DNA adducts in tumour, normal peripheral lung and bronchus, and peripheral blood lymphocytes from smoking and non-smoking lung cancer patients: correlations between tissues and detection by $^{32}$P-postlabelling and immunoassay

Erika Győrffy¹, Lívia Anna¹, Zoltán Győri², Judit Segesdi², János Minárovits², Ibolja Soltesz³, Szilárd Kostic³, Attila Csekeö³, Miriam C.Poirier⁴ and Bernadette Schoket¹,⁵

¹National Institute of Environmental Health, József Fodor National Center for Public Health, Budapest, H-1097 Hungary, ²Béla Johan National Center for Epidemiology, Budapest, H-1529 Hungary, ³Korányi National Institute of Pulmonology, Budapest, H-1529 Hungary and ⁴National Cancer Institute, NIH, Bethesda, MD 20892, USA

⁵To whom correspondence should be addressed
Email: schoketb@okk.antsz.hu

Introduction

Epidemiological studies have shown that tobacco smoking is a major causative factor for lung cancer (1). Cigarette smoke has more than 4000 chemical constituents, and of those at least 43 are animal carcinogens, while some are known to be human carcinogens. Polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BP), aromatic amines, including 4-aminobiphenyl (4-ABP), tobacco-specific nitrosamines and free radical species may contribute to the carcinogenic and mutagenic activity of cigarette smoke (2). Metabolic activation of potentially carcinogenic chemicals and the covalent binding of the reactive carcinogen metabolites to DNA are considered key events in tumour initiation (3–5). Carcinogen–DNA adduct formation results from the complex processes of carcinogen absorption, activation and detoxification as well as DNA repair and tissue turnover. Carcinogen–DNA adduct formation constitutes the biologically effective dose resulting from carcinogen exposure. Because DNA adduct formation is considered necessary for carcinogenesis, measurement of human DNA adducts provides evidence of molecular dosimetry, genotoxicity and potential cancer risk.

Molecular dosimetry of human genotoxic exposure and determination of carcinogen–DNA adducts have advanced substantially during the last two decades. The most frequently used methods for the detection of carcinogen–DNA adducts include $^{32}$P-postlabelling, immunooassays and immunohistochemistry, HPLC-electrochemical detection, mass spectrometry, fluorescence and phosphorescence spectroscopy and special combinations of separation and detection techniques (6,7). $^{32}$P-Postlabelling and the immunoassays have several advantages for monitoring human exposure to the complex chemical mixture that comprises tobacco smoke. $^{32}$P-Postlabelling has a broad specificity for bulky DNA adducts typically formed from aromatic compounds (8). (+)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-DNA chemiluminescence immunoassay (BPDE-DNA CIA) in smaller subsets of tissue samples subject to availability of DNA. Bulky DNA adduct levels ranged between 0.3 and 27.8 adducts/10⁸ nucleotides (nt) with mean adduct levels between 2.8 and 11.5 adducts/10⁸ nt. Mean PAH–DNA adduct levels were 2.6–6.2 adducts/10⁸ nt. Significantly higher bulky DNA adduct levels were detected in smokers’ lungs as compared with non-smokers’ ($P < 0.02$). PAH–DNA adduct levels appeared higher in the lungs of smokers compared with non-smokers but the difference was not significant. Lung tumour contained on average a 50% lower DNA adduct level compared with normal lung tissue. A statistically significant positive correlation was found between the DNA adduct levels of the corresponding tumour and normal lung tissue samples in both smokers and non-smokers using both methodologies. Bulky DNA adduct levels in normal lung and blood lymphocytes correlated significantly in non-smokers only ($r = 0.55$, $P = 0.023$). In lung tumour DNA samples there was a weak correlation between values obtained by $^{32}$P-postlabelling and by the BPDE-DNA immunoassay ($r = 0.27$, $P = 0.054$). However, with normal lung DNA samples, values obtained by the two assays did not correlate.

Abbreviations: BPDE–DNA CIA, (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol 9,10-epoxide–DNA chemiluminescence immunoassay; nt, nucleotides; PAH, polycyclic aromatic hydrocarbon.
DNA adduct formation in target and surrogate tissues, and there is virtually no information on the DNA adduct composition (i.e. formation of specific DNA adduct structures) in different tissues.

