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 hydrolase (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. LC patients who were recent smokers (within 30 days prior to surgery) had significantly reduced 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-nitrosamines (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. For NLC patients, 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 hydrolase (EH), glutathione S-transferase (GST), UDP-glucuronosyltransferase (UDPGT), GSH, glutathione; BP, benzo(a)pyrene.
7.4, per g of tissue, as described previously (25). The homogenate was then centrifuged at 12,000 × 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.

**Determination of Enzyme Activities in S12 Fractions.** AHH, ECDE, EH, UDPGT, and GST activities and GSH and malondialdehyde contents were measured in duplicate in coded samples. AHH activity was determined by the fluorimetric method of Nebert and Gelboin (26), with at least detection of 0.005 pmol 3-hydroxybenzo(a)pyrene/min/mg protein. ECDE activity was measured according to a fluorimetric procedure using 7-ethoxycoumarin as substrate (27); the limit of detection was 0.002 pmol 7-hydroxycoumarin/min/mg protein. EH activity was determined according to the thin-layer chromatographic method of Jerina et al. (28) with [3H]BP-4,5-oxide as substrate (29); the detection limit was 0.02 pmol BP-4,5-oxide conjugated/min/mg protein. Native UDPGT activity was measured by a fluorimetric method using 4-methylumbelliferone as substrate (29); the detection limit was 0.2 pmol 4-methylumbelliferone conjugated/min/mg protein. GST activity was measured by the method of Nemoto and Gelboin (30) with BP-4,5-oxide as substrate; the detection limit was 0.02 nmol BP-4,5-oxide conjugated/min/mg protein. These assays are performed routinely in the International Agency for Research on Cancer laboratories, mainly in animal tissues, and have been shown to be highly reproducible.

GSH content was measured as described by Saville (31), with a detection limit of 0.01 μmol/g wet tissue. Malondialdehyde content was determined as described by Uchiyama and Mihara (32) and the detection limit was 0.005 μmol/g wet tissue. The protein content of the S12 was determined according to the procedure of Lowry et al. (33).

Owing to a limited amount of resectable lung parenchyma, not all of the above assays could be performed on S12 from each patient. The limited sample size also precluded repeated determinations during storage; however, as shown previously in rat liver preparations (25), AHH activity was reduced by less than 10% when the postmitochondrial supernatant fraction was stored at below −70°C for 1 month.

No statistically significant correlation between storage time (13 ± 7 weeks) of the human lung specimens and the various enzyme activities was found for all subjects combined or for subgroups (smokers, nonsmokers, LC, NLC). The following overall correlation coefficients were obtained (r values and number of samples in parentheses): AHH (0.001; 64); ECDE (0.15; 45); EH (0.018; 68); UDPGT (0.15; 67). No correlation was evident for GSH (−0.04; 63), while for GST, a weak positive correlation was seen (0.3; 68; P < 0.001).

**Statistical Analysis.** Conventional methods of one-way analysis of variance were performed for comparison between groups (with the use of covariates when appropriate), and simple bivariate linear regression methods were used for correlation analysis (34).

**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 (comparing 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.

**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 interindividual 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

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**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>Antrhcosis</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 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>
</tr>
<tr>
<td>ECDE</td>
<td>45</td>
<td>0.038</td>
<td>0.122</td>
<td>0.002</td>
<td>0.880</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>
</tr>
<tr>
<td>UDPGT</td>
<td>68</td>
<td>3.832</td>
<td>3.172</td>
<td>0.200</td>
<td>16.970</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>
</tr>
<tr>
<td>GSH</td>
<td>64</td>
<td>0.308</td>
<td>0.220</td>
<td>0.040</td>
<td>1.200</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."
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, malondialdehyde concentration was higher (0.137 ± 0.007 \( \mu \)mol/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 correlations 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 nonsmoking LC and NLC patients, and the values for these two groups were thus combined.

### Table 4: Relationships among human lung parenchymal enzyme activities and age and overall cigarette smoke exposure (number of pack-years)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Age</th>
<th>Pack-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHH</td>
<td>-0.11</td>
<td>NS*</td>
</tr>
<tr>
<td>ECDE</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>EH</td>
<td>-0.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>UDPGT</td>
<td>-0.11</td>
<td>NS</td>
</tr>
<tr>
<td>GST</td>
<td>0.32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.18</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS, not significant; \( P > 0.05 \) (linear correlation).

