Problems in Evaluating the Effect of Secretolytic Agents on
the Mucociliary System by Means of Radioactive Particles¹,²

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Abstract. In a double-blind cross-over study the effect of two secretolytic agents on the mucociliary clearance was tested in 8 patients with mild chronic obstructive bronchitis. Clearance was assessed from the removal rate of previously inhaled sulfur colloid particles, tagged with $^{99}$TcO₄⁻. For the interpretation of the results, obtained by this method, it is essential to take into account the pattern of particle deposition. For example there was a faster clearance under placebo compared to the secretolytic drugs because of a more central deposition. To overcome this problem different approaches were therefore tested. The following constellations proved to be useful in assessing the effect of secretolytic drugs: (1) change in deposition pattern; (2) clearance rate, if no change in deposition takes place; (3) clearance rate from a peripheral area of the lung.

An attempt to apply a simple three compartment model proved to be unpracticable probably because of the complexity of the mechanism involved. One of these mechanisms could be a reversal in mucus transport, observed at least in one patient, a finding which might be of pathophysiological relevance.

Introduction

Although the mucociliary clearance mechanism in vivo has to be regarded as an unseparable system, each part can be influenced separately by drugs: strength and fre-

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way resistance [10, 11]. But in vitro measurements, using histochemical, rheological and viscosimetric methods do not reflect the actual in vivo status [6, 9, 18] and ventilatory tests are quite insensitive in evaluating minor changes concerning this particular question.

We therefore used a noninvasive technique, by which the clearance rate of inhaled radioactive particles was monitored over the lung by means of external detectors, in studying the influence of two secretolytic agents on the mucociliary clearance of patients with chronic bronchitis [2].

### Method

The aerosol was obtained by a commercially available nebulizer (Pari®, Starnberg, FRG). The nebulizer was driven with a pressure of 2.5 ata and delivered a disperse spectrum of droplets with a mean diameter of 2.8 µm, ranging from 1 to 10 µm. The substance used was sulfur colloid, tagged with $^{99m}$Tc (γ-energy ca. 140 keV, half-life time 6.04 h). The radioactivity averaged 5 mCi, dissolved in 2 ml 0.9% saline. This isotope proved to be safe for the subjects studied and favorable regarding the resolution of the recording system. The radioactivity was recorded by a dorsally placed scintillation camera (Pho Gamma, Nuclear-Chicago). The analogous data were stored on a magnetic tape, converted into digital data by help of two ADC and then fed into a computer (PDP 12 A, Digital Equipment) [4, 5]. Data processing was performed by the computer, building a storage matrix of 32×32 points. To obtain numerical data from parts of the lung, 'areas of interest' were selected using a teletype. This set-up enables one to eliminate extrapulmonary artefacts, e.g. swallowed radioactive material in the stomach. Simultaneously, polaroid photos were taken. The measurements were done, with the subjects lying prone, to avoid changes in position in front of the camera.

The two secretolytic drugs, bromhexine (Bisolvon®) and iodopropylidene glycerol (Mucantit®) – an iodinated organic compound – were tested against a placebo in a double-blind cross-over study. Eight patients with chronic bronchitis and mild airway obstruction (table 1) were examined in a stable phase of their disease on 3 days of 1 week. The drugs were given per os at 8 a.m., 12 a.m. and 2 h after the inhalation of the radioactive aerosol. This regimen was felt to be necessary in view of the fact that the processes of mucus production and ciliary motion do not act simultaneously. The clearance study regularly began at 3 p.m. Immediately following the inhalation of the radioactive particles, the subject was asked to gargle with fresh water to remove radioactive material from the mouth. The nuclear measurements were then performed continuously for 30 min and repeated after 60, 90, 120 and 240 min. The 24-hour sputum was collected for 5 days, weighed and judged macroscopically. In addition, airway resistance and lung volumes were determined on each day of the examination at 9 a.m. and 9 p.m. The patients were asked to stop smoking during the examination. Two of them were smokers (5–10 cigarettes/day), 5 were exsmokers and 1 was a nonsmoker. Besides the secretolytic agents to be tested, the patients did not receive other drugs acting on the mucociliary system.