In the present paper we document bulky DNA adduct formation in lung tumour, distal lung tissue, bronchial tissue and peripheral blood lymphocytes taken from smoking and non-smoking patients with lung malignancy. The DNA adduct levels have been determined by 32P-postlabelling with nuclease P1 adduct enrichment. In addition, PAH-DNA adduct levels have been determined in lung tumour and peripheral lung tissue by BPDE-DNA CIA. The results elucidate smoking-related primary DNA damaging processes in lung, and the use of peripheral blood lymphocytes as surrogate tissue in molecular epidemiological studies of human genotoxic exposures to complex environmental mixtures.

Materials and methods

Study population

Samples of lung tumour and histologically normal peripheral lung tissue, normal bronchial tissue and peripheral blood were obtained with informed consent from a total of 85 Hungarian patients with lung malignancy that underwent lung resection. Matched sample sets of lung tumour, normal peripheral lung, normal bronchus and peripheral blood lymphocytes were available from 32 subjects. The most prevalent histological types of lung cancer were adenocarcinoma (n = 42) and squamous cell carcinoma (n = 28). Detailed information on smoking history was obtained from the patients by self-reporting. Based on our previously published results (15), two broad smoking categories were defined in the present study. 'Smokers' included those patients who were active smoking patients with lung malignancy. The DNA adduct levels have been determined by 32P-postlabelling with nuclease P1 adduct enrichment. In addition, PAH-DNA adduct levels have been determined in lung tumour and peripheral lung tissue by BPDE-DNA CIA. The results elucidate smoking-related primary DNA damaging processes in lung, and the use of peripheral blood lymphocytes as surrogate tissue in molecular epidemiological studies of human genotoxic exposures to complex environmental mixtures.

Materials and methods

Table I. Demographics, smoking status and histological types of lung malignancies of the study population

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Number of patients (%)</th>
<th>Number of males/females</th>
<th>Age range (years)</th>
<th>Mean age ± SD (years)</th>
<th>Histological types of lung malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>85</td>
<td>53/32</td>
<td>37-79</td>
<td>56.3 ± 7.4</td>
<td>SQ/A/SC/UC/OTa</td>
</tr>
<tr>
<td>'Smokers'b</td>
<td>35/40.0%</td>
<td>21/13</td>
<td>44-72</td>
<td>55.6 ± 6.4</td>
<td>14/16/1/2/1</td>
</tr>
<tr>
<td>Short-term former smokers</td>
<td>13/15.3%</td>
<td>9/4</td>
<td>37-69</td>
<td>54.2 ± 8.8</td>
<td>4/6/1/1/1</td>
</tr>
<tr>
<td>'Non-smokers'c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term former smokers</td>
<td>23/27.1%</td>
<td>18/5</td>
<td>48-74</td>
<td>59.3 ± 7.0</td>
<td>10/10/0/2/1</td>
</tr>
<tr>
<td>Life-time non-smokers</td>
<td>15/17.6%</td>
<td>5/10</td>
<td>45-79</td>
<td>54.9 ± 8.3</td>
<td>0/10/0/0/5</td>
</tr>
</tbody>
</table>

SQ: squamous cell carcinoma; A: adenocarcinoma; SC: small cell carcinoma; U: undifferentiated carcinoma (non-small cell anaplastic carcinoma); OT: other types of lung malignancies (two mixed histological type carcinomas, two carcinoid tumours, three lung metastases, one lymphogranulomatosis).

'Smokers' are defined as the combined group of current smokers and short-term former smokers: current smokers smoked until the lung surgery; short-term former smokers gave up smoking <1 year before surgery (1 week to 1 year).

'Non-smokers' are defined as the combined group of long-term former smokers and life-time non-smokers: long-term former smokers gave up smoking >1 year before surgery (2-32 years).

