General Anesthesia Does Not Alter the Viscoelastic or Transport Properties of Human Respiratory Mucus

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Observations that mucus transport rates (MTR) are depressed in anesthetized animals and humans have led to speculation that general anesthesia depresses ciliary activity or adversely alters the physical properties of the respiratory mucus (RM). We investigated the possibility that anesthesia changes the physical properties of RM in such a way as to depress ciliary transport. We collected 33 samples of RM from the endotracheal tubes (ETTs) of 28 people aged 1 to 79 years undergoing elective surgery who had no clinical evidence of lung disease. We measured the rigidity, viscoelasticity, spinnability, and the percentage of solid composition of these specimens as well as the transport of the collected RM across the mucus-depleted frog palate. These physical properties were not significantly different from RM collected from awake volunteers using the bronchoscopy brush collection technique. Differences in spinnability, transportability, and solid content of paired mucus samples from the inside and outside of the ETTs are suggestive of altered RM hydration, but this requires further study. The decrease in MTR during general anesthesia is probably due to mechanisms other than alterations in the physical properties of mucus.

(Chest 1990; 98:101-04)

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\text{MTR} = \text{mucus transport rate; RM} = \text{respiratory mucus; ETT} = \text{endotracheal tube; NPTTR} = \text{normalized frog palate mucus transport rate; percent SC} = \text{percentage of solid composition of mucus}
\]

One of the most vexing complications of general anesthesia is mucus pooling in the lungs that can lead to atelectasis and respiratory infections. A decreased mucus transport rate (MTR) has been demonstrated in areas of postoperative atelectasis. The average tracheal mucus velocity in sheep is decreased by 36 percent following pentobarbital or thiopental anesthesia. This effect is most pronounced during deep anesthesia. Halothane, enflurane, nitrous oxide, and morphine have also been shown to decrease tracheal MTR in anesthetized animals and humans. King et al demonstrated that pentobarbital anesthesia increased the rigidity (vector sum of viscosity and elasticity) of tracheal mucus in dogs. However, to our knowledge, the effect of anesthetics employed clinically in humans on mucus rheology has not been studied.

By understanding the mechanism of this mucus clearance defect we might be able to prevent or ameliorate these complications. Because mucus transport is dependent on both ciliary function and on the physical properties of mucus, it is likely that the depressed mucus transport during and after general anesthesia is due to alterations in one or both of these systems. We report herein on a series of experiments designed to measure the viscoelastic and transport properties of respiratory mucus (RM) collected from nonsmoking patients without lung disease who required the administration of a general anesthetic for nonpulmonary surgery.

Materials and Methods

Mucus Collection

We recently developed a technique that allows us to collect sufficient RM for analysis without causing any additional discomfort to a patient receiving a general anesthetic. Briefly, the endotracheal tube (ETT) collection technique involves the removal of the mucus layer coating a freshly removed ETT from patients who require intubation as part of a general anesthetic. At the end of surgery the distal 8 to 10 cm of the patient's ETT is cut and placed into a 15-ml plastic test tube (Corning centrifuge tubes, Corning, NY) containing 8 ml of light paraffin oil (No. 0-121 Fisher Scientific, Fair Lawn, NJ). The mucus coating the tube is removed within the hour by scraping the ETT. A second sample of mucus was also collected from the inside of the ETT in eight patients. These mucus samples were coated with a layer of oil in 1.5-ml polystyrene micro test tubes (Eppendorf, Brinkmann Instruments, Westbury, NY) that were labeled and stored at \(-80^\circ\text{C}\) if the mucus specimen was not analyzed immediately. The University of Alberta Committee on Human Experimentation approved the collection of these samples and the review of patient charts.

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Physical Properties of Mucus: Definitions and Techniques

The mucus sample was allowed to reach room temperature and, except for the measurement of viscoelasticity in the microrheometer, the mucus aliquot was cleansed of surface oil by a brief immersion in petroleum ether (No. E-139B, Fisher Scientific, Fair Lawn, NJ). The petroleum ether was permitted to evaporate from the sample before beginning measurements. We have previously demonstrated that these manipulations do not alter the physical or transport properties of the mucus sample.14

The magnetic microrheometer technique was used to measure the rheologic properties of microliter quantities of mucus.15 For these measurements, a 70- to 150-μm steel ball is positioned in the sample of mucus and oscillated by means of an electromagnet at different driving frequencies. The magnitude of displacement of the sphere and its phase lag with respect to the driving force are determined to calculate the viscosity and elasticity of the mucus. The magnetic rheometer results are presented as log G* (mechanical impedance, the vector sum of viscosity and elasticity) and tangent δ (loss tangent, the ratio of viscosity and elasticity) at two frequencies, 1 and 100 rad/s.

