>
<td>1.08 (0.66)</td>
<td>0.92</td>
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<td>15 (33.3)</td>
<td>0.83 (0.48)</td>
<td>0.68</td>
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<td>18 (34.0)</td>
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<td>&gt;69</td>
<td>17 (31.5)</td>
<td>1.06 (0.50)</td>
<td>1.10</td>
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<td>16 (35.6)</td>
<td>0.78 (0.59)</td>
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<td>17 (32.0)</td>
<td>0.58 (0.50)</td>
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<tr>
<td>Cancer</td>
<td>19 (35.2)</td>
<td>1.23 (0.66)</td>
<td>1.09</td>
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<td>16 (35.6)</td>
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<td>19 (35.8)</td>
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<td>Non-neoplastic disease</td>
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<td>27 (60.0)</td>
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<td>31 (58.5)</td>
<td>0.48 (0.34)</td>
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<td>Exposure to chemicals</td>
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<tr>
<td>No</td>
<td>39 (72.2)</td>
<td>1.14 (0.66)</td>
<td>1.10</td>
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<td>33 (73.3)</td>
<td>0.87 (0.60)</td>
<td>0.69</td>
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<td>39 (73.6)</td>
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<td>Yes</td>
<td>15 (27.8)</td>
<td>1.01 (0.66)</td>
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<td>12 (26.7)</td>
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<td>14 (26.4)</td>
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<tr>
<td>Overall</td>
<td>54</td>
<td>1.10 (0.66)</td>
<td>1.04</td>
<td></td>
<td>45</td>
<td>0.82 (0.56)</td>
<td>0.68</td>
<td></td>
<td>53</td>
<td>0.54 (0.39)</td>
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</tbody>
</table>

*a* Only subjects with a definite diagnosis were included.  
*b* 16 out of 19 patients were diagnosed with a lung cancer.  
*c* $P = 0.002$ versus nasal brushing.

Table II. DNA adducts levels expressed as $10^8$ RAL in nasal brushings, bronchial biopsies and circulating lymphocytes according to genetic metabolic polymorphisms

<table>
<thead>
<tr>
<th></th>
<th>Nasal brushing</th>
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<th>Bronchial biopsy</th>
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<th>Lymphocytes</th>
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<td>$n$</td>
<td>Mean (SD)</td>
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<td><strong>GSTM1</strong></td>
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<tr>
<td>Wild-type</td>
<td>23</td>
<td>1.28 (0.6)</td>
<td>1.30</td>
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<td>19</td>
<td>0.90 (0.7)</td>
<td>0.79</td>
<td></td>
<td>23</td>
<td>0.52 (0.4)</td>
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<tr>
<td>Null</td>
<td>30</td>
<td>0.99 (0.6)</td>
<td>0.85</td>
<td></td>
<td>26</td>
<td>0.77 (0.5)</td>
<td>0.64</td>
<td></td>
<td>30</td>
<td>0.56 (0.4)</td>
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<td><strong>GSTT1</strong></td>
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<tr>
<td>Wild-type</td>
<td>44</td>
<td>1.07 (0.6)</td>
<td>1.04</td>
<td></td>
<td>37</td>
<td>0.82 (0.6)</td>
<td>0.68</td>
<td></td>
<td>44</td>
<td>0.51 (0.4)</td>
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<tr>
<td>Null</td>
<td>9</td>
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<td>1.09</td>
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<td>8</td>
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<td>0.72</td>
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<td>9</td>
<td>0.69 (0.5)</td>
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<tr>
<td><strong>CYP1A1</strong></td>
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<tr>
<td>Wild-type</td>
<td>44</td>
<td>1.19 (0.7)</td>
<td>1.09</td>
<td></td>
<td>38</td>
<td>0.83 (0.6)</td>
<td>0.51</td>
<td></td>
<td>44</td>
<td>0.53 (0.4)</td>
<td>0.46</td>
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<tr>
<td>Heterozygote</td>
<td>8</td>
<td>0.85 (0.5)</td>
<td>0.67</td>
<td></td>
<td>7</td>
<td>0.77 (0.6)</td>
<td>0.32</td>
<td></td>
<td>8</td>
<td>0.61 (0.5)</td>
<td>0.51</td>
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<tr>
<td>Mutant homozygote</td>
<td>1</td>
<td>0.16</td>
<td>0.16</td>
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</table>

Genotype, although GSTM1 and CYP1A1 wild-type subjects showed higher values of DNA adducts in nasal brushings.

The agreement between DNA adducts in the three tissues was evaluated through pair-wise correlation analysis. Significant correlation coefficients were found between the frequency of DNA adducts in nasal and bronchial mucosa ($r = 0.48, P < 0.01$) and between the frequency of DNA adducts in bronchial mucosa and PBL ($r = 0.32, P < 0.05$) (Figure 1). Moreover, the correlation between adduct levels in bronchi and nasal mucosa and lymphocytes increased when lung cancer cases were not included in the analyses ($r = 0.641, P < 0.01$ and $r = 0.430, P < 0.05$, respectively). A weaker correlation was found between adducts in PBL and nasal mucosa ($r = 0.21$, not significant).