Table II. Schematic description of the available human DNA samples for DNA adduct determination by 32P-postlabelling and BPDE-DNA CIA (total number of subjects 85)

<table>
<thead>
<tr>
<th>Method</th>
<th>Tissue type</th>
<th>DNA samples available</th>
<th>Matched DNA samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>32P-Postlabelling</td>
<td>Lung tumour</td>
<td>54</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Normal lung</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Normal bronchus</td>
<td>61</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Peripheral blood lymphocytes</td>
<td>45</td>
<td>32</td>
</tr>
<tr>
<td>BPDE-DNA CIA</td>
<td>Lung tumour</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Normal lung</td>
<td>74</td>
<td>63</td>
</tr>
</tbody>
</table>

Determination of bulky DNA adducts by 32P-postlabelling

Bulk DNA adducts were detected in DNA samples from lung tumour, normal lung, normal bronchial tissue and peripheral blood lymphocytes by the 32P-postlabelling method combined with nuclease P1 adduct enrichment, essentially as described before (15) with minor modifications. Briefly, DNA (4 µg) was digested overnight with 290 µg micrococcal nuclease (Sigma, St Louis, MO) and 1.2 µU spleen phosphodiesterase (ICN Biomedicals Inc., Irvine, CA). Nuclease P1 digestion of normal mononucleotides was performed with 1.2 U enzyme (Sigma). Radiolabelling occurred with 50 µCi carrier-free [γ-32P]ATP (end-labelling grade, ICN Biomedicals) and 6 U of T4 poly-nucleotide kinase (USB Corporation, Cleveland, OH). Multidirectional thin-layer chromatographic development of the maps of the radiolabelled DNA digests was performed on 10 × 10 cm poly(ethyleneimine)/cellulose sheets (Macherey-Nagel, Düren, Germany) with the following buffers: D1, 1 M sodium phosphate buffer, pH 6.0; D2, 3.5 M lithium formate, 8.5 M urea, pH 3.5; D3, 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0, and D4, 1.7 M sodium phosphate buffer, pH 6.0. Autoradiography of the chromatograms was at −100°C for 3-4 days. Radioactivity in the diagonal zones and individual spots, as well as in a blank area adjacent to the diagonal zone, was determined by Cerenkov counting of the excised areas of the thin-layer chromatographic maps. Background radioactivity of the blank area, corrected for the size of the excised adduct areas was subtracted from the radioactivity of the adduct areas. Quantification was calculated as described previously (15), by using specific radioactivity value of the [γ-32P]ATP, determined from radioisotope labelling of dAP. To refine quantification, an in vitro-modified BPDE-DNA standard (110 adducts/106 nucleotides (nt)) was used in duplicate as external standard in each radiolabelling assay for normalization of the DNA adduct values of the biological samples to reduce inter-assay variability (17). The standard was originally prepared for a 32P-postlabelling international trial and was kindly provided by Dr F.Beland (NCTR, Arkansas) (17). To observe the normalized DNA adduct value for the test sample, the following formula was applied: normalized adduct value of the test sample = DNA adduct value of the test sample calculated from its radioactivity measurement × known DNA adduct value of the BPDE-DNA external standard/DNA adduct value of the BPDE-DNA external standard calculated from its radioactivity measurement. Two to four replicate analyses were performed with each human DNA sample.
in separate assays. All the samples demonstrated detectable adduct levels. The assay variability was 28 ± 15% (mean ± SD). The detection limit was 0.3 adducts/10^8 nt.

**Determination of PAH-DNA adducts by competitive BPDE-DNA CIA**

PAH-DNA adducts were determined in a subset of DNA samples from lung tumour and normal lung tissue samples by BPDE-DNA CIA. There was insufficient DNA from bronchial tissue and peripheral blood lymphocytes for this assay. The BPDE-DNA CIA was performed essentially as described by Divi et al. (12). Microtitre plates were coated with BPDE-DNA modified to 0.33%, while the BPDE-DNA in the standard curve, the same standard used for the \(^{32}\)P-postlabelling assay, was modified to 110 (7R)-N\(^{-}\)2,110-(7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[g][pyrene]-yl)-deoxyguanosine (BPdG) adducts/10^8 nt. Each microtitre plate well contained either 10 μg of sample DNA or, for the standard curve wells, 10 μg of carrier calf thymus DNA. This competitive assay also employed anti-BPDE-DNA (#31, bleed 8/16/78, 1:6 dilution), biotin-avidin amplification, CDP-Star substrate and Emerald II enhancer (Tropics, Bedford, MA). Luminescence was read by TR717 microplate luminometer (PE Applied Biosystems, Foster City, CA). Samples were assayed in triplicate wells on each microtitre plate, and on one or two microtitre plates, depending on the availability of DNA sample. The 50% inhibition for the BPDE-DNA standard curve was 2.69 ± 0.22 fmol BPdG/well (mean ± SEM, n = 17) and the detection limit using 10 μg of sample DNA was 1.1 ± 0.2 BPdG adducts/10^8 nt.

