Effects of Smoking on Benzo(α)pyrene- and Glutathione-Metabolizing Enzymes in Human Lung Tissue*

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Summary. The influence of smoking on the metabolism of benzo(α)pyrene and glutathione was investigated in 190 patients with primary bronchial carcinoma and 20 patients with benign lung diseases. There were no significant differences in the activities of drug-metabolizing enzymes in the lung tissue of smokers, regardless of whether they smoked low- or high-tar and -nicotine cigarettes; former smokers; or nonsmokers; nor were there such differences between female and male patients. No significant differences existed between patients with squamous cell carcinoma and those with adenocarcinoma. Impaired detoxification due to decreased activity of glutathione S-transferases, rather induction of benzo(α)pyrene-metabolizing enzymes, may disturb the delicate balance between the generation and detoxification of reactive metabolites. This impairment may lead to the accumulation of these compounds in the cell.

Key words: Smoking – Polycyclic aromatic hydrocarbons – Benzo(α)pyrene metabolism – Glutathione metabolism – Lung cancer – Nicotine – Tar

Smoking is the most important cause of lung cancer, but the involuntary inhalation of tobacco smoke is also considered to be an important risk factor for nonsmokers [4, 13, 15, 37]. Many constituents of cigarette smoke are known to have carcinogenic or mutagenic properties [12]. Among these are polycyclic aromatic hydrocarbons (PAHs), such as benzo(α)pyrene (BP), which need enzymatic activation to reactive metabolites that may bind to cellular macromolecules. Detoxification of carcinogenic intermediates is preferably by conjugation with glutathione [7, 19, 22, 29].

The relative risk of lung cancer increases with the number of cigarettes per day and the duration of smoking [2, 14]. There is controversy over whether high-tar and high-nicotine cigarettes more likely favor the development of bronchial carcinoma than those with low amounts of these compounds [10, 16, 18, 25].

Patients and Methods

Patients

All patients included in this study underwent surgery because of malignant lung tumors or benign lung diseases. Histopathological classification was according to the criteria laid down by the World Health Organization [35].

Of 137 smokers, 98 (71%) stopped smoking 1 to 2 weeks before surgery, after admittance to the hospital; 27 (20%) did not give up smoking at all; and only 12 patients (9%) had been abstinent for 1 to 5 months. High-T/N cigarettes were those without filter containing 1.5 mg nicotine and >19 mg tar; low-T/N cigarettes contained <0.6 mg nicotine and ≤9 mg tar. Ex-smokers were those who gave up smoking at least two years prior to surgery.

There were 190 malignant tumor patients (165 males, 25 females) aged 32–77 years (mean age 57 ± 9 years), of whom 137 (15 females) were smokers, 35 (1 female) were ex-smokers (abstinence > 2 years), and 18 (9 females) had never smoked. The histopathological distribution of these patients is shown in Figure 1.

Of the 20 controls (11 males, 9 females, age 21–67 years), 11 were smokers, one was an ex-
Table 1. Differentiation of benzo(a)pyrene-metabolizing enzyme activities according to sex and histological type (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Male patients</th>
<th>Female patients</th>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Ethoxycoumarin (pmol·mg⁻¹·min⁻¹)</td>
<td>0.90 ± 0.06</td>
<td>1.02 ± 0.16</td>
<td>0.83 ± 0.07</td>
<td>1.13 ± 0.16</td>
</tr>
<tr>
<td>O-deethylase (nmol·mg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Epoxide hydrolase (nmol·mg⁻¹·min⁻¹)</td>
<td>1.05 ± 0.03</td>
<td>0.94 ± 0.09</td>
<td>1.04 ± 0.04</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>Glutathione S-transferases (nmol·mg⁻¹·min⁻¹)</td>
<td>100.7 ± 3.40</td>
<td>94.6 ± 7.4</td>
<td>96.6 ± 4.4</td>
<td>99.3 ± 5.9</td>
</tr>
<tr>
<td>Glutathione reductase (nmol·mg⁻¹·min⁻¹)</td>
<td>21.1 ± 0.7</td>
<td>18.9 ± 2.4</td>
<td>21.0 ± 1.02</td>
<td>21.1 ± 13.9</td>
</tr>
</tbody>
</table>

smoker, and eight had never smoked. Eleven had bronchiectases, two had cysts, two had fibrolipomas, and five had other benign lung diseases.

**Assays**

Lung tissue from the periphery of the lobe without macroscopic signs of malignancy was frozen in liquid nitrogen immediately after surgery and stored at −80°C until use. Subcellular fractions were prepared as described by Oesch et al. [21]. Microsomal monooxygenase activity (7-ethoxycoumarin O-deethylase, ECDE) was determined according to Ullrich and Weber [31] with the modification by Oesch et al. [21]. Epoxide hydrolase (EH) activity was measured with [3H]-benzo(a)pyrene-4,5-(K-region)-oxide as substrate [27]. Cytosolic glutathione S-transferases (GST) were measured using 1-chloro-2,4-dinitrobenzene as substrate [9]. Activity of glutathione reductase was determined according to Mize and Langdon [20] with a change of the final NADPH concentration to 0.11 mM.

**Statistical Analysis**

Variances were tested by an F-test for homogenous distribution. In case of homogeneity, data were compared with an unpaired Student’s t-test; otherwise a Wilcoxon U-test was performed. Correlations were tested by the Spearman rank evaluation [26].

**Results**

The histological type of adenocarcinoma was frequently diagnosed in female patients and in nonsmokers of both sexes, whereas squamous cell carcinoma, small cell carcinoma, and adenosquamous carcinoma were primarily associated with a history of smoking (Fig. 1). Pulmonary drug-metabolizing enzyme activities, however, were similar in both sexes and did not differ significantly between patients with squamous cell carcinoma and adenocarcinoma, although a slightly higher activity of ECDE was observed in the latter group (Table 1). Evaluation of enzyme activities in other histological types was not possible as these groups represented only a small number of patients with great interindividual differences concerning age, sex, and smoking habits.

Most patients had smoked about 20 cigarettes/day for 30–40 years with a tendency to more excessive smoking, especially in former smokers (Fig. 2).