A synoptic description of DNA adduct levels in the three tissues by various indices of tobacco smoking is shown in Table III. Current and former smokers were grouped together as ever smokers since several smokers changed their habits shortly before sampling, stopping or decreasing cigarette smoking. The choice of collapsing these two groups was further supported by the evidence that levels of DNA adducts were quite similar, i.e. in nasal brushing, current smokers mean RAL ± SD = $1.15 ± 0.69$ versus former smokers $1.22 ± 0.62$; in bronchial biopsy, current smokers $0.92 ± 0.66$ versus former smokers $0.84 ± 0.55$; in PBL, current smokers $0.58 ± 0.40$ versus former smokers $0.61 ± 0.44$.

Ever smokers showed higher levels of DNA adducts than never smokers in all tissues, but this was more evident in nasal brushings and PBL, with a 44% ($P < 0.05$) and a 81% increase ($P < 0.05$), respectively.

DNA adduct levels in smokers appeared to be associated with the number of cigarettes smoked per day in nasal brushings and lymphocytes. A significant increase was evident for the individuals smoking >10 cigarettes/day over non-smokers, with a significant dose–response linear trend ($P < 0.05$).

The results of the multivariate analysis based on the log-normal regression model confirmed the increase in DNA adduct levels in the group of smokers when compared with those who had never smoked [nasal brushing, FR (95% CI) $1.68 (1.01–2.77)$; bronchial biopsy, $1.28 (0.77–2.10)$; lymphocytes, $1.99 (1.11–3.60)$]. The increase appeared to be dose-related in nasal mucosa and especially in lymphocytes, with increased levels in smokers of 1–10 (1.67, 0.99–2.80)
and >10 cigarettes/day (2.06, 1.12–3.78), respectively, when compared with never smokers (test for linear trend $P < 0.05$).

A diagonal radioactive zone (DRZ) and/or distinct DNA adduct spots were detected in positive samples (data not shown). The intensity of the adduct patterns, i.e. the DRZ, was generally stronger in the chromatograms of smokers compared with those of non-smokers. The above DRZ adduct pattern has previously been described in human lung and other tissues of cigarette smokers (6). This adduct profile is characteristic of complex PAH and other aromatic compound exposure and indicates that such DNA adducts are primarily formed by complex mixtures of these chemicals (6,16). Similar chromatographic profiles were generally observed in the maps of bronchial, nasal and lymphocyte samples, however, the intensity of DNA adducts was lower in the chromatograms of lymphocyte samples.

To test the role of metabolic polymorphisms as effect modifiers of the association between smoking habit and DNA adduction, a multivariate approach was performed. Ad hoc models were fitted for each tissue and gene, adjusting for confounders. An effect modification occurred for the $GSTM1$ polymorphism in nasal and bronchial mucosa, although the corresponding interaction term did not reach statistical significance ($FR = 1.98$ and $1.86$, respectively, for the comparison of smokers versus non-smokers in the subgroup with the null genotype).

Evaluation of the $GSTT1$ gene was impossible because no never smokers were found in the group with the null genotype. Finally, in the strata of heterozygote and homozygote mutated $CYP1A1$ gene subjects ever smokers had higher FR values when compared with never smokers ($FR = 3.56$ in bronchial mucosa, $4.52$ in nasal mucosa and $6.92$ in lymphocytes), although the small number of these subjects (6 smokers and 3 non-smokers) makes interpretation of this finding difficult.

**Discussion**

The results of this study show that exposure to inhalable carcinogens may be detected not only by measuring the level of DNA adducts in bronchial tissue and lymphocytes, but also in cells from the nasal mucosa, where the yield of DNA adducts seems to be particularly elevated. A dose-related increase in adduct level in ever smokers when compared with those who had never smoked was found not only in PBL but also in the nasal mucosa. Another major finding of this study was a correlation between the levels of DNA adducts in the target cells of the bronchial mucosa and in both surrogate tissues. No major effect of genetic metabolic polymorphisms on DNA adduct frequencies was found.

This study also provided some novel information about the use of nasal mucosa as a source of DNA for biomonitoring purposes. A major advantage of using cells from this tissue is...
Indeed, the levels of cytochrome P450 enzymes required to adduct formation in target and surrogate tissues may in part with previous studies reporting a similar correlation between some effects of the disease itself. These findings are in keeping respectively), indicating that the production of DNA adducts in bronchi and in the two surrogate tissues, e.g. the bronchoscopy. Interestingly, the correlation between adduct levels in bronchi and in the general population, with different benign bronchial pathologies, such as asthma and chronic bronchitis, characterized by important inflammatory processes. Such inflammatory phenomena have been shown to significantly influence the formation of aromatic DNA adducts in lung target cells by increasing the biologically effective dose of PAHs (30). Thus, increased production of DNA adducts in the bronchi of never smokers with inflammatory pathologies may have masked the effects of cigarette smoke, decreasing the differences between smokers and non-smokers. This hypothesis is consistent with a previous DNA adduct-based case-control study where the levels of aromatic DNA adducts in subjects with inflammatory diseases were significantly higher than those without such inflammatory conditions (31).

