Long-Lasting Effects of Tobacco Smoking on Pulmonary Drug-metabolizing Enzymes: A Case-Control Study on Lung Cancer Patients

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ABSTRACT

Lung tissue specimens were taken during surgery from middle-aged men with either lung cancer (LC, n = 54) or a nonneoplastic lung disease (n = 20). Aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin O-deethylase (ECDE), epoxide hydroxylase (EH), glutathione S-transferase (GST), and UDP-glucuronosyltransferase (UDPGT) activities and glutathione and malondialdehyde contents were determined in 12,000 × g supernatant fractions from nontumorous parenchymal tissues.

Interindividual differences in enzyme activities ranged from 11- to 440-fold, and glutathione content varied by 17-fold; the values showed unimodal distributions. AHH, ECDE, EH, and UDPGT activities were significantly and positively correlated to each other; a significant negative correlation was found between GST and the other enzymes. A relationship between enzyme activity and number of cigarettes smoked (pack-years) was found only for GST. Ignoring detailed smoking histories in the 6-month period preceding surgery, no difference was found in enzyme activities or glutathione content between LC and nonneoplastic lung disease patients or between smokers and nonsmokers. However, when the number of days since stopping smoking was considered, in smokers a significant increase was found for AHH, EH, and UDPGT activities and a significant decrease was found for GST activity, as compared to nonsmokers. LC patients who had smoked until the day before surgery had higher activities of AHH, ECDE, EH, and UDPGT than non-smokers, while GST activity was reduced by one-third. The activities of these enzymes returned to the basal level found in nonsmokers within 59 days (AHH), 108 days (EH), 67 days (UDPGT), and 40 days (GST). LC patients who were recent smokers (within 30 days prior to surgery) had significantly higher activities of AHH, ECDE, EH, and UDPGT than nonsmokers, while GST activity was reduced by one-third. The activities of these enzymes returned to the basal level found in nonsmokers within 59 days (AHH), 108 days (EH), 67 days (UDPGT), and 40 days (GST). LC patients who were recent smokers (within 30 days prior to surgery) had significantly induced AHH and ECDE activities when compared with smoking nonneoplastic lung disease patients. These results show that pulmonary drug metabolism can be altered by tobacco smoking and that these effects can last 40 to 108 days after cessation of smoking. These new findings should be considered in studies on the role of carcinogen-metabolizing enzymes in determining susceptibility to lung cancer.

INTRODUCTION

Although lung cancer is clearly associated with tobacco smoking (1), host factors may play a role in individual susceptibility to cancer (2). In particular, variations in drug-metabolizing enzymes in target cells of the lung may be important (3).

Cigarette smoke has been shown to alter several metabolic functions of the lung in both experimental animals (4-7) and humans (8). Many carcinogens in tobacco smoke, such as PAH (9) and N-nitrosoamines (10), require metabolic activation before exerting their carcinogenic activity (11). In the lung, as in other tissues, PAH and other carcinogens undergo a series of metabolic transformations under the control of cellular enzymes. These may generate not only nontoxic forms of the compounds but also electrophiles that are thought to be responsible for the onset of carcinogenesis. For example, pulmonary metabolism of PAH occurs via a two-phase process (12); phase I, in which enzymes such as AHH and EH activate the parent compounds to mutagenic and carcinogenic diol-epoxides (13, 14), and phase II, in which conjugating enzymes such as UDPGT and GST produce various nontoxic, water-soluble metabolites (15). Several of these enzyme activities may themselves be altered by cigarette smoke (16-19): conflicting results, mostly from animal studies, have been obtained showing enzyme induction as well as inactivation after acute exposure to smoke (18, 20, 21). It is reasonable to assume that the balance between activation and detoxification affects individual susceptibility to carcinogens present in cigarette smoke (12), and variations in the activities of AHH and GST have been proposed as determinants of the susceptibility of human smokers to lung cancer (22, 23).

The aim of this study was 3-fold: (a) to investigate whether recent exposure to tobacco smoke can affect the activity (inducibility) of PAH-metabolizing enzymes in human lung parenchyma; (b) to investigate whether differences in inducibility exist between lung cancer cases and non-cancer controls; and (c) to study, using the same patient and lung tissue material, the effect of tobacco smoke on pulmonary metabolism of mutagens. The latter data have been reported separately (24).

