Comparison of DNA Adducts and Sister Chromatid Exchange in Lung Cancer Cases and Controls


ABSTRACT

In a molecular epidemiological study of lung cancer cases (n = 81) and noncancer controls (n = 67), polycyclic aromatic hydrocarbon (PAH)- DNA adducts were evaluated in peripheral blood leukocytes from all subjects and in a smaller number of lung tissue specimens collected prior to or at surgery. Sister chromatid exchanges (SCE) in lymphocytes were also studied in a subset of cases and controls. Questionnaire, medical record, or tumor registry data provided a family history of cancer, as well as information on cigarette smoking, dietary and occupational exposure to PAHs, and other factors related to SCEs. In both cases and controls PAH-DNA adducts in leukocytes measured by an enzyme-linked immunosorbent assay were not significantly related to age, sex, ethnicity, amount of cigarette smoking, passive smoking, dietary charcoal, or caffeine consumption. Nor did family history of cancer or histological type of cancer significantly affect adduct levels. However, when subjects were stratified according to smoking status (current, former, and nonsmoker), lung cancer cases who were current smokers had significantly higher levels of covalent adducts than current smoker controls. A seasonal variation was observed in PAH-DNA binding, with a peak in adduct levels during July-October. This peak corresponds to that seen in a prior study of aryl hydrocarbon hydroxylase inducibility by other investigators. The finding of significant levels of PAH-DNA adducts in former smokers and nonsmokers supports an earlier observation that this marker is not smoking specific but reflects a pervasive and variable "background" exposure to PAH. These results are consistent with a genetically determined enhancement of PAH-DNA adduct formation in leukocytes of lung cancer cases which is evident in current smokers. The results in lung tissue are limited by the small number of samples. Adduct levels were not significantly increased in lung tissue of smokers compared with nonsmokers. An inverse linear correlation was seen between adduct values in lung tissue and age of the donors. SCEs were significantly related to pack years of smoking. However, there was no difference in the frequency of SCE between cases and controls; nor were SCE and DNA adducts significantly correlated in this small sample.

INTRODUCTION

Lung cancer has recently become the leading cause of cancer deaths in both men and women in the United States (1). While the great majority of lung cancer cases are believed to be attributable to cigarette smoking, other environmental exposures (products of fossil fuel combustion including PAH, arsenic, nickel, radon, etc.) are causally related to the disease. There is evidence that genetic factors may, in the presence of carcinogenic exposures, operate to confer greater susceptibility to lung cancer. A strong familial factor (i.e., increased lung cancer incidence with at least one affected first degree relative) has been observed (2, 3). Speculating that genetic factors influence individual susceptibility to carcinogens, many investigators have evaluated the role of genetically regulated metabolism of PAHs by the AHH microsomal enzyme system. In experimental systems (inbred strains of mice), a relationship has been demonstrated between AHH inducibility and susceptibility to lung cancer (4, 5). However, the results of human studies have been conflicting (4, 6-9) possibly because of the different methods used to assay AHH induction or because individual enzymes are operating in a complex metabolic network (10). The complexity of this dynamic metabolic system is compounded by the observation of a strong seasonal variation in AHH inducibility, which was independent of smoking status (11). A recent study showing a higher level of AHH in lung tissue of lung cancer cases who were recent smokers compared to smoking controls supports the concept that response to AHH induction by tobacco smoke is linked to an increased susceptibility to lung cancer (12).

Formation of covalent DNA adducts may be a more relevant marker of risk of PAH carcinogenesis than AHH inducibility since they represent the net effect of competing metabolic activation and detoxification processes as well as DNA repair (10, 13-17). We emphasize, however, that, despite the important role that adduct formation plays in cancer risk, it is clear that covalent binding is a necessary but not sufficient step in carcinogenesis and that subsequent events may also be rate limiting (18). In a large number of experimental studies, formation of the covalent adducts by BP and other carcinogens has been associated with tumorigenic potency (19, 20) and generally, but not always, with target tissue susceptibility in sensitive species (21). In humans, levels of PAH-DNA adducts have been significantly elevated in PBL of exposed workers (22-25) but not in PBL of cigarette smokers compared to nonsmokers (26). PAH-DNA adducts have also been measured in lung tissue (17) and placental tissue (27, 28) but were not correlated with amount of cigarette smoking. In all of these studies, considerable interindividual variation in binding has been observed.

