Bronchoscintigraphic visualization of the acute effect of tobacco exposure and terbutaline on mucociliary clearance in smokers

J. Mortensen, S. Groth, P. Lange, N. Rossing

ABSTRACT: The aim of this study was to examine the acute effect of tobacco smoke exposure and inhaled terbutaline on mucociliary clearance in 9 healthy smokers. It was based on a recently described method for scintigraphic visualization of the bronchi (bronchoscintigraphy). After an initial bronchoscintigram had been made by having the subjects inhale aerosolized $^{99m}$Tc-albumin, they inhaled either terbutaline or placebo from a metered-dose inhaler. Subsequently, data acquisition for production of bronchoscintigrams was repeated at 10 min intervals for 120 min, and the mucociliary clearance was estimated from the movement of radioactivity in the series of bronchoscintigrams thus obtained. On two study days the subjects remained tobacco abstinent, while on two occasions they chain-smoked during the examination. Acute tobacco exposure resulted in an increased clearance rate in the lobar bronchi of 8 of the 9 smokers ($p<0.03$), while in the main bronchi and the trachea the effect was inconsistent. In all subjects terbutaline systematically increased the clearance rate in all visible bronchial structures compared to placebo ($p<0.04$). The combination of smoking and terbutaline caused a faster clearance rate in the lobar bronchi in most subjects than tobacco smoke or terbutaline alone. It is concluded that both acute tobacco exposure and terbutaline increase mucociliary clearance in healthy smokers.

Keywords: Bronchoscintigraphy; drug effects; mucus; physiology; smoking; terbutaline.

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Cigarette smoke may cause profound changes in the composition and function of the airway epithelium in both a time and dose-dependent manner [1, 2]. If mucociliary defence is affected it may imply disease [3]. There have been reports indicating a significantly slower mucociliary clearance in the central airways of asymptomatic smokers than of nonsmokers, while clearance in the peripheral airways seems to be the same [4, 5]. However, as to the acute effect of tobacco exposure on mucociliary clearance results are conflicting [6-11]. Different cilioactive compounds of cigarette smoke have been shown to settle on and affect the ciliated epithelium of different generations of the bronchial tree in a non-uniform manner [12]. In previous studies of the acute effect of tobacco smoke on mucociliary clearance the evaluation has often been confined to arbitrarily defined regions of interest rather than to selected airway generations. Thus, regional effects of tobacco smoke on mucociliary clearance may have been overlooked, and may explain the conflicting results.

Recently a method has become available that allows regional mucociliary clearance to be visualized by the movement of $^{99m}$Tc-albumin in the airways by means of bronchoscintigraphy [13]. The aim of this study was to employ bronchoscintigraphy to examine whether the effect of cigarette smoke on mucociliary clearance in the large airways is so pronounced that it can be demonstrated during acute tobacco exposure of otherwise healthy smokers. The technique was also used to determine whether inhaled terbutaline has an acute effect on mucociliary clearance in healthy smokers.

We chose healthy, smoking subjects because we wanted to study airways in which the ciliated epithelium was not yet expected to be grossly disturbed, and because we wanted to perform the examinations without too much interference from coughing due to hypersecretion.

Methods

Subjects

Nine healthy smokers participated. Their age, sex, percentage predicted forced expiratory volume in one second ($\text{FEV}_1$), percentage predicted forced vital capacity ($\text{FVC}$), and smoking history are listed in table 1. All subjects had normal FEV₁ and FVC. The study was approved by the local Ethical Committee.
The radioaerosol procedure

The radioaerosol generation and inhalation procedure have been described in detail previously [13]. A technetium-bound human albumin aerosol was generated ultrasonically. The mass median aerosol diameter (MMAD) was 3.5 μm, geometric standard deviation (GSD) 1.9. Subjects inspired slowly (inspiratory flow rate ≤15 L/min) starting at residual volume, until 500 ml had been inspired. Exhalation was by maximal forced expiration. Inhaling were made until a count rate of 2,000 counts•s•1 was achieved. On average this was reached after 20 inhalations. Subsequently, the subjects gargled 3 times and finally swallowed some water. In between the following gamma camera acquisitions, they cleared the oesophagus with water.

