Impact of Smoking on the Concentration and Activity of Alpha-1-antitrypsin in Serum in Relation to the Urinary Excretion of Hydroxyproline

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(Received December 5, 1986)

The impact of active and passive smoking on the serum levels of $\alpha_1$-AT, the trypsin inhibitory capacity (TIC), the trypsin inhibitory activity (TIA) and the urinary hydroxyproline to creatinine ratio (HOP-ratio) was studied. The subjects used in the study on active smoking were 167 healthy adult men and in the study on passive smoking 189 healthy primary school children. Serum levels of $\alpha_1$-AT in active smokers were significantly higher than those in non-smokers. The TIC as well as the TIA in active smokers decreased with increasing number of cigarettes smoked. The urinary HOP-ratio increased significantly with increasing number of cigarettes smoked. On the other hand, in the case of passive smokers a significant difference was obtained only for the HOP-ratio. The correlations between all markers in active smokers were significant. Less significant correlations were found in the case of passive smokers. These results suggest that the urinary excretion of hydroxyproline can be considered as a marker for the imbalance between proteases and anti-proteases as a result of smoking.

(Key Words: Smoking, $\alpha_1$-antitrypsin, Trypsin Inhibitory Capacity, Urinary Hydroxyproline)

INTRODUCTION

Cigarette smoking is the major risk factor of chronic bronchitis and emphysema (4). Eriksen was the first to describe a relation between $\alpha_1$-antitrypsin ($\alpha_1$-AT) deficiency and the occurrence of emphysema (17). The underlying theory was the existence of an imbalance between proteases and anti-proteases. This "protease anti-protease" theory was later supported by a number of studies (27,28,32) and is now a widely accepted concept of the pathogenesis of emphysema (24). $\alpha_1$-AT is the most important inhibitor of proteases in human serum and in the lower respiratory tract (19,25) and is essential in preventing autodigestion of the lung by inhibiting a.o. elastases and collagenases.

Oxidation renders this inhibitor inactive (14,26). Cigarette smoke contains many potent oxidants which can reduce the functional activity of $\alpha_1$-AT (8,15) and turn the existing balance with lung proteases (e.g. elastase and collagenase) into an imbalance resulting in the degradation of connective tissue in the lung. However, studies on the functional activity of $\alpha_1$-AT in serum and broncho alveolar lavage fluid (BAL) of smokers compared with that of non smokers, have given conflicting results (1,3,5—7,10,12,16,18,20,22,30,31,44).

Hydroxyproline (HOP) is a specific imino acid of collagen and elastin and is excreted into the urine as an end product of collagen and elastin metabolism. A number of epidemiological studies were done which showed a relation between the urinary HOP to Creatinine ratio (HOP-ratio) and active and passive smoking (34—36,47). However no studies have been reported which clarify the mechanism by which smoking causes an elevated urinary excretion of HOP. Considering the above mentioned "protease anti-protease" theory, elevated excretion of HOP in both active and passive smok-
ers could be the result of collagen and elastin breakdown due to a disturbed balance between proteases and their anti-proteases.

The purpose of the present study is to clarify the impact of smoking on the connective tissue in the lungs by measuring 1) parameters related to the inhibition of proteolysis and 2) the excretion of HOP which is a measure for the extent of proteolysis in the connective tissue.

METHODS

The active smoking subjects participating in this study were 167 middle aged men (range 31—48 yrs) who were diagnosed to be healthy after medical examination at the department of Automated Multiphasic Health Testing and Services of the Tokai University Hospital in 1983. Information on smoking habits of the subjects was obtained during the health examination. The passive smokers participating in this study were 189 children randomly selected from the Fujimigaoka primary school in Suginami, Tokyo. They were tested healthy at health examinations regularly held at the same school. The smoking habits of their family were obtained as part of the ATS-DLD questionnaires which had to be completed by the parents during a previous study.

Blood samples were obtained by venipuncture. Serum $\alpha_1$-AT was measured by the Immunodiffusion plate kit (43) and the Trypsin Inhibitory Capacity (TIC) was measured according to Oshima's improved method (39). The functional activity of $\alpha_1$-AT, or Trypsin Inhibitory Activity (TIA) was calculated by dividing the TIC by the $\alpha_1$-AT concentration. Because of difficulties in collecting 24-hours urine, fasting urine samples were collected in which the hydroxyproline and creatinine concentrations were measured. After the hydroxyproline to creatinine ratio was applied by Allison (2) and Whitehead (46) it has been widely accepted as a representative value of the urinary hydroxyproline excretion within 24 hours. Urinary hydroxyproline was measured by the improved method of Matsuki which is adapted for analysis by autoanalyzer (37). The urinary creatinine concentration was also determined by autoanalyzer (45). Serum and urine samples were kept at $-40^\circ$C until analysis.

RESULTS

The distributions of $\alpha_1$-AT, TIC, TIA and the urinary HOP-ratio in both study populations were almost normal. Active smokers were classified into four groups by their smoking habits; non-smokers (61), 1-10 cig./day (42), 11-20 cig./day and above 21 cig./day (42). Passive smokers were classified as non-passive smokers and passive smokers.