The filancemeter (SEFAM, Nancy, France) measures large-amplitude elastic deformation or spinnability as thread formation measured in millimeter units. This measurement was performed with a 15- to 20-μl mucus sample and a distraction velocity of 10 mm/s.14

We used a drying apparatus and a microbalance to calculate the wet weight and dry weight for samples of mucus larger than 10 mg from which the percentage of solid composition (percent SC) was calculated. The mucus sample, free of oil, was weighed in a microbalance on a previously weighed glass slide. This slide was then dried in a microwave oven (500 W for 30 minutes) and allowed to cool. The dried sample was then reweighed and the percent SC calculated from the ratio of wet to dry weights of the mucus.

Finally, samples of mucus as small as 1 μl were placed on the mucus-depleted palate of the leopard frog, Rana pipiens, and the transport rate of these samples was normalized to the transport rate for collected endogenous frog mucus (NFPR) giving us a biologic measurement of the transport properties of the mucus sample across a ciliated epithelium.19

Statistical analysis was performed using a statistics package (StatView II, Abacus Concepts, Inc, Berkeley, Calif) and a computer (Macintosh II, Apple Computer, Inc, Cupertino, Calif). A two-tailed unpaired t-test was used to compare mucus collected by the bronchoscopy brush technique with the ETT mucus and a paired t-test was used to compare the properties of mucus collected from the inside and outside of the same endotracheal tube. Results were considered significant at the p<0.05 level.

Results

We collected 33 samples of ETT mucus from 25 people (16 male and nine female) aged 1 to 79 years (mean, 27.5 ± 22.7 years). Patients were excluded from the study if they had any history of pulmonary disease. None of the patients suffered from either respiratory allergies or from a respiratory tract infection in the two weeks preceding surgery. There were 20 nonsmokers and five exsmokers who had stopped tobacco consumption for at least 4 years before the study. Although we have demonstrated that active smokers have changes in the physical properties of their RM as compared with nonsmokers,16 no differences were found on any measurement of mucus properties between nonsmokers and exsmokers in this group. A variety of nonthoracic surgical procedures were performed on these patients (Table 1). Values for the physical, viscoelastic, and transport properties of this mucus are shown in Table 2 and compared with published values for mucus collected by the bronchoscopy brush technique.17

The duration of anesthesia was 25 to 390 minutes (mean, 135 ± 99 minutes). There was a trend toward slower normalized transport rate on the mucus-depleted frog palate (NFPR) associated with increased anesthesia time (r = 0.354, p = 0.08). Anesthesia duration correlated with the type of surgery (p = 0.0001). Male subjects had a longer duration of anesthesia than female subjects (196 vs 92 minutes, p = 0.04) largely due to the number of short, gynecologic procedures in the female subjects. There were no sex-related differences seen in the properties of the mucus.

Correlations With Anesthetic Technique

The age of the patients was directly correlated with the wet weight or volume collection (r = 0.52, p = 0.005) and inversely correlated with mucus spinnability (r = 0.40, p = 0.05). As an "oral pediatric premed mixture," which included atropine and diazepam, was given to all children younger than six years of age in this study, there was a strong correlation between the age of the patient and administration of atropine (p = 0.0001) or diazepam (p = 0.0001). For similar reasons, the age of the patient was inversely correlated with the use of narcotic induction (p = 0.001). There was a smaller volume of mucus with the preanesthetic use of atropine (p = 0.04) or diazepam (p = 0.07) and spinnability was greater when diazepam was given (45.7 mm vs 30.9 mm, p = 0.05) and was lower when a narcotic induction was used (28.6 mm vs 43.3 mm; p = 0.03).