Since only 10-15% of heavy smokers ultimately develop lung cancer, it is of interest to know whether there are constitutional differences between lung cancer patients and controls (persons without cancer) in DNA adduct formation resulting from PAH exposure. In a series of studies, Rudiger et al. (10, 29) have observed a genetically determined enhancement of BP-DNA adduct formation in vitro in monocytes of lung cancer patients compared to normal subjects, which was insensitive to individual smoking habits. This difference was most pronounced in patients with an early onset of the disease (<46 years) who smoked.

The present study was intended to expand upon an earlier...
pilot investigation suggesting an increase in PAH-DNA adduct formation in lung cancer cases compared with normal subjects (17).

We selected PAH-DNA adducts for study because of the extensive database on these complexes as well as their environmental and public health relevance (30–35). Antibodies elicited against BPDE-I-DNA were used as a promising marker of the biologically effective dose of this group of carcinogens (36–38).

SCEs were also evaluated as a complementary non-chemical-specific indicator of the genetic effect of mutagens/carcinogens (39). Prior studies have shown significant differences in SCE frequency between smokers and non-smokers (26, 39) but not between cancer cases and controls (40).

SUBJECTS AND METHODS

Subjects, Samples, and Questionnaire Data

Peripheral Blood Study. Over a 3-year period, we enrolled a total of 81 lung cancer patients at the Columbia-Presbyterian Medical Center from whom we obtained a sample of blood upon admission to the hospital for surgery. In each case, the diagnosis of primary lung cancer was confirmed by pathology report. These were 33 adenocarcinomas, 16 squamous cell carcinomas, and 32 undifferentiated large cell carcinomas. Our controls were 67 noncancer patients who were being treated at Columbia-Presbyterian Medical Center for orthopedic conditions (excluding fracture) or for benign lung disease. None of the controls had a prior history of cancer.

We were able to obtain detailed questionnaire data at interview from 77% of our subjects. The standardized instrument, which was administered by trained personnel, covered lifetime active smoking. It asked about exposure during the past 2 years to passive smoking, dietary PAH and caffeine, PAH in the workplace, and various other factors related to SCE induction. The questionnaire also elicited a family history of cancer. Specifically, smoking status was determined as current smoker (within last 2 months), former smoker, or nonsmoker.

Indices of active smoking were: (a) lifetime duration (sum of mg tar/cigarette for each brand of cigarette smoked during lifetime based on Federal Trade Commission tar values for cigarettes 1956–1984 (40) x number of cigarettes smoked of that brand/day x 365 x number of years smoked that brand; (b) current smoking levels [PPD]; (c) pack-years [PPD x years smoked]; and (d) tar levels of current brand smoked (41). A passive smoking score reflecting cumulative residential exposure during the previous 2 years was calculated (number of persons smoking x their PPD x number of hours of subject exposure/day x number of months living with smokers in past 2 years). The average total daily hours that individuals were exposed to passive smoke at home and work during the preceding 2 years was also determined. A “dietary charcoal exposure score” was calculated for each subject, reflecting average intake of broiled or smoked meat and fish during the previous 2 years. Caffeine consumption was quantified in terms of average daily servings of caffeine-containing beverages over the preceding 2 years since caffeine is associated both with SCE induction and with DNA adduct formation (42). In addition to these quantitative scores, participants were qualitatively rated on area of residence (urban versus rural) as a surrogate measure of outdoor air pollution; occupational and environmental exposures to PAHs or to other substances capable of influencing SCE formation (e.g., petroleum products, tar, and ethylene oxide) and other forms of smoking (e.g., cigars, pipes, and marijuana). Information on alcohol, X-rays, and health status was collected to identify potential confounders in the SCE analysis.