### Results and Discussion

Neither the ventilatory studies (airway resistance and lung volumes) nor the examination of the 24-hour sputum changed significantly. Also the patients when asked did not prefer any particular drug which would have improved their symptoms.

The interpretation of the results, obtained with the clearance rate of the radioactive
particles, proved to be problematic. From previous studies it is known that the clearance is quite reproducible in the same subject. Examinations in monozygotic twins led Camner et al. [7] to the conclusion that the tracheobronchial clearance rate is determined to a great extent constitutionally. Because of large intra-individual differences, drugs affecting the mucociliary system can only be tested if the individual subject serves himself as control. If the pattern of deposition of the radioactive particles remains the same, a change in clearance rate has to be interpreted as a function of mucus transport [3].

In the present study, the deposition pattern was defined for each patient as the ratio of the initial counts, recorded over a central area of the right lung (fig. 2) to the initial counts over the total right lung. Under placebo, the ratio central/total ranged between 20 and 78% (table II). The mean clearance rate of all 8 patients with chronic bronchitis was for the first 60 min under the treatment with both secretolytic drugs slower compared to the placebo (fig. 1). These paradoxical findings can be explained by the different deposition patterns of the radioactive particles in the lung (table II). Under treatment with both drugs, the ratio central to total counts decreased significantly, indicating a deeper penetration of the particles into the peri-
Table II. Deposition pattern (ratio central/total counts, see fig. 2) immediately after inhalation of the radioactive particles; the difference in deposition pattern between placebo and bromhexine was statistically significant \( t = 2.42; p < 0.05 \)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Placebo</th>
<th>Bromhexine</th>
<th>Mucantil®</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.37</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>0.61</td>
<td>0.62</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>0.23</td>
<td>0.20</td>
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<tr>
<td>4</td>
<td>0.78</td>
<td>0.37</td>
<td>0.49</td>
</tr>
<tr>
<td>5</td>
<td>0.63</td>
<td>0.35</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>0.43</td>
<td>0.34</td>
<td>0.45</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>0.41</td>
<td>0.42</td>
</tr>
<tr>
<td>8</td>
<td>0.71</td>
<td>0.62</td>
<td>–</td>
</tr>
<tr>
<td>Mean ( \pm SD )</td>
<td>0.53 ( \pm 0.21 )</td>
<td>0.39 ( \pm 0.16 )</td>
<td>0.40 ( \pm 0.18 )</td>
</tr>
</tbody>
</table>

Fig. 3. Deposition pattern in patient No. 1 immediately after inhalation of the radioactive particles. Polaroid photos taken at 3 different days immediately after inhalation. a Placebo. b Mucantil®, c Bromhexine.

Brownian motion [22]. With the droplet size, used in the present study, deposition takes mainly place by impaction in the larger airways and probably by sedimentation in the periphery of the lung [27]. Inertial impaction increases with higher laminar and turbulent flow. In normal persons, there is already turbulent flow in the trachea and the mainstem bronchi. Morphological changes in the bronchial wall – roughness and irregularities within in the lumen, strings of mucus and narrowing of the lumen – will exaggerate this mechanism [12, 13]. The fact that the deposition is changed from a more 'central' to a more peripheral pattern under the treatment with both secretolytic drugs could be interpreted by a ‘smoothing’ effect on the central airways, which in turn changed the mechanism of particle deposition from impaction to sedimentation. This means that the initial deposition pattern in a previously untreated bronchitic subject can be regarded as an expression of the distorted geometry of his bronchial tree and that this condition can be reversed partially by treatment.
On the other side, a ‘peripheral pattern of deposition’ will cause a delay in clearance [8]; (1) the mean transfer path for the particles to the glottis becomes longer; (2) the ciliary action seems to be inherently slower in the peripheral airways, and (3) the number of ciliated epithelial cells decreases with decreasing airway diameter [13]. According to these considerations there was a statistically significant difference in the activity remaining in the lung after 240 min in all patients when comparing their most peripheral with their most central initial deposition pattern (45.9 vs. 37.9%, \( t = 2.49, p < 0.05 \)).