SUBJECTS AND METHODS

Study Subjects. Seventy-four male patients (mean age ± SD, 52.1 ± 9.7 years) who were undergoing surgery for LC (n = 54) or other thoracic disease (NLC; n = 20) at the University Hospital, Pisa, Italy, were studied. None of the patients were receiving drugs known to be human enzyme inducers, and none of the LC patients had received X-ray treatment or chemotherapy prior to surgery. A standardized, 140-variable questionnaire was completed for each patient the day before surgery to collect data regarding pulmonary and/or extrapulmonary diseases, pulmonary symptoms, environmental conditions at home and at work, and smoking habits. Particular attention was paid to recent smoking history, and detailed information was collected about the number and type of cigarettes smoked per day during the 6 months previous to the day preceding surgery. Patients were divided into smokers and nonsmokers; the latter were defined as subjects who had never smoked or who had refrained from smoking for more than 6 months.

Preparation of Lung Parenchymal Postmitochondrial Fractions. Specimens of peripheral lung parenchyma (and, for 10 patients, bronchial tissue samples) were obtained at surgery from each patient. In LC patients, specimens were taken as remote as possible from the tumor. Each specimen was kept in cold 0.15 M KCl solution until homogenization, which was always carried out within 1-2 h after surgery, sequentially, using a Polytron blender and then a Potter-Elvehjem homogenizer in 3 ml of 0.25 M sucrose-50 mM Tris-HCl buffer, pH 7.4.

Received 12/16/87; revised 5/4/88; accepted 5/17/88.

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1 This work was supported by the C.N.R. of Italy Special Oncology Project, Contract 8600438.44.

2 To whom requests for reprints should be addressed, at the International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France.

3 The abbreviations used are: PAH, polynuclear aromatic hydrocarbons; LC, lung cancer; NLC, nonneoplastic lung disease; AHH, aryl hydrocarbon hydroxylase; ECDE, ethoxycoumarin O-deethylase; EH, epoxide hydroxylase; GST, glutathione S-transferase; UDPGT, UDP-glucuronosyltransferase; GSH, glutathione; BP, benzo(a)pyrene.

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7.4, per g of tissue, as described previously (25). The homogenate was then centrifuged at 12,000 \times g for 15 min, and the resulting S12 supernatant was immediately frozen in liquid nitrogen and stored at −70°C until assay. Frozen samples were dispatched to the International Agency for Research on Cancer for analysis of enzyme activities and malondialdehyde and GSH contents. Malondialdehyde was measured as an indicator of lipid peroxidation.

**RESULTS**

LC patients (54.5 ± 7.1 years old) were significantly older (P < 0.001) than NLC patients (45.7 ± 12.7 years old), and the mean number of pack-years (corresponding to the number of cigarettes smoked per day per year of smoking/20) was significantly higher (P < 0.001) in LC (48.5 ± 26.3) than in NLC patients (27.7 ± 21.6). The pathological conditions of the lung cancer cases and of the non-cancer controls studied are listed in Table 1. Among the 54 lung cancer cases 43 were smokers (i.e., they had been smoking one or more cigarettes per day until 6 months before operation) and 11 were exsmokers; among the 20 NLC subjects 9 were smokers, 7 were exsmokers, and 4 were nonsmokers. The age-adjusted (Mantel-Haenszel) odds ratio for smoking (combining exsmokers and nonsmokers) is 6.77. This approximately 7-fold increase in risk of lung cancer in smokers with respect to nonsmokers is well in agreement with the estimates of risk from previous studies (1).

Comparisons of enzyme activities in parenchymal and bronchial tissue (both LC and NLC) from the same patients (n = 10) revealed no statistically significant difference for enzyme activities, but the mean GSH content was 4 times higher in lung parenchyma (data not shown). Thus, only the data for parenchymal tissue specimens were analyzed in detail.

**Table 1 Pathological conditions of 74 adult males undergoing lung resection for lung cancer or non-cancer lung diseases**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pathological condition</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>Squamous cell carcinoma</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Undifferentiated large cell carcinoma</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Undifferentiated small cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bronchioalveolar carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Squamous cell + large cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>NLC</td>
<td>Hamartoma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Carcinoid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bullous emphysema</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Nodular fibrosis</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bronchiectasis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chondromyxoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mediastinal lipoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bronchogenic cyst</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hemorrhagic pseudotumoral consolidation</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rounded atelectasis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Abcess</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Anthracosis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Intraparenchymal lymph node hyperplasia</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2 Levels of AHH, ECDE, EH, UDPGT, and GST activities and GSH content in human lung parenchyma**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of samples</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Fold variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHH</td>
<td>65</td>
<td>0.025</td>
<td>0.037</td>
<td>0.005</td>
<td>0.280</td>
<td>56</td>
</tr>
<tr>
<td>ECDE</td>
<td>45</td>
<td>0.038</td>
<td>0.132</td>
<td>0.002</td>
<td>0.880</td>
<td>440</td>
</tr>
<tr>
<td>EH</td>
<td>69</td>
<td>0.180</td>
<td>0.092</td>
<td>0.050</td>
<td>0.540</td>
<td>11</td>
</tr>
<tr>
<td>UDPGT</td>
<td>68</td>
<td>3.832</td>
<td>3.172</td>
<td>0.200</td>
<td>16.970</td>
<td>85</td>
</tr>
<tr>
<td>GST</td>
<td>69</td>
<td>0.110</td>
<td>0.080</td>
<td>0.020</td>
<td>0.343</td>
<td>17</td>
</tr>
<tr>
<td>GSH</td>
<td>64</td>
<td>0.308</td>
<td>0.220</td>
<td>0.040</td>
<td>1.200</td>
<td>30</td>
</tr>
</tbody>
</table>