The mean concentration of $\alpha_1$-AT, and the mean TIC and TIA and HOP-ratio as they were measured in active smokers and passive smokers are shown in Figures 1, 2 and 3 respectively. The concentrations of $\alpha_1$-AT in active smokers who smoke more than 10 cig./day are significantly higher than in non-smokers (Figure 1). In active smokers the mean TIC as well as the mean trypsin inhibiting activity of $\alpha_1$-AT were significantly lower than in non smokers (Figures 2 and 3). The mean urinary HOP-ratio was significantly higher in smokers than in non smokers (Figure 4). On the contrary, the only significant difference between the passive and non-passive smokers was a higher HOP-ratio in the former group. The correlation coefficients between $\alpha_1$-AT, TIC, TIA, urinary HOP-ratio and the number of cigarettes smoked are shown in Tables 1 and 2 for the active and passive smokers respectively. In the active smokers group the correlation between all markers were significant except for the correlation between $\alpha_1$-AT and TIC. In the case of the passive smokers similar results were obtained, although less correlation coefficients were significant. Simple linear regression of each marker on the number of cigarettes smoked gave the following equations:

Active smoking:

\[
\alpha_1\text{-AT} = 1.964 \text{ cig./day} + 190.2
\]

TIC = $-0.00942 \text{ cig./day} + 1.285$

TIA = $-0.0965 \text{ cig./day} + 6.76$

HOP-ratio = $0.322 \text{ cig./day} + 18.91$

Passive smoking:

\[
\alpha_1\text{-AT} = -0.0818 \text{ cig./day} + 233.5
\]

TIC = $-0.00214 \text{ cig./day} + 1.074$

TIA = $-0.0102 \text{ cig./day} + 4.75$

HOP-ratio = $0.2383 \text{ cig./day} + 94.04$

By means of these equations it is possible to predict within a certain confidence interval, the value of each marker if the number of cigarettes (passively or actively) smoked are known. For
example, one person who smokes 20 cigarettes per day has the predicted values: $\alpha_1$-AT = 229.5, TIC = 1.097, TIA = 4.83 and urinary HOPratio = 25.35. In the case of the passive smokers group which consists of children, it is very likely that the regression coefficient of the regression of HOP on the number of cigarettes passively smoked, will change if a factor for growth, a potential confounding factor, is being introduced. However we did not dispose of such data.

![Graph 1](image1)

** p<0.01 : Compared with non smokers or non passive smokers

Fig. 1  Serum $\alpha_1$-antitrypsin concentration in active and passive smokers

![Graph 2](image2)

** p<0.01 : Compared with non smokers or non passive smokers

Fig. 2  Trypsin inhibitory capacity in active and passive smokers
** Fig. 3  Trypsin inhibitory capacity to $\alpha_1$-antitrypsin ratio in active and passive smokers

** Fig. 4  Urinary hydroxyproline to creatinine ratio in active and passive smokers
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Table 1  Correlation coefficients among serum α1-antitrypsin (α1-AT), trypsin inhibitory capacity (TIC), TIC/α1-AT ×1000 (TIC/α1-AT), urinary hydroxyproline to creatinine ratio (HOP-ratio) in active smokers  N = 167

<table>
<thead>
<tr>
<th></th>
<th>α1-AT</th>
<th>TIC</th>
<th>TIC/α1-AT</th>
<th>HOP ratio</th>
<th>cig./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-AT</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIC</td>
<td>0.0179</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIC/α1-AT</td>
<td>-0.7761**</td>
<td>0.5936**</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOP ratio</td>
<td>0.2086**</td>
<td>-0.2740**</td>
<td>-0.3306**</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>cig./day</td>
<td>0.3819**</td>
<td>-0.4288**</td>
<td>-0.5677**</td>
<td>0.4318**</td>
<td></td>
</tr>
</tbody>
</table>

**: p<0.01

Table 2  Correlation coefficients of serum α1-antitrypsin (α1-AT), trypsin inhibitory capacity (TIC), TIC/α1-AT ×1000 (TIC/α1-AT), urinary hydroxyproline to creatinine ratio (HOP-ratio) in passive smokers  N = 189

<table>
<thead>
<tr>
<th></th>
<th>α1-AT</th>
<th>TIC</th>
<th>TIC/α1-AT</th>
<th>HOP ratio</th>
<th>cig./day</th>
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<tr>
<td>α1-AT</td>
<td>—</td>
<td></td>
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<tr>
<td>TIC</td>
<td>0.2218**</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIC/α1-AT</td>
<td>-0.5319**</td>
<td>0.6937**</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOP ratio</td>
<td>0.1859**</td>
<td>-0.0499</td>
<td>-0.0184</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>cig./day</td>
<td>-0.0726</td>
<td>-0.1688*</td>
<td>-0.1906**</td>
<td>0.5146**</td>
<td></td>
</tr>
</tbody>
</table>

**: p<0.01  *: p<0.05

DISCUSSION

The data from the present study suggest that active smoking has a significant impact on the concentration and activity of α1-AT in serum as well as on the urinary excretion of hydroxyproline. The data concerning passive smoking reveal less consistent results, except for the urinary excretion of hydroxyproline.