For all subjects, age, sex, cigarette smoking history, histology, and familial history of lung cancer in first degree relatives were also ascertained by medical records and/or the Columbia-Presbyterian Medical Center Tumor Registry. A prior study has shown that these secondary sources of data are in good agreement with information obtained at interview (17). Because the two groups (questionnaire and nonquestionnaire) did not differ significantly with respect to age, socioeconomic status, sex distribution, smoking history, diagnosis, or assay results, they were combined in analysis.

Each participant donated 35 ml of blood which was collected in heparinized tubes and immediately coded. For the DNA assays, buffy coat cells, RBC, and plasma were separated by centrifugation and frozen at −70°C. Nuclei were isolated from cells treated with 0.32 M sucrose, 1 mM potassium phosphate, 1.5 mM CaCl2 and 1% Triton X-100 pH 7.5, by centrifugation at 2000 x g. Nuclei were suspended in 10 mM Tris-1 mM EDTA-0.4 M NaCl, pH 7.9, and briefly sonicated. DNA was purified by phenol/chloroform extraction (43) and RNase treatment.

Lung Tissue Study. We obtained lung (tumor and/or tumor adjacent) tissue from a total of 29 lung cancer patients, 13 of whom were enrolled in the PBL study. The cases included 8 adenocarcinomas, 8 squamous cell carcinomas, 12 undifferentiated large cell carcinomas, and 1 small cell carcinoma. One control tissue was normal lung from a lung surgery case with benign disease; the remaining 9 samples were from autopsy cases without a history of lung cancer. None of the tissue controls were also participants in the PBL study. Following collection, tissues were coded and frozen at −70°C. DNA was extracted from 10% homogenates in 10 mM Tris-1 mM EDTA-0.4 M NaCl-0.2% sodium dodecyl sulfate, pH 7.9, using standard phenol/chloroform procedures (43).

PAH-DNA Adduct Measurements

Coded samples were assayed by competitive enzyme-linked immunosorbent assay, essentially as described previously (17), with fluorescence detection. Briefly, 96-microwell black plates (MicroFLUOR “B”; Dynatech Laboratories, Alexandria, VA) were coated with 0.5 ng BPDE-1-DNA (5 adducts/10^6 nucleotides or 15 pmol/µg). A previously characterized rabbit polyclonal antiserum (36) was used at a 1:800,000 dilution. A standard curve was constructed by mixing 50 µl diluted antibody with BPDE-1-DNA modified to a level of 1.5 adducts/10^6 nucleotides (4.5 fmol/µg) with [3H]BPDE-1 (kindly provided by Ainsley Weston, NIH, Bethesda, MD) in carrier nonmodified calf thymus DNA such that 50 µl contained 0.25–25 fmol BPDE-1-deoxyguanosine adduct in 50 µg DNA. All human samples were also assayed at 50 µg DNA/well after sonication and denaturation by boiling for 3 min and cooling on ice. A goat anti-rabbit IgG-alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) was used at 1:400 dilution. The substrate, 4-methylumbelliferyl phosphate (100 µl, 50-µg/ml 0.1 M diethanolamine, pH 9.6) becomes fluorescent after phosphate removal.

Fluorescence was read on a MicroFLUOR reader (Dynatech). Samples with greater than 20% inhibition were considered positive. For analytical purposes, samples with assays showing less than 20% inhibition were classified as “negative” (i.e., nondetectable) and each was assigned a value of 0.05 fmol/µg, an amount midway between the lowest positive fmol/µg concentration and zero. Results are the mean of single or double assays with triplicate wells. The average coefficient of variation between triplicate wells was 9.2%; the average coefficient of variation between repeat assays on the same sample was 36%.

