Different Susceptibility to Smoking-induced DNA Damage among Male and Female Lung Cancer Patients

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Abstract

The levels of aromatic/hydrophobic DNA adducts were analyzed in normal lung tissue from 63 lung cancer patients and examined in relation to exposure and genetic factors. Adduct levels were significantly higher in smokers than in nonsmokers, but among smokers the number of cigarettes smoked per day had only low significance for the variation in adduct levels. An inverse correlation was found between years of smoking and DNA adduct levels (r = 0.52, P = 0.001). Thus, patients with high adduct levels generally had shorter duration of smoking and/or lower smoking dose before the clinical onset of the disease, which fits expected behavior of cancer susceptible individuals. The data indicated an excess of individuals with glutathione S-transferase M1 deficiency among male patients with high adduct levels. Among females the DNA adduct levels were higher than in males when adjusted for smoking dose. There was a highly significant difference in the distribution of males and females when smokers were divided into quartile groups according to adducts per pack year (trend test: 2-sided P = 0.005). This may indicate that women are at greater risk of tobacco-induced lung cancer.

Introduction

Several studies have indicated that individuals may differ in their susceptibility to carcinogenic compounds in the tobacco smoke (1, 2), and roughly only 1 of 10 lifetime smokers contracts lung cancer. A further characterization of biochemical/genetic features of these patients is important in order to understand disease development, particularly at low dose exposure. Large interindividual variations have been found in the activity of a number of enzymes involved in the biotransformation of carcinogens, including AHH (3) and GSTs (4). It is therefore suggested that the ability to activate/detoxify carcinogens may modulate an individual’s risk for lung cancer (1, 2). Similar importance has been attributed to the enzymes in the repair of damaged DNA (5).

Tobacco smoke contains at least 40 known carcinogens, many of which exert their biological effects after biotransformation and through the formation of DNA adducts in target tissues (6). Some studies have shown a correlation between tobacco smoke exposure and DNA adduct levels in the respiratory tract of lung cancer patients (7–9). However, the individual variation in adduct levels may be large even at similar exposure doses, since this level also is affected by the metabolism of mutagens as well as the repair of damaged DNA. Animal studies have indicated that there is no simple relation between adduct level and cancer risk; however, for a particular carcinogen, high levels of stable adducts in the target tissue appear to be related to the probability that tumors will occur (10). In the light of the high complexity of carcinogenic compounds in tobacco smoke and their metabolism in the body, the DNA adduct level may be a simple but potentially informative parameter in the study of predisposition to lung cancer. In the present study we have examined the relationship between lung DNA adduct level, exposure, and genetic factors in a group of lung cancer patients.

Materials and Methods

Samples of normal lung tissue were obtained from 63 previously untreated lung cancer patients undergoing surgery. The tissues were snap-frozen and stored at −80°C until DNA extraction. A questionnaire was filled out for each patient by the treating physician where information on smoking habits, other relevant exposures, occupation, and family history of cancer were recorded. All the tumors were non-small cell lung carcinomas. DNA were extracted from powdered nontumorous tissues by protease K/RNase digestion and phenol/chloroform extraction.

The DNA adducts were measured by 32P-postlabeling analysis with the nuclease P1 digestion described previously (6). This procedure detects aromatic and/or hydrophobic DNA adducts, predominantly those formed by PAHs (7). Thin layer chromatography maps showed the diagonal band of radioactivity characteristic of DNA adduct formation by a complex mixture of compounds. All samples were analyzed in triplicate on separate days. The adduct maps were quantitatively reproducible and the quantitative variability between assays was <15%.

The GSTM1 genotype was determined by polymerase chain reaction amplification as described previously (11). The control group in the genotype study comprised 177 males with the same ethnic background as the patients. The mean age and smoking dose for the controls was 54 ± (SD) 13 years and 17.1 ± 10.2 pack years, respectively. Data for the cases are given in Table 1.

Results and Discussion

DNA was isolated from normal lung tissue from 63 patients with primary non-small cell lung carcinoma and analyzed for the presence of DNA adducts using 32P-postlabeling with nuclease P1 digestion modification. The adduct levels were significantly higher (Wilcoxon test, P < 0.0001) among smokers (10.52 ± 7.88 adducts/108 nucleotides) than among a group consisting of never-smokers and exsmokers of 2 or more years of abstinence (2.24 ± 1.14; Table 1). These values should be regarded as minimum values because of inherent uncertainties in the efficiency of labeling, and hence the absolute quantitation, of adducts formed by complex mixtures. A tendency towards higher adduct levels in female than in male smokers was also observed (Table 1). When the smokers were divided into quartiles according to adduct levels, there was a significant trend in the direction of more females in the high adduct quartiles (linear rank trend test: 2-sided P = 0.043, data not shown), even without taking account of the fact that the women had, on average, smoked significantly less (Table 1). When the data are adjusted for smoking dose and the individuals are divided into quartiles according to total adducts per pack year, there was a highly significant difference in the distribution of males and females (Fig. 1) (trend test: 2-sided P = 0.005). Similar results were
SUSCEPTIBILITY TO SMOKING-INDUCED DNA DAMAGE