Table 1. - Anthropometric data, lung function and smoking history

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>FEV₁ %pred</th>
<th>FVC %pred</th>
<th>Cig-day⁻¹</th>
<th>yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>42</td>
<td>103</td>
<td>107</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>39</td>
<td>106</td>
<td>112</td>
<td>20-25</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>29</td>
<td>102</td>
<td>101</td>
<td>15-20</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>26</td>
<td>97</td>
<td>100</td>
<td>10-15</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>24</td>
<td>93</td>
<td>114</td>
<td>15</td>
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<tr>
<td>6</td>
<td>M</td>
<td>37</td>
<td>96</td>
<td>110</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>26</td>
<td>94</td>
<td>95</td>
<td>15</td>
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<tr>
<td>8</td>
<td>F</td>
<td>24</td>
<td>85</td>
<td>81</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>46</td>
<td>112</td>
<td>115</td>
<td>10-15</td>
</tr>
</tbody>
</table>

Median: 29 yrs; Range: (24-46) yrs
FEV₁: forced expiratory volume in one second; FVC: forced vital capacity.

Immediately after inhalation of the aerosol, the subjects were placed in the supine position, with their back against a gamma camera. A static acquisition was repeated every 10 min for 120 min to follow mucociliary clearance of ⁹⁹mTc-albumin. The acquisition time remained unchanged apart from being corrected for the physical decay of ⁹⁹mTc. In this way it was intended that changes in scintigrams contained biological information only.

The acute effect of terbutaline and tobacco exposure on mucociliary clearance

The subjects were studied by two series of examinations, each consisting of two investigations at least 48 h apart. Subjects abstained from smoking at least 2 h before the examination. In the first series (days A and B), the subjects inhaled, in a randomized double-blind crossover fashion, either 5 puffs of placebo or terbutaline from a metered-dose inhaler, immediately after the first gamma camera acquisition. The days of placebo treatment are called days A, and terbutaline treatment days B. The second series (called days C and D) was a repeat of the first series but after the terbutaline or placebo administrations, the subjects started smoking. They were instructed to smoke at random in a relaxed state as normal and on average they smoked 7 cigarettes (range 5-9 cigarettes) during the next 120 min. Smoking was interrupted only during data acquisition. The number of smoked cigarettes was held constant in each subject on days C and D. The subjects used their own brand of cigarettes (all filter-tipped). Only one of the subjects coughed a few times (non-productive) while chain-smoking. Therefore, it was not necessary to correct for cough as an additive clearance mechanism.

Data analysis

The number of different bronchi that could be identified on the bronchoscintigrams was counted and the time after inhalation when they could no longer be visualized was noted. The scintigrams were evaluated independently, by two readers and the mean of their values was used. The comparison of mucociliary clearance between the four different days was performed by a Friedman two-way analysis of variance [14]. The Wilcoxon matched pairs signed rank test (two-tailed test) was used to evaluate the significance of the effect of either terbutaline or tobacco smoke exposure to placebo (or tobacco abstinence).

Fig. 1. - An example of a bronchoscintigram. The large airways from the trachea to the segmental bronchi are visualized.
Results

Figure 1 is an example of the quality of the morphological information that may be obtained by the technique for bronchoscintigraphic imaging. Individual bronchial structures are easily identified, but peripheral to the segmental bronchi, the structures merge. The radioactivity in the stomach below the left lung comes from the aerosol, which was originally deposited in the mouth. A small bolus in the oesophagus is visualized.

A typical example of the series of bronchoscintigrams that was used to evaluate mucociliary clearance is seen in fig. 2. The deposition of the radioactivity in the lung immediately after inhalation is seen on the uppermost scintigram (left panel). The initial images appeared similar on all 4 days, indicating a high degree of reproducibility of the deposition pattern. The major part of the retained activity was deposited on the large airways.

These cases are indicated in table 2 by the value >120 min. The Friedman test revealed a significant difference in the mucociliary clearance rate between all visualized structures on the four study days. The differences were most pronounced in the lobar bronchi (p<0.001), which always became scintigraphically indiscrete faster than both the main bronchi (p<0.01) and the trachea (p<0.001).

The acute effect of cigarette smoke

The acute effect of cigarette smoke was assessed by comparing the regional mucociliary clearance on the days of placebo treatment (day A) and placebo+tobacco smoking (day C) (table 2). The lobar bronchi were cleared significantly faster in 8 of 9 subjects while chain-smoking (p<0.03). There was no significant acute effect of tobacco smoke on the mucociliary clearance of the main bronchi or the trachea.