**Statistical analysis**

For calculations of the levels of DNA adducts measured by CIA, samples with values lower than the limit of detection were assigned a value half way between zero and the limit of detection. The statistical analyses were performed with GraphPad Prism 3.0 software. Tests used for statistical analysis included the Mann–Whitney test for assessing the difference of DNA adduct levels of each tissue type between smokers and non-smokers, one-way ANOVA for comparisons of the DNA adduct levels among the various tissues, and Spearman correlation test for calculating the correlation between DNA adducts.

**Bulky DNA adducts, determined by \(^{32}\)P-postlabelling, related to smoking status**

Levels of bulky DNA adducts, as determined by \(^{32}\)P-postlabelling, were stratified by smoking status and are presented in Table III. Bulky DNA adduct levels exhibited large inter-individual variation, from 0.3 to 27.8 adducts/10^8 nt in smokers, and from 0.3 to 14.4 adducts/10^8 nt in non-smokers. Compared with non-smokers, smokers had 1.7–2.4-fold higher DNA adduct levels, detected by \(^{32}\)P-postlabelling, in lung tumour, normal lung and normal bronchial tissues (P < 0.02). In peripheral blood lymphocytes, there was no smoking-related increase in bulky DNA adducts.

A set of matched samples that included all four tissues was available for 17 smokers and 15 non-smokers for DNA samples analysed by \(^{32}\)P-postlabelling (Figure 2). The relative differences between tissues and assays were similar to values for the complete sample set shown in Table III. In the matched set of samples, DNA adducts in lung tumour, normal lung and bronchus were significantly higher in smokers compared with non-smokers (P = 0.016, 0.0003 and 0.0006, respectively). In peripheral blood lymphocytes the difference between smokers and non-smokers was marginally significant (P = 0.054). In the individually matched DNA sample sets, in general, there were similar relative differences in the DNA adduct levels as among the unmatched tissue samples.

**PAH–DNA adducts, determined by competitive BPDE–DNA CIA, related to smoking status**

Levels of PAH–DNA adducts, determined by competitive BPDE–DNA CIA in lung tumour and normal lung tissue DNA, were stratified by smoking status and are presented in Table III. PAH–DNA adduct levels exhibited large inter-individual variation, from 0.4 to 22.6 adducts/10^8 nt in smokers, and 0.4–10.8 adducts/10^8 nt in non-smokers. Undetectable PAH–DNA adduct levels were observed in 6% of samples. PAH–DNA adduct levels were 1.2–1.4-fold higher in lung tissues from smokers, compared with non-smokers, however, the smoking-related increase was not statistically significant.

**DNA adduct levels found in individuals with different cancer type and gender**

In the two smoking categories, the DNA adduct levels in different tissues, determined by both \(^{32}\)P-postlabelling and BPDE–DNA CIA, were compared for adenocarcinoma and squamous cell carcinoma. For lung tumour in non-smokers by \(^{32}\)P-postlabelling only the difference between DNA adducts in adenocarcinoma (2.3 ± 1.4 adducts/10^8 nt, n = 14), and squamous cell carcinoma (3.9 ± 1.3 adducts/10^8 nt, n = 6) was significant (P = 0.036). For normal lung the same difference was not significant (P = 0.067). There was no significant difference in the DNA adduct levels of any tissue, regardless of the method, between males and females after stratification for histological type and smoking category (data are not shown).
Table III. Levels of DNA adducts, as determined by $^{32}$P-postlabelling and competitive BPDE-DNA CIA, in tissues from lung cancer patients in two main smoking categories$^{a-d}$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$^{32}$P-Postlabelling</th>
<th>BPDE-DNA CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers DNA adducts/10^8 nt</td>
<td>Non-smokers DNA adducts/10^8 nt</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD (n)</td>
<td>Median</td>
</tr>
<tr>
<td>Lung tumour</td>
<td>4.7 ± 3.1 (29)</td>
<td>3.9</td>
</tr>
<tr>
<td>Normal lung</td>
<td>11.0 ± 6.9 (35)</td>
<td>8.8</td>
</tr>
<tr>
<td>Normal bronchus</td>
<td>11.5 ± 6.8 (34)</td>
<td>11.1</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>6.0 ± 3.9 (24)</td>
<td>4.8</td>
</tr>
</tbody>
</table>

$^a$Smokers and non-smokers are defined in Table I.