In 3 of the 8 bronchitics studied, the pattern of deposition did not change substantially under treatment (less than 10%). In these 3 subjects, the mean clearance rate became faster when treated with bromhexine as well as with Mucantil® (fig. 4). These findings are in agreement with a recently published study of Thomson et al. [29], who found that the rate of removal of previously inhaled particles tagged with radioisotopes became significantly faster after the administration of bromhexine 16 mg three times daily for 14 days.

To overcome the difficulties in the interpretation of clearance curves with different deposition patterns, another approach was tried:

At first, the trachea was taken as representative for the mucociliary system in analogy to the studies of Sacker et al. [25], who examined the removal of teflon particles from the tracheal mucosa by direct observation through a bronchoscope. But the attempt to follow a ‘bolus of radioactivity’ from the carina to the glottis proved to be unpracticable, mainly because the ‘bolus’ activity did not differ statistically significant from the ‘surrounding’ radioactivity.

The second approach was based upon the idea that the percent disappearance rate of the radioactivity in the periphery of the lung should be independent upon the deposition of the radioactive particles. The two-dimensional numerical print-out of the right ‘lung’ was therefore divided into three areas: a central area (identical with the central area
in figure 2), an intermediate and a peripheral area (obtained by dividing the remaining external area of figure 2 into two equally spaced areas). The central area should then represent mainly the large central airways, the intermediate area mainly the smaller airways and the peripheral area the terminal airways with and without ciliated epithelium and the alveolar space. Surprisingly, there was at least in one patient under placebo a marked initial increase of radioactivity in the peripheral area of the lung (fig. 5). Similar findings were reported by Sanchis et al. [27] in studying normal persons with $^{131}$I-labeled albumin particles. He tried to interprete this phenomenon by redistribution via lymphatic channels. In previous studies with $^{99}$Tc$^m$ sulfur colloid particles used in the present study, no evidence could be found that they were metabolized or cleared by lung perfusion and/or lymphatic drainage during the measurement to a significant degree [21]. One can therefore conclude that there can be a reversal of the mucus transport in patients with chronic bronchitis. In studying the mucus transport directly with a stereoscopic microscope in bronchitic rats, Iravani and van As [15] and Iravani [16] indeed observed ciliary incoordination and alteration of the effective ciliary beat, resulting in a mucus transport into the caudal direction in some areas of the bronchial mucosa. Therefore, it does not seem permissible to compare the mucociliary system with a simple, one-directional escalator[23].

Excluding the patient with the ‘reversal transport phenomenon’, the mean clearance rate in the peripheral area of the right lung was – in contrast to the mean clearance rate of the total lung (fig. 1) – in the remaining patients faster under treatment with both secretolytic drugs compared to placebo (fig. 6).

In a third approach it was tried to apply a three-compartment transit analysis, using the three areas of the lung described above. Assuming that the disappearance rate in the peripheral area is only a function of the inherent clearance system, the disappearance rate of the intermediate area a function of the
particle transport from the peripheral area and to the central area and the disappearance rate of the central area a function of the particle transport from the intermediate area and to the glottis, one should be able to describe the transit of the particles through the whole system independent upon the pattern of deposition. The results were disappointing, giving no useful statistical regressions at all. This might be due to the fact that the model assuming only one-directional mucus transport does not take into account the rather complex mechanisms, taking place in one compartment, at least in bronchitics (see above). It might prove useful to apply such a model in normal subjects.

References


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