The production of DNA adducts in bronchi was significantly correlated with that in nasal mucosa ($r = 0.477$) and, to a lesser extent, with that in PBLs ($r = 0.320$). Obviously, the subjects were not randomly selected from the general population but came from people who were candidates for diagnostic bronchoscopy. Interestingly, the correlation between adduct levels in bronchi and in the two surrogate tissues, e.g. the nasal mucosa and lymphocytes, increased when lung cancer cases were not included in the analyses ($r = 0.641$ and 0.430, respectively), indicating that the production of DNA adducts in different tissues of lung cancer cases could be influenced by some effects of the disease itself. These findings are in keeping with previous studies reporting a similar correlation between adduct levels in lung and in other tissues, including lymphocytes, bladder and liver (10,15,46,47). Differences in DNA adduct formation in target and surrogate tissues may in part reflect different exposure and differences in the rates at which DNA adducts are formed and repaired in different cell types. Indeed, the levels of cytochrome P450 enzymes required to metabolize the carcinogens into reactive metabolites, capable of forming DNA adducts, are higher in bronchi than in lymphocytes (48,49). Specific studies aimed at assessing the relevance of different tissues for biomarker research requires the investigation of a larger number of subjects.

The role of individual susceptibility is commonly reported in many papers as the most likely explanation of non-consistent results from biomonitoring studies. To take this aspect into account we screened the study group for metabolic genetic polymorphisms, a trait that is known to play a major role in modulating the genotoxic effect of many carcinogens.

In a recent study by Teixeira et al., the levels of DNA adducts in the lymphocytes of smokers were found to be dependent on a MspI polymorphism of CYP1A1 ($P < 0.05$), whereas no effect was observed for GSTM1, GSTT1 and GSTP1 (32). Despite this evidence, the literature on this topic is contradictory, and contrasting results on the effect of CYP1A1 polymorphisms on the level of DNA adducts have been reported (33–36). The GSTM1 null genotype has been associated with the level of PAH–DNA adducts in lung tissue and sometimes in blood cells in a number of studies (37–44). There are also a number of studies showing that increased levels of aromatic DNA adducts in subjects, including smokers, who do not have the capacity to detoxify carcinogens via the GSTM1 pathway are associated with low plasma levels of dietary antioxidants (18,44,45).

In the present study a slight effect of cigarette smoking on DNA adduct levels was found in subjects with the GSTM1 null genotype in the respiratory tissues but not in lymphocytes. An increase was also consistently found in the three tissues in smokers with the mutated CYP1A1 genotype. These results are suggestive and deserve to be further evaluated in a larger dataset.

Another potential source of individual variability in the response to genotoxic damage is polymorphism of DNA repair enzymes (50). In particular, the xeroderma pigmentosum group D (XPD) and X-ray repair complementing group (XRCC) 1 and 3 genes have been reported to be polymorphic and potentially involved in human carcinogenesis (50–55). Analysing three genetic polymorphisms in different DNA repair genes, XRCC1 Arg399Gln (exon 10), XRCC3 Thr241Met (exon 7) and XPD Lys751Gln (exon 23), in 41 nasal mucosa biopsies, we found that a difference exists among the XRCC3, XRCC1 and XPD polymorphisms for nasal mucosa DNA adducts (unpublished data). Stratifying by smoking habit, we observed opposite trends for the latter two polymorphisms, with the variant homozygotes showing increased levels in non-smokers and lower levels in smokers. Conversely, DNA adducts were always higher across the different smoking groups for XRCC3 Met241 homozygotes. The same effect on DNA adduct levels for the same three DNA repair polymorphisms has been found in the leukocytes of an Italian cohort of the collaborative European project known as the European Prospective Investigation into Cancer and Nutrition (EPIC) (52). It is possible that in both target and surrogate tissues cigarette smoking may influence the amount of DNA damage activating and up-regulating repair enzymes. Indeed, heavy smokers have been shown to have more proficient DNA repair capacity in lymphocytes than lighter smokers, indicating that DNA repair enzymes may be stimulated in response to DNA damage induced by cigarette carcinogens (56). Further investigations are needed to investigate the possible interaction between these DNA repair polymorphisms, cigarette smoking and other risk factors.
In conclusion, this study confirmed the reliability of the classic surrogate tissue for assessing exposure to inhalable carcinogens, but also showed that the use of an innovative source of DNA, nasal mucosa, appeared to be a suitable procedure. The significant correlation between the levels of DNA adducts in nasal mucosa and lymphocytes with the corresponding values in bronchial mucosa strongly supports this conclusion. The effect of cigarette smoking in the nasal mucosa is evident and a quantitative relationship with the number of cigarettes smoked was determined. The role of polymorphisms of metabolic genes seems to be of limited importance, although smokers with GSTM1 null genotypes had increased levels of DNA adducts in respiratory tissues.

The good sensitivity of the assay and the acceptability of nasal mucosa brushing support the suitability of this approach for biomonitoring populations and require further research.

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