Table 2 shows the individual results for the longest time at which the lobar bronchi, the main bronchi, and the trachea were seen on the series of bronchoscintigrams on the four study days. In some cases the trachea was not seen at the end of examination (120 min) while the main bronchi still contained so much radioactivity that they could be visualized. The “disappearance” of the trachea could, therefore, only be temporary since the radioactivity would reach the trachea at a later point.

The effect of chain-smoking was also assessed by comparing differences in clearance on days B (terbutaline) and D (terbutaline and tobacco). Again, the lobar bronchi were cleared faster on the day of tobacco smoke exposure in all but one subject (p<0.02), and again there was no significant effect on mucociliary clearance of the main bronchi or the trachea. The acute effect of tobacco smoke exposure on mucociliary clearance thus seems to be most pronounced in the lobar bronchi.
The acute effect of terbutaline

Terbutaline significantly increased mucociliary clearance in the lobar bronchi (p<0.02), the main bronchi (p<0.04), and the trachea (p<0.00) regardless of the subjects being tobacco abstinence (day A versus day B) or chain-smoking (day C versus day D).

Discussion

In vitro tests are not necessarily good predictors of events taking place in the intact respiratory system, but can provide a means for specific evaluation of the effect of individual constituents of cigarette smoke on the mucociliary apparatus (ciliary activity, viscoelastic properties and quantity of mucus and the periciliary layer). Several of the more than 2,000 compounds that have been identified in tobacco smoke, have been shown to affect ciliary beating although the effectiveness and duration of their action differ [12, 15-17]. For instance, small nicotine doses slightly stimulate ciliary co-ordinated activity, while higher concentrations seem to cause a decline [18, 19].

In man, observations have been conflicting. In young smokers CAMMER et al. [6] and ALBERT et al. [7] have found a stimulatory effect from chain-smoking on lung mucociliary clearance. GOODMAN et al. [8] and YEATES et al. [9], on the other hand, have found no acute effect on tracheal mucus velocity of the smoking of up to 12 cigarettes, while NAKHOSTEN et al. [10] reported a decrease in tracheal mucus velocity in a two-case study after acute tobacco exposure. PAVIA et al. [11] found in 22 long-term smokers (19 pack-yrs) that smoking 2-5 cigarettes decreased clearance in 10 subjects, while in the others there were no significant effects.

There may be many explanations for the discrepancy between the different observations, the most important perhaps being related to the methods employed, in particular as regards correction for deposition of tracer material on nonciliated airways and for coughing. Recently, a new method was introduced [13] that allowed visualization of the clearance process on a semi-morphological basis, thus enabling studies of regional clearance defects that might easily be overlooked by a conventional analysis of arbitrarily defined regions.

In the present study of the acute effect of tobacco smoke exposure on mucociliary clearance, the method provided evidence of an acute stimulatory effect on an airway generation basis, but also that this effect is confined to the lobar bronchi. This might easily have been overlooked in conventional analysis of arbitrarily defined regions of interest. The reason for this observation is unclear. It has been demonstrated, however, that individual compounds of tobacco smoke settle on different locations in the airways [12]. It is possible, therefore, that our findings of an increased clearance rate in the lobar bronchi of chain-smoking subjects might be associated with a predominant deposition in these airway generations of the ciliaactive substances of the cigarette smoke. The stimulatory effect of acute tobacco smoke exposure on the mucociliary clearance of the large airways is interesting in as much as the study group consisted of young healthy smokers whereas after long-term smoking, mucus hypersecretion often ensues. The results of the present study may, therefore, be an expression of a general stimulatory effect of cigarette smoke on the airway epithelium.