Table 1 PAH adducts and smoking-related parameters for males and females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (mean ± SD)</th>
<th>Females (mean ± SD)</th>
<th>P (Wilcoxon rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adducts/10^6 nucleotides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>(N = 38) 9.75 ± 7.86</td>
<td>(N = 11) 13.55 ± 7.89</td>
<td>0.058</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>(N = 7) 2.72 ± 1.23</td>
<td>(N = 7) 1.70 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>Cigarettes/day^b</td>
<td>18.6 ± 8.4</td>
<td>15.6 ± 5.2</td>
<td>0.264</td>
</tr>
<tr>
<td>Smoking years^b</td>
<td>45.0 ± 9.5</td>
<td>32.9 ± 9.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pack years^b</td>
<td>42.0 ± 20.2</td>
<td>26.4 ± 12.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Age^b</td>
<td>62.8 ± 9.3</td>
<td>56.7 ± 11.0</td>
<td>0.093</td>
</tr>
</tbody>
</table>

^a Never-smokers and ex-smokers of 2 or more years of abstinence.
^b Data for smokers only.

Fig. 1. Distribution of males and females in quartiles according to adducts per pack year. The parameter “adducts per pack year” was calculated for all smokers and ranked according to its size. After the group was divided into 4 equal parts, the percentage of males and females in each group was determined. The range of adducts per pack year were 0.033—0.130, 0.131—0.220, 0.221—0.440, and 0.441—2.300 for groups 1 to 4, respectively.

Fig. 2. Scatterplot of adduct level against the inverse of smoking years. Males and females are indicated with different symbols. The regression line is for male patients only (n = 37, r = 0.52, P = 0.001).

Table 2 GSTM1 genotypes in relation to adduct level in male lung cancer patients compared to healthy controls

<table>
<thead>
<tr>
<th>GSTM1 genotype</th>
<th>Adducts^a (mean ± SD)</th>
<th>Adduct level</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High^c</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>10.58 ± 8.52</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Positive</td>
<td>8.48 ± 6.80</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

^a Adducts/10^6 nucleotides. Wilcoxon test of null versus positive genotype, P = 0.028.
^b Males with adduct levels lower and higher than the median (7.63/10^6 nucleotides) are grouped. The number of patients with each genotype are indicated.
^c Fisher exact test of patients with high adducts versus healthy controls: P = 0.043; odds ratio, 2.77; 95% confidence interval, 0.96—8.02.
genotype and detectable DNA adduct levels. The GSTM1 genotype may therefore have some effect on the disposition for high adducts at least for male lung cancer patients, but its penetrance is probably not high. It is also interesting that our results indicated an excess of GSTM1-deficient individuals only among patients with high adduct levels.

It has been hypothesized that cancer risks may be related to metabolic imbalance between activating and detoxifying pathways for carcinogens (16). Several PAHs are converted to reactive epoxides by AH-deficient individuals only among patients with high adduct levels. The GSTM1 genotype of AHH has been reported among recent smoking lung cancer patients than in cancer-free controls with a similar smoking history (17, 18). A positive, linear correlation between DNA adduct levels and AH activity has also been described (19, 20). The involvement of genetic factors in DNA adduct formation is further supported by familial studies of adduct variation (21).

In studying lung cancer patients, there may be a selection of individuals with high sensitivity to both PAH and other carcinogenic compounds in the tobacco smoke. The current study indicates that patients with high DNA adduct levels developed lung cancer after lower smoking dose and/or shorter duration than patients with low adduct levels. This fits the expected behavior of susceptible individuals. Our results suggest that susceptibility to DNA damage caused by PAH-like compounds may be higher among women than among men. This may be related to (unknown) differences in metabolism of PAH and other carcinogens in tobacco smoke and/or different accumulation of these carcinogens in the body. Our results provide biochemical evidence to support recent epidemiological data indicating that women are at higher risk (1.5—2-fold) than men of developing smoking-induced lung cancer (22, 23). This may be significant for predictions of future lung cancer incidence in women, based on current smoking trends.

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References

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