Osman's observations and our data suggest that smoking may inhibit elastin-repair in the lungs. Thus, abnormalities in elastin structure and function could be further aggravated by smoking, causing accelerated alveolar destruction leading to pulmonary emphysema.

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Smokers Do Not Have Less Functional Alpha,-Protease Inhibitor in Their Lower Respiratory Tracts Than Nonsmokers*


Cigarette smoking is the major risk factor in the development of pulmonary emphysema, a condition thought to result from an imbalance between the elastase and antielastase levels in the lungs. An increased number of neutrophils and alveolar macrophages in the lungs of smokers could account for an elevation of their elastase burden. A decreased level of functional alpha,-protease inhibitor (alpha,-PI) has been reported in the bronchoalveolar lavage (BAL) fluid from smokers as compared with nonsmokers. 1,2

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We determined binding and inactivation of H-porcine pancreatic elastase and 3H-human neutrophil elastase by alpha,-PI in BAL from volunteers; we also measured the inhibition by BAL of porcine pancreatic elastase-catalyzed solubilization of H-elastin. The mean levels of functional alpha,-PI in the BAL of smokers were comparable to those of nonsmokers.

METHODS

Two groups were studied: 8 nonsmokers (1 woman, 7 men), ages 27 ± 1 yr (mean ± 1 SE) and 17 smokers (8 women, 9 men), ages 25 ± 1 yr (mean number of pack-years smoked was 9 ± 1). Aliquots of the BAL fluid obtained from these individuals were assayed 3,4 for functional alpha,-PI in BAL fluid by adding 3H-human neutrophil elastase (3H-HNE) and H-porcine pancreatic elastase (H-PPE), prepared as previously described. 3,4

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FIGURE 1. Functional alpha,-protease inhibitor in bronchoalveolar lavage (BAL) fluid of nonsmokers (SM) and smokers (SM). After passage through gauze and centrifugation at 300 × g to remove cells, the BAL supernatant was made 0.02% in NaN3 and stored at 4°C under N2. Protein was measured by the dye-binding method of Bradford, 15 using bovine serum albumin as a standard. Functional alpha,-PI was measured by incubating aliquots of BAL fluid (5 min × 22°C) with 1 ml of buffer A (0.05 M tris, pH 7.6, 0.14 M NaCl, 0.05% NaN3) and 10 μg of 3H-porcine pancreatic elastase (3H-PPE) or 3H-human neutrophil elastase (3H-HNE). The mixtures were chromatographed on Sepharose 6B molecular sieve columns with buffer A for PPE mixtures and, to minimize adsorption losses, buffer A + 0.46 M NaCl for HNE mixtures. The presence of active enzyme in the 25,000-29,000 dalton elution volume indicated saturation of available functional alpha,-PI. 3,4 The amount of eluting enzyme bound to alpha,-PI (confirmed by immunoprecipitation and absence of elastolytic activity of the 70,000 dalton complex) was calculated from the radioactivity of the original enzyme preparation expressed per μg and corrected for the recovery of radioactivity from the column which was similar for both groups. Functional alpha,-PI, H-PPE-specific (PPE-SPEC) or 3H-HNE-specific (HNE-SPEC), was calculated by multiplying the μg of elastase bound to alpha,-PI by the molecular weight ratio of alpha,-PI to 3H-PPE or 3H-HNE (2.12 and 1.83, respectively) and then expressed as a percentage of total protein or as total functional alpha,-PI per BAL. The long horizontal bars represent the mean and the short bars, ± 1 SE; the means for the SM and SM groups are not statistically different.
RESULTS AND DISCUSSION

The mean values of functional α1-PI in unconcentrated BAL fluid from smokers and nonsmokers were not statistically different, but unconcentrated BAL fluid from smokers contained 140% as much PPE-specific α1-PI and 135% as much HNE-specific α1-PI as did BAL fluid from nonsmokers (Fig 1). The level of functional α1-PI measured using 3H-PPE correlated well with that measured using 125I-HNE (r = 0.99).