We found a significantly faster clearance in asymptomatic smokers after inhalation of terbutaline than after placebo administration, just as we have previously observed in healthy nonsmokers [13]. In comparison with the results of the nonsmokers in the previous study, the mucociliary clearance of the smokers was not significantly slower after placebo. After terbutaline, the increase in mucociliary clearance of the nonsmokers was larger

### Table 2 - Longest time (min) at which the different anatomical structures were seen on the bronchoscinograms, expressed as the mean values for the trachea, the main bronchi and the lobar bronchi, respectively, on study days A, B, C and D

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Trachea</th>
<th>Main bronchi</th>
<th>Lobar bronchi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>&gt;120</td>
<td>100</td>
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<td>&gt;120</td>
</tr>
<tr>
<td>9</td>
<td>&gt;120</td>
<td>80</td>
<td>120</td>
</tr>
</tbody>
</table>

Median: >120; 90; 120; 100; 115; 70; 100; 70; 114; 65; 70; 30

Range: 80-; 40-; 50-; 30-; 40-; 26-; 20-; 20-; 44-; 22-; 13-; 10-; 120-; 106; 116; 62

>120: the structure will be visualized after the examination has stopped.
than the increase in the smokers. After a combination of terbutaline and cigarette smoking, however, the clearance rate in the smokers further increased to reach similar values as seen in the nonsmokers after terbutaline. In a previous study of Foster et al. [20], employing an open design, there was a similar effect of beta-agonists on mucociliary clearance in healthy smokers. To obtain a similar effect of a dose-response to placebo, and to our knowledge, therefore, the present study is the first controlled study to demonstrate a stimulatory effect of an inhaled beta-agonist on mucociliary clearance in healthy smokers. The possibility that the aerosolized freon propellants contained in the placebo (and terbutaline) canister *per se* might have an effect on the mucociliary clearance has been dealt with in a study by Sackner et al. [21]. They were unable to demonstrate any effect.

The additive effect of terbutaline and tobacco smoking on the mucociliary clearance need not necessarily be mediated via the same mechanism. Thus, exposure to cigarette smoke stimulates mucus discharge [22, 23], inhibits epithelial ion movement in *vivo* and in *vitro* [24, 25], initiates biochemical changes in the composition of mucus [23] and alters ciliary beat frequency [12, 15-19]. Beta-agonists, on the other hand, increase ciliary beating in a dose-related manner [18, 36] and change the composition of the periciliary fluid by affecting ion transport [25, 27]. Bronchodilatation can facilitate mucus transport by altering the depth and the viscoelastic properties of the mucus layer. Furthermore, mucus discharge may be stimulated by beta-agonists [23, 28].

Another explanation of the additive effect may be that we have not reached the top of the dose-response by giving 1.25 mg terbutaline or having the subjects chain-smoking. Systematically performed dose-response studies analysing the effect of inhaled terbutaline and tobacco smoke on mucociliary clearance are still required.

To conclude, both acute tobacco exposure and inhaled terbutaline increase mucociliary clearance in healthy smokers.

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References


RÉSUMÉ: L'objectif de cette étude est d'examiner les effets aigus de l'exposition à la fumée de tabac et à l'inhalation de terbutaline sur la clearance muco-ciliaire chez 9 fumeurs bien portants. Elle s'est fondée sur une méthode récemment décrite pour la visualisation scintigraphique des bronches (broncho-scintigraphie). Après un broncho-scintigramme initial, réalisé par l'inhalation de $^{99m}$Tc-albumine en aérosol, les sujets ont inhalé, soit de la terbutaline soit un placebo par un aérosol doseur. Ulterieurement, les données acquises pour la production du broncho-scintigramme ont été répétées à des intervalles de 10 minutes pendant 120 minutes, et la clearance muco-ciliaire a été estimée par le déplacement de la radioactivité dans les séries de broncho-scintigrammes obtenus de la sorte. Les sujets sont restés sans fumer pendant deux jours d'étude, alors que, pendant deux examens, ils ont fumé à la chaîne pendant toute la durée de l'examen. L'exposition aiguë à la fumée de tabac a entraîné un taux de clearance accru dans les bronches lobaires de 8 des 9 fumeurs ($p<0.03$), alors que l'effet sur les bronches principales et la trachée était inconstant. Chez tous les sujets, la terbutaline augmentait systématiquement le taux de clearance dans toutes les structures bronchiques visibles, par comparaison avec le placebo ($p<0.04$). La combinaison de la fumée et de la terbutaline a entraîné une accélération du taux de clearance dans les bronches lobaires chez la plupart des sujets, par rapport à l'effet isolé du tabac ou de la terbutaline. L'on conclut que, aussi bien l'exposition aiguë au tabac que la terbutaline en aérosol, augmentent la clearance muco-ciliaire chez les fumeurs bien portants.

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