Although originally elicited against BPDE-1-DNA, the antibody was later shown to cross-react with DNA modified by diol epoxides of other PAHs which form adducts with stereochemistry similar to BPDE-1-DNA, such as benz(a)anthracene (with a 5- to 10-fold lower affinity than BP) and chrysene (with a higher affinity) (37, 38). These PAHs are found in the same sources as BP, in fairly similar concentrations (e.g., benz(a)anthracene, 40–70 ng/cigarette; chrysene, 40–60 ng/cigarette) (32). Thus, positive reaction with the antibody may indicate the presence of multiple PAH-DNA adducts. However, because the standard curve is constructed using BPDE-DNA adducts, the modification level is expressed in terms of fmol BPDE-1-deoxyguanosine adduct which would cause similar inhibition per µg DNA.

Sister Chromatid Exchanges

After separation of leukocytes from the plasma, duplicate 72-h cultures per subject were prepared as described previously (44). Bromodeoxyuridine was added at 24 h. Coded slides were prepared and stained according to the method of Goto et al. (45). Fifty metaphases were analyzed per culture and the average number of SCEs per metaphase was calculated.
DNA ADDUCTS AND SISTER CHROMATID EXCHANGE IN LUNG CANCER

Statistical Analysis

Associations were evaluated between host factors and exposure variables (age, sex, ethnicity, familial history of lung cancer, type of lung cancer, cigarette smoking, dietary charcoal and caffeine consumption, occupational exposure to PAH, season of blood sample collection, whether case or control) and laboratory measurements. Outcome variables (adducts and SCEs) were first evaluated for normality of distribution. Adducts were not normally distributed (P < 0.01 by the Kolmogorov-Smirnov test) but the normality assumption for SCEs was questionable (P = 0.13 by the same test). Therefore, for consistency, nonparametric methods of analysis were used for both adducts and SCEs. Discrete data were cross-tabulated and analyzed by the Wilcoxon rank sum test and (for SCEs only) the two sample t test. Correlation coefficients were calculated for continuous data using Pearson’s and Spearman’s correlation procedures. Associations were further assessed by linear regression and analysis of covariance.

We used two approaches in analyzing adduct data: (a) using all the values for adduct levels (assigning 0.05 as the value for a “negative” sample); and (b) using values for positive samples only. Trends were similar using both approaches. Since greater reliance can be placed on the quantitation of positive samples and since the percentage of positive samples was generally comparable in cases and controls, we have presented those results, noting the percentage of positive samples in each instance.

RESULTS

Demographic characteristics and cigarette smoking exposures are summarized in Table 1 for cases and controls who gave a PBL or lung tissue sample, respectively. The two groups (cases and controls) were generally comparable in terms of age and sex distribution but cases reported significantly higher levels of cigarette smoking (PY, PPD, packs per day, cumulative cigarette intake). Most subjects were from the urban/suburban New York Metropolitan area. Of the individuals who provided information on occupation during the last 2 years, 15% reported having worked in occupational settings with possible PAH exposure (14% of the lung cancer patients and 16% of controls). Thirteen % of lung cancer patients (8 of 64) and 11% (7 of 62) of controls reported a family history of lung cancer in a first degree relative.

Adducts in PBL. Age, sex, ethnicity, amount of cigarette smoking, passive smoking, dietary charcoal and caffeine, occupational exposure, family history of cancer, and histological type of cancer were not significantly associated with adduct levels either by linear regression or analysis of covariance (used as appropriate). Significant relationships were seen only with group (case or control) status, smoking status, and season as described below.

Since only 2 of 5 lung cancer cases in the nonsmoker group were positive and there were no differences in adduct levels between former smokers and nonsmokers, the two groups were combined. As shown in Fig. 1 and Table 2, lung cancer cases who were current smokers had significantly higher levels of covalent adducts than controls (P = 0.01, Wilcoxon rank sum test). In the former and nonsmoker group, only a small increase was seen in cases.