Several workers found in smokers higher serum levels of α1-AT than in non-smokers (3,5,7,20,30,31) while others did not (6,12). The functional activity of α1-AT, expressed as the Trypsin Inhibitory Activity (TIA), is lower in smokers than in non-smokers, which was also found by Higashi (20), but not by Bridges (7) and Cox (16). Some studies used the Elastase Inhibitory Activity (EIA) as a marker for the functional activity of α1-AT. Janoff found a decreased EIA (22) in the serum of smokers whereas other workers were not able to find such a difference (6,7,16). Oxidation of α1-AT may occur by two mechanisms. 1) The oxidants inhaled along with cigarette smoke can oxidize α1-AT (8,15) and 2) inhalation of cigarette smoke recruits alveolar macrophages (AM) and polymorphonuclear leukocytes (PMN) (11,21,31). The recruitment of PMNs is suggested to be due to the chemotactic factors which are secreted by activated AMs (21). The activation of AMs is suggested to be caused by phagocytosis of smoke particles (21). On the other hand AMs are attracted by breakdown products of collagen and debris (38,41). Both PMNs and AMs secrete oxidants which can cause inactivation of α1-AT (9,13,38,40,48).

An increasing α1-AT concentration in smokers' serum could be explained as an attempt of the body to compensate for the loss of functional activity of α1-AT in order to restore the Trypsin Inhibitory Capacity (TIC). However we have found that 1 ml serum of smokers inhibit-
ed significantly less trypsin than that of non-smokers. The increase in the \( \alpha _1 \)-AT concentration is apparently not enough to neutralize the hazardous effect of smoking. A reduced TIC was also found by Chowdhury (12), but not by Higashi (20) and Bridges even found an increased TIC and EIC (Elastase Inhibitory Capacity) in smokers' serum (7).

In contrast with serum only a few studies on broncho alveolar lavage fluid (BAL) have been performed (1, 6, 10, 18). Three of these studies revealed no difference in the concentration of \( \alpha _1 \)-AT in BAL between smokers and non-smokers (6, 10, 18), while one study did not give data on the \( \alpha _1 \)-AT content of serum (1). In addition Gadek and Carp found a decreased EIA in smokers' BAL (10, 18). They also found that \( \alpha _1 \)-AT in smokers' BAL was only about 50 per cent active as compared to non-smokers.

Our data on the concentration and functional activity of \( \alpha _1 \)-AT in serum of smokers compared with that of non-smokers suggest that smoking has a negative effect on \( \alpha _1 \)-AT, notwithstanding attempts of the body to compensate this effect by increasing the \( \alpha _1 \)-AT concentration. Several other studies on serum as well as BAL point in this direction. Because of the inactivation of \( \alpha _1 \)-AT the inhibition of proteolytic enzymes is decreased. This disturbs the balance between protease and anti-proteases resulting in breakdown of connective tissue in the lungs. Nevertheless we can not deny the results of other studies which revealed other results.

Besides an increase in oxidants, recruitment and activation of PMNs and AMs results in an increase in the elastolytic activity of BAL and serum because of the intensified release of elastases (23, 42). All studies have been concentrated on elastases and the breakdown of elastin in relation to emphysema (24), but PMNs and AMs also secrete collagenases (29, 38). The increase in collagenase levels in the lungs and the possibility that certain elastases are able to breakdown specific types of collagen, could also lead to an increased collagenolytic activity of BAL and serum. This additional effect of smoking is not compensated by either an increase in the inhibition potential of serum which means another disturbance of the balance between proteases and anti-proteases.

The breakdown of collagen and to a lesser extent elastine results in the excretion of hydroxyproline containing peptides, into the urine. The HOP-ratio in the urine therefore provides another aspect in studying effects on connective tissue of the lungs by smoking. If the balance between proteases and anti-proteases is disturbed, breakdown of collagen and elastin will occur with a subsequent increased urinary excretion of hydroxyproline containing peptides. Indeed in the present study the hydroxyproline excretion in smokers is significantly higher than in non-smokers, a result which we also found in some of our previous studies (34–36, 47). Additionally this study showed significant correlations between the excretion of hydroxyproline and the concentration and activity of \( \alpha _1 \)-AT. These correlations support the postulation that the urinary HOP-ratio is a marker for the disturbance of the balance between proteases and anti-proteases, which can be caused by smoking.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Toshiaki HIGASHI Dept. of Preventive Medicine and Public Health, School of Medicine, Keio University for his valuable comments on this paper. And also we are grateful to Mrs YAMAMOTO. Her contributions to this paper was great and we take pleasure in acknowledgement.

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