* AHH, ECDE, and UDPGT activities are expressed as pmol/min/mg of protein; EH and GST activities are expressed as nmol/min/mg of protein; GSH content is expressed as µmol/g of wet tissue; for limits of detection see “Materials and Methods.”

**Drugs**

Drug-metabolizing enzyme activities and GSH content of the lung parenchyma are shown in Table 2. Although AHH activity showed a 56-fold variation, all patients but one had activity levels between 0.005 and 0.076 unit, giving only a 15-fold variation. The individual variation in ECDE activity was exceptionally high (440-fold); however, all patients but two had ECDE levels between 0.002 and 0.098 unit, thus giving a 49-fold variation. Other enzyme activities varied between 11- and 85-fold.

The frequency distribution of the enzyme activities considered looked asymmetrical and skewed to the right. After logarithmic transformation of the data, a unimodal, close to normal distribution was evident for the enzyme activities and for the GSH content (not shown). Further correlation analyses were performed on the whole data set, taking the logarithms into consideration, whereas untransformed data were used when subgroups of patients were compared. All of the enzyme activities were significantly correlated to each other (Table 3). In particular, a positive correlation was found among AHH, ECDE, EH, and UDPGT activities (P < 0.05), whereas a negative correlation was found between these enzymes and GST (P < 0.05 or less). In contrast, none of the enzyme activities correlated with GSH content. Although the concentration of malondialdehyde varied from 0.02 to 0.22 µmol/g wet tissue, it
showed no apparent correlation with any other variable investigated in this study. However, in recent smokers, i.e., those who stopped smoking within 30 days before surgery, malondi- aldehyde concentration was higher (0.137 ± 0.007 µmol/g) than in smokers who stopped smoking over 30 days before surgery (0.116 ± 0.07; P < 0.05).

No significant correlation was found between AHH, ECDE, and UDPGT activities or GSH content and age or number of pack-years (Table 4). A weak negative correlation was found between EH activity and age (r = -0.30) and a weak positive correlation was found between GST activity and age (r = 0.32) or pack-years (r = 0.22).

A detailed record of smoking habits of cases and controls over the last 6 months before surgery allowed two kinds of data analysis to be performed. When details of the patients' recent smoking histories were simply disregarded, no significant difference was found between LC patients and NLC patients for any enzyme activity or for GSH content (Table 5), using either untransformed values or their logarithms, or after adjustment for age or number of pack-years of smoking.

However, when the detailed information of the smoking habits of patients within the last 6 months before surgery were taken into account, a different picture emerged. Plotting enzyme activities versus the number of days since stopping smoking (Fig. 1; time zero is the day of surgery) gave significant correlation for all enzymes except ECDE. In particular, an inverse relationship was found for AHH (r = -0.31, P < 0.05), EH (r = -0.37, P < 0.01), and UDPGT (r = -0.25, P < 0.05); a positive correlation was found for GST (r = 0.28, P < 0.05). No relationship was found for ECDE (r = -0.12). This was also the case for GSH (r = 0.09). The time (in days) that would have elapsed until these regression lines intercepted the respective basal enzyme activities of nonsmokers (Fig. 1) was calculated for each enzyme using a linear regression analysis and was found to be 59 for AHH, 108 for EH, 67 for UDPGT, and 40 for GST. This basal enzyme activity did not differ in non- smoking LC and NLC patients, and the values for these two groups were thus combined.

Induced enzyme activities [values above the mean of non-smokers (Fig. 1)] were seen in a greater proportion of LC patients who had given up smoking only recently [<30 days before surgery] than in smoking NLC patients: 14 of 24 versus 1 of 5 for AHH; 11 of 24 versus 0 of 4 for ECDE; 22 of 24 versus 5 of 7 for EH; and 10 of 22 versus 2 of 6 for UDPGT. Although the differences in patient proportions did not reach statistical significance, possibly because of the small numbers of NLC cases available, the data suggest differential inducibility of AHH and ECDE in LC and NLC patients. This was confirmed when the actual level of enzyme activities (mean ± SEM) for subjects who gave up smoking within 30 days before surgery were compared in LC and NLC patients. In the LC group both ECDE (0.070 ± 0.041) and AHH (0.042 ± 0.011) activities were significantly higher (P < 0.01) than in NLC groups (ECDE, 0.008 ± 0.004; AHH, 0.017 ± 0.004), while differences in EH, UDPGT, and GST or GSH were not statistically significant. Thus, for ECDE and AHH activities, the time elapsed from stopping smoking appears to markedly influence the enzyme inducibility.