Mucus from the Inside of the ETT Compared with That from the Outside of the ETT

In eight of the patients (six nonsmokers and two exsmokers) we were able to collect paired mucus samples from the inside of the tube exposed to anesthetic gas flow and from the outside of the ETT. In addition, we collected paired samples from nine patients who were active cigarette smokers. The paired data from the smoking patients was used only to examine the differences between inside and outside mucus and are not included for analysis in any other

Table 1—Type of Surgery Performed and Anesthetic Duration

<table>
<thead>
<tr>
<th>Type of Surgery</th>
<th>N</th>
<th>Duration of Anesthesia, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial</td>
<td>5</td>
<td>271</td>
</tr>
<tr>
<td>Abdominal/gynecologic</td>
<td>16</td>
<td>111</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>59</td>
</tr>
</tbody>
</table>

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Table 2—Physical, Viscoelastic, and Transport Properties of Mucus Collected by ETT Technique Compared With That Collected by Bronchoscopy Brush Technique*

<table>
<thead>
<tr>
<th>Property Measured</th>
<th>Bronchoscopy Mucus (N = 33)*</th>
<th>ETT Mucus (N = 33)</th>
<th>p Value: Unpaired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log G* 1 rad/s</td>
<td>2.19 ± 0.32</td>
<td>2.09 ± 0.36</td>
<td>0.78</td>
</tr>
<tr>
<td>Tangent δ 1 rad/s</td>
<td>0.28 ± 0.05</td>
<td>0.32 ± 0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>Log G* 100 rad/s</td>
<td>2.58 ± 0.33</td>
<td>2.48 ± 0.39</td>
<td>0.80</td>
</tr>
<tr>
<td>Tangent δ 100 rad/s</td>
<td>0.95 ± 0.26</td>
<td>0.76 ± 0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Spinnability, mm</td>
<td>not measured</td>
<td>35.7 ± 17.5</td>
<td></td>
</tr>
<tr>
<td>NFPTTR</td>
<td>not measured</td>
<td>0.9 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Percent Solids (percent SC)</td>
<td>not measured</td>
<td>15.96 ± 7.0</td>
<td></td>
</tr>
</tbody>
</table>

ETT = endotracheal tube; NFPTTR = normalized frog palate mucus transport rate.
*Data taken from Jeanneret-Grosjean et al.17

portion of this study. The age range of these 17 patients was 3 to 65 years (mean, 37.4 years). Duration of anesthesia was 55 to 386 minutes (mean, 141 minutes). Viscoelastic and transport properties of these paired samples are shown in Table 3.

Although there were no significant changes in viscoelasticity between the inside and outside mucus, NFPTTR was depressed an average of 29 percent in RM samples from the inside of the ETT as compared with samples taken from the outside of the ETT (p = 0.0035) and spinnability was increased an average of 41 percent for the inside mucus (p = 0.005). There was also a 39 percent decrease in percent SC for the RM samples from the inside of the ETT (p = 0.032).

**DISCUSSION**

Mucociliary clearance is the first line of pulmonary defense and mucus retention can lead to atelectasis and pulmonary infection. Studies in both man and animals have shown significant reductions in pulmonary mucus clearance with the administration of general anesthesia either by barbiturates or by volatile gases,1-7 although there appears to be no reduction in nasal clearance rates in beagle dogs under halothane or phenobarbital anesthesia if the plane of anesthesia is light enough.18

Mucus clearance is dependent on both ciliary beating and on the viscoelastic properties of mucus.10,11 One study has examined the effect of pentobarbital anesthesia on mucus rheology in six dogs.8 In that study there was a threefold increase in both viscosity and elasticity with pentobarbital anesthesia, but no change in tangent δ, the viscoelastic ratio, which has been shown to correlate more closely with ciliary transport than either viscosity or elasticity.10 The NFPTTR fell 19 percent with pentobarbital but this was not a large effect given the magnitude of viscoelastic change. These studies suggest that barbiturate anesthesia may alter mucus properties to a greater extent than inhalational agents.3,8

We found that the viscoelastic properties of the mucus in the patients we studied were not statistically different from mucus samples obtained from awake human volunteers who had mucus collected on a bronchoscopy brush placed against the respiratory epithelium.17 There were no significant differences in the viscoelastic properties of mucus seen with different anesthetic agents or with different durations of surgery. Differences in the volume of mucus collection with different induction agents could be accounted by the age of the patient and the surface area of the endotracheal tube used.