Aliquots of BAL fluid from 6 of the nonsmokers and 6 of the smokers were concentrated for measurement of immunoreactive α1-PI and albumin, as well as functional α1-PI and total protein (Table 1). Statistically significant differences were not found between the mean values for the 2 groups. This was so whether total functional α1-PI levels were directly compared or were expressed as a percentage of immunoreactive α1-PI, albumin or total protein (Table 1). The PPE-inhibitory activity of concentrated BAL was assessed as described by Gadek et al., the amount of α1-PI present in BAL fluid required to achieve 100% inhibition of 1.0 µg of PPE was 3.3 ± 0.4 µg (mean ± SE) for smokers and 4.5 ± 1.1 µg for nonsmokers (NS). By this measurement, α1-PI from smokers was at least as effective in inhibiting PPE as α1-PI from nonsmokers. We were unable to find oxidatively inactivated α1-PI whose functional elastase-binding activity could be restored by chemical reduction of the BAL fluid samples from 2 smokers who had smoked 1 cigarette 15 min before the lavage procedure. The level of 125I-HNE-specific α1-PI was not significantly increased by incubation of an aliquot of the original sample at 37°C for 30 minutes with 125I-HNE, as compared with 22°C for 5 minutes, suggesting the absence of oxidized α1-PI.

The mean of the 2 values for PPE-specific functional α1-PI as a percentage of immunoreactive α1-PI (inhibitory efficiency) in smokers and nonsmokers combined is 67% (Table 1). For the 12 concentrated BAL fluid samples in our study, a mean value of 3.8 µg of immunoreactive α1-

Table 1—Functional Alpha1-Protease Inhibitor in Concentrated Bronchoalveolar Lavage Fluida

<table>
<thead>
<tr>
<th>Measured by Binding of:</th>
<th>H-PPE</th>
<th>HNE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
<td>SM</td>
</tr>
<tr>
<td>Total per sample (µg)</td>
<td>129 ± 18</td>
<td>123 ± 37</td>
</tr>
<tr>
<td>% of Immunoreactive Alpha1-PI</td>
<td>66 ± 7%</td>
<td>66 ± 8%</td>
</tr>
<tr>
<td>% of Albumin</td>
<td>3.5 ± 0.6%</td>
<td>4.3 ± 0.9%</td>
</tr>
<tr>
<td>% of Protein</td>
<td>2.5 ± 0.3%</td>
<td>1.9 ± 0.3%</td>
</tr>
</tbody>
</table>

aAliquots of BAL fluid samples from nonsmokers (SM) and smokers (SM) were concentrated at 2°C under N2 (Amicon cell, UM-10 membrane) after addition of buffer A (5% by volume), and aliquots were stored under N2 at 4°C and also in liquid N2 (storage at -20°C lowered the concentration of functional α1-PI). Levels of immunoreactive α1-PI and albumin were measured with radial immunodiffusion plates and a serum standard (Calbiochem-Behring Corp). Functional α1-PI and protein were measured as described in the legend of Figure 1. Data are mean ± SE.

PI was required to eliminate the elastolytic activity of 1 µg of pancreatic elastase or a 56% inhibitory efficiency. For purified human α1-PI and PPE Cohen et al. have found a 57% inhibitory efficiency, presumably due to proteolysis; Satoh et al. found 59% efficiency. The inhibitory efficiency of α1-PI in smoker BAL samples can be calculated from the data of Gadek et al. and Carp et al. as 76 ± 6% and 68 ± 4%, respectively, values comparable to ours; the calculated values for their nonsmoker BAL samples were 125 ± 4% and 118 ± 3%, respectively, values larger than ours. The reasons for the discrepancy in the functional α1-PI levels in the BAL fluid of nonsmokers and smokers in our study and that of the 2 previously published studies is not clear. The ratios of functional to immunoreactive α1-PI were similar in smoker samples in all 3 studies and were comparable to the values we obtained from nonsmokers. However, both published studies reported much higher ratios of functional to immunoreactive α1-PI from the nonsmoker samples. Further studies are required to clarify the role of decreased levels of functional pulmonary α1-PI in the pathogenesis of emphysema in smokers.

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