$^b$Mann-Whitney test, P values for statistical significance, for difference between smokers and non-smokers, by $^{32}$P-postlabelling: for lung tumour, 0.019; for normal lung, <0.0001; and for normal bronchus, <0.0001.

$^c$One-way ANOVA with Bonferroni’s multiple comparison post test for log-transformed adduct values, P values for statistical significance, difference between tissues for smokers assayed by $^{32}$P-postlabelling: lung tumour versus normal lung, <0.001; lung tumour versus normal bronchus, <0.001; normal lung versus lymphocytes, <0.05; and normal bronchus versus lymphocytes, <0.01.

$^d$Mann–Whitney test, P values for statistical significance, difference between tissues for smokers assayed by BPDE–DNA CIA: for smokers: lung tumour versus normal lung, 0.0086; and for non-smokers: lung tumour versus normal lung, 0.0001.
These relative differences were statistically significant for smokers by 32P-postlabelling and BPDE in the complete set of samples, for smokers and non-smokers combined, tumour tissues contained 1.6-fold higher DNA adducts, measured by both 32P-postlabelling and BPDE in normal lung or bronchial tissue compared with blood lymphocytes (Figure 3A and C). In non-smokers, there was no remarkable difference in bulky DNA adduct levels between the normal lung or bronchial tissue compared with blood lymphocytes (Figure 3B and D). For the combined population of smokers and non-smokers, there was a statistically significant positive correlation between bulky DNA adduct levels in normal lung and peripheral blood lymphocytes ($r = 0.45$, $P = 0.006$, $n = 36$), as well as between normal bronchial tissue and peripheral blood lymphocytes ($r = 0.47$, $P = 0.004$, $n = 35$). However, stratification by smoking revealed a statistically significant correlation between bulky DNA adduct levels in blood lymphocyte DNA and normal lung DNA in non-smokers ($r = 0.55$, $P = 0.023$) (Figure 3B), whereas no such correlation was observed in smokers ($r = 0.25$, $P = 0.30$) (Figure 3A). Bulky DNA adducts in bronchial tissue were correlated with those in blood lymphocytes in non-smokers ($r = 0.55$, $P = 0.026$) (Figure 3D) but not in smokers ($r = 0.31$, $P = 0.20$) (Figure 3C).

**Correlation between bulky DNA adduct values obtained by 32P-postlabelling and PAH–DNA adduct values obtained by competitive BPDE–DNA CIA**

The only DNA samples subjected to PAH–DNA adduct determination by BPDE–DNA CIA were normal lung and lung tumour, as there was insufficient DNA from normal bronchus and lymphocytes. For those tissues, comparison of values obtained showed similar relative differences between the DNA adduct levels measured by both 32P-postlabelling and BPDE–DNA CIA. There were 50 lung tumour DNA samples and 62 normal lung DNA samples assayed by both methods. There was a weak positive correlation between the two methodologies for the same set of lung tumour DNA samples ($P = 0.054$, Figure 4A); however, there was no correlation when normal lung DNA samples were measured by both methods ($P = 0.96$). When the matched 50 normal lung samples were analysed for the correlation between the two methods (Figure 4B), the correlation did not change substantially (Spearman correlation coefficient $r = 0.11$, $P = 0.45$).

**Discussion**

This study is the first one, in which 32P-postlabelling and BPDE–DNA CIA were used together for the DNA adduct determinations in the same human DNA sample set. In the present study we compared carcinogen–DNA adduct levels in lung tumour and normal lung tissue between smokers and non-smokers, and determined the correlation between the tissues and the two DNA adduct methods. We also compared carcinogen–DNA adduct levels in normal lung and bronchial tissue with those in peripheral blood lymphocytes, and established exposure dose-related correlation between them.