Seasonal variation was significant with a peak seen in samples collected during the months of July–October (see Fig. 2). The mean value for positive samples [0.47 ± 0.54 (SD); 42 of 70 positive] for July–October [Season 1] was significantly higher than that for November–February [Season 2; 0.20 ± 0.12] and March–June [Season 3; 0.23 ± 0.16] combined [P = 0.05 by the Wilcoxon test]. For all cases (July–October) the mean was 0.59 ± 0.62; for controls (July–October) it was 0.36 ± 0.43. An increase in cases was particularly pronounced in the current smokers. During Season 1, the current smoker lung cancer cases greatly exceeded the current smoker controls (P = 0.005 by the Wilcoxon test); whereas in Seasons 2 and 3, no differences were seen between the two groups (see Fig. 3 and Table 3).

Among former smokers and current smokers combined, the cases sampled during the “high” season (Season 1) exceeded that for controls (P = 0.01, Wilcoxon rank sum test).

Table 1. Study population: with data on adducts in PBL and lung tissue

<table>
<thead>
<tr>
<th></th>
<th>Sex (F:M)</th>
<th>Age (yr)</th>
<th>Packs/day*</th>
<th>Pack-yr's*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral blood cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (81)</td>
<td>41:40</td>
<td>63.7 (10.0)</td>
<td>1.2 (0.6)</td>
<td>49.2 (30.7)</td>
</tr>
<tr>
<td>Controls (67)</td>
<td>36:31</td>
<td>59.3 (12.6)</td>
<td>0.7 (0.9)</td>
<td>21.2 (31.6)</td>
</tr>
<tr>
<td><strong>Lung tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (29)</td>
<td>10:19</td>
<td>65.5 (9.9)</td>
<td>1.24 (0.81)</td>
<td>43.2 (27.3)</td>
</tr>
<tr>
<td>Controls (10)</td>
<td>7:3</td>
<td>56.1 (21.1)</td>
<td>0.71 (0.56)</td>
<td>31.3 (25.1)</td>
</tr>
</tbody>
</table>
* Mean (SD).

Fig. 1. PAH-DNA adducts in peripheral WBC. Values plotted are the means for positive samples; numbers above the bars, ratio of positive samples to all samples assayed. Among current smokers, the mean level of adducts for cases exceeded that for controls (P = 0.01, Wilcoxon rank sum test).

Table 2. PAH-DNA adducts in peripheral blood leukocytes*

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fraction of samples positive</strong></td>
<td>Mean PAH-DNA ± SD</td>
<td>Mean PAH-DNA ± SD</td>
</tr>
<tr>
<td>Current smokers</td>
<td>19/38</td>
<td>0.35 ± 0.33*</td>
</tr>
<tr>
<td>Former smokers and nonsmokers</td>
<td>21/43</td>
<td>0.44 ± 0.61</td>
</tr>
</tbody>
</table>

* Values given are the means for positive samples. The means and SD for all samples (positives and "negatives") are given in parentheses.

* Cases > controls: P = 0.01, Wilcoxon test.
lung cancer patients < 55 years of age (0.35 ± 0.07; 3 of 5 = 0.08), both in two-tailed tests. The mean adduct value for

(r = -0.46; P = 0.04) and by Spearman’s method (r = -0.4; P

correlation or analysis of covariance. However, age was inversely correlated with adduct levels by Pearson’s correlation procedure

consumption (PPD, PY, total tar, or recency) by linear regres

nonsmoker cases precludes seasonal comparisons between cases

(Seasons 2 and 3). Unfortunately, the very small number of

smokers combined) exceeded that in former and current smoker controls (P =

FIG. 4. PAH-DNA in WBC of cases and controls (current smokers and former

CS, current smokers; FS, former smokers; NS, nonsmokers.

* Values given are the means for positive samples.

a CS, current smokers; FS, former smokers; NS, nonsmokers.

b Cases > controls: P = 0.005, Wilcoxon test.

c Cases > controls: P = 0.02, Wilcoxon test.

d Cases > controls: P = 0.14, Wilcoxon test.