### Table 5: Levels of AHH, ECDE, EH, UDPGT, and GST activities and GSH content in lung parenchyma from LC and NLC

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group</th>
<th>No. of samples</th>
<th>Mean</th>
<th>SD</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHH</td>
<td>LC</td>
<td>49</td>
<td>0.026</td>
<td>0.041</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>16</td>
<td>0.022</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>ECDE</td>
<td>LC</td>
<td>34</td>
<td>0.049</td>
<td>0.151</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>11</td>
<td>0.007</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>LC</td>
<td>51</td>
<td>0.187</td>
<td>0.097</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>18</td>
<td>0.159</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>UDPGT</td>
<td>LC</td>
<td>50</td>
<td>3.882</td>
<td>3.294</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>18</td>
<td>3.689</td>
<td>2.894</td>
<td></td>
</tr>
<tr>
<td>GST</td>
<td>LC</td>
<td>51</td>
<td>0.114</td>
<td>0.084</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>18</td>
<td>0.094</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>LC</td>
<td>48</td>
<td>0.300</td>
<td>0.231</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NLC</td>
<td>16</td>
<td>0.318</td>
<td>0.191</td>
<td></td>
</tr>
</tbody>
</table>

*AHH, ECDE, EH, UDPGT, and GST activities and GSH content are expressed as described in the legend to Table 2.
*\( F \) value not significant (NS) if \( P > 0.05 \).

Induced enzyme activities [values above the mean of nonsmokers (Fig. 1)] were seen in a greater proportion of LC patients who had given up smoking only recently \([\leq 30 \text{ days before surgery}]\) 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 occupational 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 time, is consistent with data in animals that these enzyme activities are under a common genetic control. Genetic control of these enzymes by the Ah locus has been firmly established in inbred mice (43). Recently, a statistically significant correlation was reported between AHH and ECDE activities in human lung tissue and pulmonary AHH and the inducibility ratio of AHH in lymphocytes of the same patients \( r = 0.62 \) (44).

The large interindividual variations we observed in all the enzyme activities determined may explain, at least in part, the absence of significant differences between LC and NLC patients in our study and in similar investigations (40, 45), except when detailed smoking histories in the preceding 6 months were considered (see below). Probably for the same reasons, our data revealed no relationship among any of the enzyme activities measured and overall exposure to smoke, with the exception of a very weak correlation between GST activity and number of pack-years. A similar lack of correlation was reported previously for human lung monoxygenase activities (25, 38).

We found in this study, however, that the activities of some PAH-metabolizing enzymes are strongly influenced by recent exposure to tobacco smoke. Thus, EH activity was much higher in smokers, i.e., patients who were smoking 6 months before surgery, than in nonsmokers, whereas no difference was seen for the other enzymes or for GSH content. However, by plotting enzyme activities versus the number of days since stopping smoking, an inducing effect of smoke on AHH, ECDE, EH, and UDPGT activities became evident. All enzyme activities (except GST, which decreased) increased in smokers over the level of nonsmokers in a time-dependent fashion. The closer to the date of surgery the patients were smoking the higher were the enzyme activities. LC patients who had smoked until 1 day before lung tissue was taken had a 2-fold induction of AHH, a 7-fold induction of ECDE, a 2.5-fold incubation of EH and a 1.6-fold induction of UDPGT. Such increases and the time course indicative of enzyme induction by tobacco smoke have not previously been reported for human pulmonary drug-metabolizing enzymes (25, 46), although this effect is well documented in animal studies. In the present study, we found that LC who were recent smokers (i.e., within 30 days prior to surgery) had significantly higher AHH and ECDE activities than smoking NLC.

In accord with our observation are the findings of a study done in parallel (24) on an increased inactivation of direct-acting mutagens (such as ICR 191 and sodium dichromate) by pulmonary \( S_{12} \) fractions, seen only in very recent smokers.

Several lines of evidence demonstrate that drug-metabolizing enzymes in other human tissues are also induced by tobacco smoking: smokers have decreased antipyrine half-lives \textit{in vivo} (47); and the level of hepatic 7-ethoxyresorufin-O-deethylase (48), but not that of AHH (48, 49), in liver tissue is enhanced by tobacco smoking; in human placenta, AHH activity is increased by maternal tobacco smoking (50).

Induced enzyme activities persisted for an unexpectedly long time: from 59 days for AHH to 108 days for EH. This effect might plausibly be due to deposition in the lung of tar (51), which slowly releases enzyme inducers such as PAH. Our data show some striking similarities with a rat model (52). After intratracheal instillation of methylcholanthrene crystals, a regimen that produced lung cancer in rats, the activity of PAH-metabolizing enzymes in lung microsomes remained induced for more than six weeks.

Due to the presence of isoenzymes for GST and EH, assessing each of these forms would be desirable, preferably using immunochemical methods. Using conventional enzyme assays, we have not detected significant differences in GST and EH activities in LC versus NLC patients.

The approximately 30% inhibition of GST activity by recent cigarette smoking is a novel finding and suggests that heavy smokers have a lower capacity to detoxify smoke constituents. Some inhibition of PAH-metabolizing enzyme activities by smoke has been reported in rats (20). Preliminary observations on human pulmonary macrophages have confirmed an inducing effect of cigarette smoke on EH and an inhibitory effect on GST activities, using the same enzyme substrates as in this study (53).

In our study, we found that exposure to tobacco smoke 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, responsiveness to AHH induction is linked to an increased susceptibility 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 debrisoquine 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 determinant 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. Wrisz for typing the manuscript.

REFERENCES


6. Karhi, T., Rantala, A., and Toivonen, H. Pulmonary inactivation of S-


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

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