We have demonstrated lower DNA adduct levels in the lung tumour tissues than in the histologically normal tissues, in both smokers and non-smokers by both 32P-postlabelling and immunoassay. In an earlier comparative study of lung tumour and non-tumour lung tissues by van Schooten et al. (18), there was an indication of lower adduct levels in the tumour than in the normal tissue. Tang et al. (19), however, did not detect significant difference between PAH–DNA adduct levels of tumour and non-tumour tissues using a BPDE–DNA ELISA. The difference between DNA adduct levels in tumour and histologically normal tissues may reflect changed transport of...
the xenobiotics into the tumour cells, altered metabolic capacity, altered DNA repair and/or ‘adduct-dilution’ in the rapidly proliferating tumour. In the literature the relative differences in DNA adduct levels in the tumour and non-tumour tissues vary. In larynx (20,21), breast (22), hepatocellular carcinoma (23), gastric adenocarcinoma (24) and intrahepatic cholangiocarcinoma (25) observations are inconsistent suggesting that the relative differences in aromatic DNA adduct formation in human tumour and corresponding non-tumour tissues are largely organ-specific.

Smoking-related elevation of bulky DNA adduct levels in human lung and in other solid tissues has been reported in many studies since the early 1990s (13–15,26). The present investigation serves to confirm the published findings. However, smoking-related elevation of bulky DNA adduct levels in white blood cells is not consistent in the literature (27–30). It is likely that leukocyte DNA adduct levels may be influenced not only by smoking, but also by PAH exposures from the ambient environment, as well as diet (31).

Those few human biomonitoring studies that provide correlations between biomarkers of exposure in target and surrogate tissues are valuable for estimation of genotoxic doses in environmental and occupational exposure situations in healthy populations. We detected statistically significant positive correlation between bulky DNA adduct levels, determined by 32P-postlabelling, in normal lung or bronchial tissues and peripheral blood lymphocytes in lung cancer patients, which supports the results by Wiencke et al. (32). However, in our study population, after stratification for smoking status, it was only the non-smokers for whom the correlation was significant. Also in lung patients, a positive correlation was observed between smoking-related N7-methylguanine-DNA adduct levels in normal bronchus and peripheral blood lymphocyte DNA (33). No correlation was found between normal lung and white blood cell PAH-DNA adducts by van Schooten et al. (18,19). Other studies with human bronchoalveolar lavage cells (34), nasal mucosa (35), larynx (21) and skin (36) also suggest that the correlation between DNA adduct levels in white blood cells and the corresponding tissue may be influenced by the internal dose of the genotoxic agents. The dose-dependence of correlation is also supported by animal studies, in which linear dose-response relationships were found for adduct formation at low dose treatments, and the saturation of DNA-binding potency occurred at high doses (37).

**Fig. 3.** Correlation between bulky DNA adduct levels, determined by 32P-postlabelling, in peripheral blood lymphocyte and normal lung (A and B), and in peripheral blood lymphocyte and normal bronchial DNA (C and D), respectively, in smokers and non-smokers. Each circle represents one individual, n is the number of individuals and r is the Spearman correlation coefficient.
The activation of a xenobiotic depends on the metabolic capacity of the corresponding tissues, which should be considered in dose–response extrapolations from surrogate tissue to target tissue in risk assessment.

In molecular cancer epidemiology and in biomonitoring of human exposures to complex mixtures of PAHs, 32P-postlabelling and immunoassays are the most suitable methods for DNA adduct detection. The present study is the first one in which both 32P-postlabelling and BPDE-DNA CIA are used for the same in vivo-modified human DNA samples. 32P-Postlabelling has the advantage of high sensitivity for bulky DNA adducts, on the other hand, without prior chemical structure-selective adduct enrichment it is not suitable for the identification of specific DNA adduct structures. The BPDE–DNA CIA is the most sensitive and reproducible BPDE–DNA competitive immunoassay and the smallest amount of DNA is required for the determination by using the same rabbit antiserum as for the previous BPDE–DNA immunoassays (12). It detects BPDE-DNA adducts in experimental samples following treatment with BP in vivo and in vitro, and is selective for PAH–DNA adducts in tissues exposed to or treated with complex mixtures of PAHs. In the past, our laboratories and others applied and compared 32P-postlabelling and BPDE–DNA ELISA or DELFIA (dissociation-enhanced lanthanide fluorimunoassay) in biomonitoring studies of PAH-exposed populations (38–41). 32P-Postlabelling and the immunoassays resulted in similar qualitative evidence of the relative exposure differences among the compared groups in most of the studies (39–41). In the present study, the same ranges of DNA adduct levels were obtained by both methods. The good agreement may largely be attributed to the fact that the same in vitro-modified BPDE–DNA adduct standard was used as external standard for normalization of the adduct levels (17) in each 32P-postlabelling assay, and for the calibration standard curve in the CIA measurements.