Here also, no difference was seen between former smokers plus

case versus control status; nor did age, sex, race, caffeine, or season have any significant effect on SCEs (see Table 4). There was, however, a significant relationship between SCE frequency and cigarette pack years (P = 0.05 by linear regression). SCEs and DNA adducts were not significantly correlated by linear regression.

DISCUSSION

These results are consistent with prior studies in that PAH-

DNA adduct formation in PBL (unlike SCEs) appears to be unrelated to amount of cigarette smoking (PY, PPD, cumulative cigarette tar). Instead, they seem to be an integrated measure of environmental PAH, reflecting both cigarette smoking and other background sources (diet, ambient air, drinking water, workplace, passive smoking, etc.). However, when we compared current smokers (cases versus controls) we observed a significant increase in PBL adduct formation in lung cancer cases, whereas former smokers and nonsmoker cases and controls did not differ with respect to adduct levels. We speculate that since our assay is a crude one in the sense that it is detecting multiple adducts (from a number of structurally related PAHs), comparisons between groups experiencing exposure to different sources and mixtures of PAHs (e.g., current smokers and nonsmokers) are less valid than those between groups with at least the same predominant exposure (e.g., cigarette smoking). Thus it is possible that in the smokers and nonsmokers we are measuring different combinations of adducts, which can be expected to vary in their biological effect. We stress that our results are limited by the relatively small number of current smokers with positive data. We did not include biochemical validation of current smoking status (e.g., serum cotinine) because prior studies have shown that data derived from validated questionnaires regarding current smoking status are reliable (26, 46).

The second finding of interest was the strong seasonal effect on adduct formation, with blood samples collected during the late summer and early fall showing higher levels of DNA binding than those collected during the other two seasons. This

Here also, no difference was seen between former smokers plus current smoker cases and controls during the “low” seasons (Seasons 2 and 3). Unfortunately, the very small number of nonsmoker cases precludes seasonality comparisons between cases and controls.

Adducts in Lung Tissue. Adducts were not significantly related to sex, race, family history of lung cancer, or cigarette consumption (PPD, PY, total tar, or recency) by linear regression or analysis of covariance. However, age was inversely correlated with adduct levels by Pearson’s correlation procedure (r = -0.46; P = 0.04) and by Spearman’s method (r = -0.4; P = 0.08), both in two-tailed tests. The mean adduct value for lung cancer patients < 55 years of age (0.35 ± 0.07; 3 of 5 positive) exceeded the mean for older lung cancer patients (0.17 ± 0.06; 13 of 24) (P = 0.01, Wilcoxon test). There were not enough subjects to permit an evaluation of the effect of season. The small number of control samples did not permit case-control comparisons in the various smoking categories. The mean (positive samples including both tumor and nontumorous tissue) for all cases was 0.21 ± 0.09 (16 of 29) compared to 0.23 ± 0.15 (4 of 8) for controls. Of the 37 individuals with lung tissue measurements whose smoking history was known, the smokers (current plus former) did not have significantly higher binding levels than nonsmokers (0.23 ± 0.12 and 13 of 30 versus 0.16 ± 0.03 and 5 of 5).

SCES in PBL. In our sample of 28 lung cases and 18 controls, no association was seen between SCEs and group (case versus control status) nor did age, sex, race, caffeine, or season have any significant effect on SCEs (see Table 4). There was, however, a significant relationship between SCE frequency and cigarette pack years (P = 0.05 by linear regression). SCEs and DNA adducts were not significantly correlated by linear regression.

FIG. 3. PAH-DNA in WBC of current smokers. Values plotted are the means for positive samples; numbers above the bars, ratio of positive samples to all samples assayed. Among samples collected in Season 1, those from lung cancer cases had a higher mean adduct level than those from controls (P = 0.005, Wilcoxon test).

Fig. 4. PAH-DNA in WBC of cases and controls (current smokers and former smokers; all subjects). Values plotted are the means for positive samples; numbers above the bars, ratio of positive samples to all samples assayed. In Season 1 samples, the mean adduct level in lung cancer patients (former and current smokers combined) exceeded that in former and current smoker controls (P = 0.02, Wilcoxon test).