Tracheal mucus velocity is dependent on the degree of systemic hydration. Studies in animals have shown a fall in MTR with dehydration that then returned to normal with rehydration.19 However, excessive sys-

Table 3—Viscoelastic and Transport Properties of Paired Mucus Samples Taken from the Inside and Outside of the ETT*

<table>
<thead>
<tr>
<th>Property Measured</th>
<th>Inside ETT Mucus (N = 17)</th>
<th>Outside ETT Mucus (N = 17)</th>
<th>P Value: Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log G* 1 rad/s</td>
<td>2.14 ± 0.33</td>
<td>1.97 ± 0.28</td>
<td>0.074</td>
</tr>
<tr>
<td>Tangent δ 1 rad/s</td>
<td>0.33 ± 0.10</td>
<td>0.31 ± 0.07</td>
<td>0.47</td>
</tr>
<tr>
<td>Log G* 100 rad/s</td>
<td>2.52 ± 0.34</td>
<td>2.35 ± 0.28</td>
<td>0.10</td>
</tr>
<tr>
<td>Tangent δ 100 rad/s</td>
<td>0.71 ± 0.13</td>
<td>0.76 ± 0.14</td>
<td>0.18</td>
</tr>
<tr>
<td>Spinnability, mm</td>
<td>39.8 ± 11.0</td>
<td>23.6 ± 9.1</td>
<td>0.005</td>
</tr>
<tr>
<td>NFPTTR</td>
<td>0.9 ± 0.45</td>
<td>1.26 ± 0.51</td>
<td>0.0035</td>
</tr>
<tr>
<td>Percent Solids (percent SC)</td>
<td>10.77 ± 4.1</td>
<td>14.95 ± 6.8</td>
<td>0.032</td>
</tr>
</tbody>
</table>

ETT = endotracheal tube; NFPTTR = normalized frog palate mucus transport rate.
*Data taken from Jeanneret-Grosjean et al.17
termic hydration in association with antigen-induced bronchoconstriction has also been demonstrated to reduce MTR, suggesting that there is a critical range of mucus hydration for optimal tracheal transport. Puchelle et al recently measured the NFPT and spinnability with either high or low inspired humidity in anesthetized dogs. They, too, collected mucus from the outside end of an ETT. The NFPT was significantly lower with decreased humidity (mean reduction, 21.2 percent) while spinnability was not significantly affected by humidity. In the patients studied here, standard anesthetic practice dictated the insertion of an intravenous line with infusion of maintenance amounts of intravenous solution and replacement of only significant blood volume loss.

We found no significant changes in viscoelasticity between the RM taken from the inside and paired samples from the outside of the ETT. Compared with the outside samples, the inside mucus had a higher spinnability, and lower percent SC and NFPT. We have previously noted an inverse relationship between NFPT and spinnability in nonpurulent mucus specimens which is consistent with these observations. The difference in percent SC suggests that the inside mucus was better hydrated than the outside RM samples. This higher water content could be due to increased humidity of the inspired air or salivary contamination of the samples during surgery. The inside samples did not appear to be contaminated on visual inspection, although this observation should be confirmed by measuring markers of salivary admixture (such as amylase) in the collected samples. An alternative explanation for the observed difference is that the presence of the ETT increases the content of cellular debris in the outside mucus, thus increasing the solid content without altering the mucus glycoprotein content or mucus viscoelasticity. The depression of NFPT with inside mucus is puzzling unless we assume that this is related to mucus hyperhydration or to a direct effect of the anesthetic gas accumulated in the sample on frog palate ciliary activity.

Because the physical properties of RM are not altered by general anesthesia in healthy individuals, it is likely that the observed decrease in MTR is either due to ciliary depression as a direct effect of anesthetic gases or that the effect of anesthesia on MTR is seen only in some individuals. Animal studies indicate that the MTR depression of pure barbiturate anesthesia may be more severe than that observed using inhalational agents.

REFERENCES
3 Bridger CP, Proctor DF. Mucociliary function in the dog's larynx and trachea. Laryngoscope 1972; 82:218-24
5 Forbes AR. Halothane depresses mucociliary flow in the trachea. Anesthesiology 1976; 45:59-63
7 Forbes AR, Gamsu G. Mucociliary clearance in the canine lung during and after general anesthesia. Anesthesiology 1979; 50:26-29
8 King M, Engel LA, Macklem PT. Effect of pentobarbital anesthesia on rheology and transport of canine tracheal mucus. J Appl Physiol 1979; 46:504-10
10 King M. Relationship between mucus viscoelasticity and ciliary transport in guanin gel/frog palate model system. Biorheology 1980; 17:249-54
11 King M. Interaction between mechanical properties of mucus and mucociliary transport: effect of pharmacologic interventions. Biorheology 1979; 16:57-68
16 Rubin BK, Ramirez O, King M. Changes in the respiratory mucus of asymptomatic smokers are comparable to those observed in animal models of tobacco smoke exposure. Am Rev Respir Dis 1989; 139:4334