32P-Postlabelling was more sensitive than the BPDE–DNA CIA for recognizing the effect of smoking status on the DNA adduct levels in the pulmonary tissues. The smoking-related increases in adduct levels were statistically significant for the DNA adduct values obtained by the 32P-postlabelling assay, but were not statistically significant when measured by CIA. A similar methodology-dependent recognition of effect was observed by Culp et al. (42) who compared 4-ABP-DNA adduct levels in human peripheral lung by immunoochemical, 32P-postlabelling and GC/MS detection. No difference in the DNA adduct levels was found comparing smokers and long-term ex-smokers when the DNA adduct levels were determined by 4-ABP–DNA-specific immunoassay, but a statistically significant difference was shown when 32P-postlabelling was applied. Such method-dependent differences in the recognition of exposure categories may primarily originate from the dissimilar overall adduct specificity of the two assays and from the complexity of semi-quantification.

In earlier human biomonitoring studies in PAH-exposed study populations, there was a weak negative correlation or a lack of correlation between corresponding DNA adducts by 32P-postlabelling and BPDE–DNA CIA. The applied DNA adduct correlation between tissues and methods

![Fig. 4](https://academic.oup.com/carcin/article-abstract/25/7/1201/2390790)

Fig. 4. Correlation between DNA adduct levels, determined by 32P-postlabelling and BPDE-DNA CIA, in matched lung tumour (A) and normal lung tissue (B) DNA samples. Each circle represents one individual, n is the number of individuals and r is the Spearman correlation coefficient.

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DNA adduct correlation between tissues and methods

A) Lung tumour

B) Normal lung

<table>
<thead>
<tr>
<th>BPDE-DNA CIA</th>
<th>32P-Postlabelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
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<table>
<thead>
<tr>
<th>PAH-DNA adducts/10^6 nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

n=50, r=0.27, P=0.054

n=50, r=0.11, P=0.45
```
adduct levels in lung tumour and normal lung by both DNA adduct methods. This finding suggests that it might be possible to approximate DNA adduct levels in this study population between normal lung and lung tumour using either 32P-postlabelling or BPDE-DNA CIA. Correlation between DNA adducts in lung tissue and in peripheral blood lymphocytes was observed only in non-smokers; the lack of correlation in smokers suggests significant differences in metabolism, and perhaps exposure dose in the two tissues. This finding implies that carcinogen-DNA adduct formation in the peripheral blood lymphocytes, a potential surrogate for normal lung and bronchial tissues, may be used for exposure assessment for the lung tissues at low exposure conditions only. There was a positive correlation between the two DNA adduct methods in lung tumour tissues, and lack of correlation in normal tissue, which may suggest that qualitatively different DNA adduct patterns are formed in the tumour and in the normal lung tissue, respectively. Further studies are needed to better characterize the qualitative composition of the smoking-related DNA adducts in human tissue samples and white blood cells.

Acknowledgements

The technical assistance of K.Lévy and G.Papp and the secretarial assistance of A.Karácsonyi and G.Fleischer are gratefully acknowledged. The technical advice and support of Dr R.L.Diví in the execution of immunohistoassays are highly appreciated. The research project has been supported by the Hungarian OTKA T 034616 and ETT 003/2001 research grant, and in part by the UICC International Cancer Technology Transfer Fellowship to B.S. (ICRET No. 605/2002) with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract NO2-CO-91012.

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*Received October 20, 2003; revised January 12, 2004; accepted February 20, 2004*