Table 3 PAH-DNA adducts in peripheral blood leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Fraction of samples positive</td>
</tr>
<tr>
<td>CS</td>
<td>0.55 (0.38)</td>
<td>9/16</td>
</tr>
<tr>
<td>CS + FS</td>
<td>0.60 (0.64)</td>
<td>19/36</td>
</tr>
<tr>
<td>NS</td>
<td>0.47 (0.59)</td>
<td>2/2</td>
</tr>
<tr>
<td>All</td>
<td>0.59 (0.62)</td>
<td>21/38</td>
</tr>
<tr>
<td></td>
<td>Season 2 + 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Fraction of samples positive</td>
</tr>
<tr>
<td>CS</td>
<td>0.18 (0.12)</td>
<td>10/22</td>
</tr>
<tr>
<td>CS + FS</td>
<td>0.20 (0.11)</td>
<td>19/40</td>
</tr>
<tr>
<td>NS</td>
<td>0.20 (0.11)</td>
<td>0/3</td>
</tr>
<tr>
<td>All</td>
<td>0.20 (0.11)</td>
<td>19/43</td>
</tr>
</tbody>
</table>

* Values given are the means for positive samples.

Table 4 SCEs in lung cancer cases and controls

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Lung cancer cases (n)</th>
<th>Controls (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>11.1 ± 4.2*</td>
<td>11.5 ± 6.7</td>
</tr>
<tr>
<td>Former</td>
<td>10.3 ± 4.1</td>
<td>10.1 ± 1.8</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>10.2 ± 0.0</td>
<td>11.1 ± 6.2</td>
</tr>
<tr>
<td>Total</td>
<td>10.7 ± 4.0</td>
<td>10.7 ± 4.4</td>
</tr>
</tbody>
</table>

* Mean ± SD.
increase was highly significant in the current smokers but was also seen in current and former smokers combined. This seasonal effect is consistent with the observation of Paigen et al. (11), of a peak in AHH inducibility during this period. The heightened response during the season of high AHH inducibility could be attributable to the fact that during this period intra-individual variation is minimized and the range of inter-individual variation is greatest, allowing constitutional differences to be observed. Taken together, these results are consistent with the concept that in lung cancer cases there may be a genetically determined enhancement of PAH-DNA adduct formation in PBL of lung cancer cases which is most pronounced in cigarette smokers.

The lack of a case-control difference in SCEs [here controlling for cigarette smoking (PY)] was consistent with prior studies (26, 39, 40). Unfortunately, the number of subjects with both SCEs and PAH-DNA adducts in peripheral blood was too small to allow definitive comparison. However, by linear regression the SCEs and DNA adducts were not significantly correlated. This is not surprising given both the small sample size and the different life span of the two markers.

The results of the PAH-DNA adduct study in lung tissue are limited by the small numbers of samples (a total of 39). Of interest was the significant inverse linear correlation between adduct values and age of the donors as well as the higher mean adduct level for younger (<55 years) lung cancer patients. Such an age effect was not seen in adduct levels in peripheral blood in this study. However, other investigators have reported that in vitro BP-DNA binding was elevated in patients with an early onset of lung cancer (<46 years), possibly evidencing a genetic predisposition (29).

These are preliminary results from an ongoing study which also includes evaluation of oncogene activation as a marker of biological effect. Although limited, they serve to illustrate the complexity of such molecular epidemiological studies and the fact that the use of biological markers can introduce both greater precision and a new set of design considerations (such as the possible need to account for season of blood sample collection). Unfortunately, our small number of subjects with both PBL and lung tissue measurements precludes conclusions about the relationship between adducts in the "target" (lung) and the surrogate tissue used for biomonitoring (PBL). However, a correlation might be expected based on evidence for a systemic regulation of AHH induction (9) and the observation of similar levels of BP-DNA binding in lymphocytes and lung of experimental animals (47).

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REFERENCES

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