## DISCUSSION

The levels of most of the activating/detoxifying enzymes that were measured in this study have been reported to vary markedly between individuals (12, 25, 35–40), and we have confirmed this. Variations in activity comparable to those found here were previously reported for monooxygenases (25, 40), EH (40), and UDPGT (41); our study subjects showed slightly wider variations in GST levels than that reported by Oesch et al. (40), but a different substrate was used in the enzyme assay. Moreover, the frequency distribution of AHH, ECDE, EH, and GST activities (not shown) looked similar to that reported in a comparable group of patients (40). The highest activities for AHH (0.28 unit), ECDE (0.88 unit), and UDPGT (16.97 units) were found in the same individual, a 52-year-old patient who underwent surgery for adenocarcinoma of the lung. He had an overall smoking history of 67 pack-years and continued to smoke up to the day of surgery; he had also had severe occu- pational exposure to fibrous glass for 24 years.

The 30-fold variation in GSH content may be related to the reportedly heterogeneous distribution of GSH in human lung cells (42). The positive correlation between AHH, ECDE, EH, and UDPGT activities in human lung, reported here for the
Fig. 1. Linear correlation analysis of AHH, ECDE, EH, UDPGT, and GST activities and GSH content as a function of days since stopping smoking. Individual values for LC patients and for NLC patients are plotted and lines represent the best fit to all points. Shaded areas, mean value ± SEM for enzyme activities and GSH content in nonsmokers (both LC and NLC patients). ns, not significant.
first of these forms would be desirable, preferably using im-
show some striking similarities with a rat model (52). After
which slowly releases enzyme inducers such as PAH. Our data
increased by maternal tobacco smoking (50).
metabolizing enzymes in lung microsomes remained induced
intratracheal instillation of methylcholanthrene crystals, a reg-
smoking: smokers have decreased antipyrine half-lives in vivo
enhanced in human placenta, AHH activity is in
(48), but not that of AHH (48, 49), in liver tissue is enhanced
pulmonary S)2 fractions, seen only in very recent smokers.
acting mutagens (such as ICR 191 and sodium dichromate) by
done in parallel (24) on an increased inactivation of direct-
surgery) had significantly higher AHH and ECDE activities
 LC patients who had smoked until 1 day
extensively modified pulmonary enzyme activities, with a time-
dependent effect up to three months after cessation of smoking.
Consequently, this parameter, ignored in all previous studies,
was taken into account when comparing enzyme activities in
LC patients and NLC patients. The results, although limited
by the small number of smoking NLC patients who gave up
smoking within 30 days before surgery, nevertheless indicate
that LC cases have significantly higher levels of AHH and
ECDE activities than smoking NLC patients. This observation,
which requires confirmatory studies, is somewhat similar to
that made in an animal model. In inbred mouse strains, respons-
siveness to AHH induction is linked to an increased suscepti-
bility to PAH-induced cancer (43). In rodents, induction of
AHH is mediated by the Ah receptor (43); recently, this receptor
was also detected in lung cytosol from humans and showed a
great variation in its concentration (54).
Earlier studies (a) indicate that higher induced levels of AHH
occur frequently in lymphocytes of patients with cigarette
smoke-associated lung cancer (22, 50) and (b) suggest that LC
patients are preponderantly extensive metabolizers of debriso-
quine as compared to matched smoking controls (55). These
findings and our new results are compatible with the hypothesis
that genetic factors may be a primary control mechanism in
oxidative metabolism of xenobiotics, including tobacco-related
carcinogens, lending support to the existence of a genetic de-
terminant of susceptibility to lung cancer in tobacco smokers.
Most importantly, the long-lasting effects of tobacco smoke
on pulmonary carcinogen-metabolizing enzymes must be taken
into account when studying drug and carcinogen/mutagen
metabolism and its relationship to lung cancer susceptibility in
smokers. Using the same patient and lung tissue material of
the present study, the effect of very recent tobacco smoke
exposure on pulmonary metabolism of mutagens has also been
demonstrated (24).

ACKNOWLEDGMENTS

The authors wish to thank E. Heseltine for editorial help and M.
Wrisez for typing the manuscript.

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