Mucus Rheology and Transport in Neonatal Respiratory Distress Syndrome and the Effect of Surfactant Therapy*

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Background: Neonatal respiratory distress syndrome (RDS) is caused by a deficiency of pulmonary surfactant. Alveolar collapse and obstruction of conducting airways leads to mismatch of ventilation and perfusion and profound hypoxemia. We postulated that surfactant deficiency could alter the properties of respiratory mucus in such a way that it would be poorly cleared from airways and promote airway luminal obstruction and that these changes might be reversed by the exogenous administration of a synthetic surfactant preparation.

Methods: Respiratory mucus coating an endotracheal tube (ETT) or suction catheter was collected from 14 neonates (gestational age, 24 to 36 weeks; birth weight, 600 to 2,400 g) with RDS who required tracheal intubation and ventilation. Eight of these neonates received 5 ml/kg of an intratracheal artificial surfactant preparation (Exosurf), given between 2 and 10 h of age and six neonates received 5 ml/kg of air. Mucus viscoelasticity, hydration (percentage of solid composition of mucus), and mucociliary clearability (NFPTCR) were measured for each specimen.

Results: The total volume of mucus collected from the surfactant-treated and control infants was similar, but mucus hydration was significantly less in babies with RDS who did not receive Exosurf (percentage of solid composition of mucus 18.7 vs 11.4; p = 0.013). Ciliary transportability was also less in the untreated babies (NFPTCR 0.39 vs 0.86; p = 0.018) and this mucus was more rigid (increased viscoelasticity: log G' 1 rad/s 2.28 vs 1.50; p = 0.0001).

Conclusions: These data suggest that airway obstruction in RDS may be due, in part, to abnormal mucus properties and impaired ciliary transport. Surfactant therapy appears to improve mucus clearability. Exogenously administered surfactant may also be beneficial for the treatment of other selected respiratory conditions associated with impaired mucus clearance. (Chest 1992; 101:1080-85)

More than 30 years ago, Avery and Mead demonstrated that deficiency of pulmonary surfactant is the cause of hyaline membrane disease or respiratory distress syndrome of the newborn (RDS). Surfactant deficiency leads to mismatch of ventilation and perfusion, and hypoxemia that may be due in part to mucus obstruction of the airways. We postulated that surfactant deficiency might also alter the surface tension of airway mucus causing poor mucociliary coupling or increased mucus adherence to the epithelium, thus promoting airway obstruction. We further postulated that mucus abnormalities might be ameliorated by the administration of surfactant to the airways of babies with RDS.

Since 1980, the topical application of surfactant preparations has been studied for therapy of RDS. One such synthetic product, an artificial surfactant preparation (Exosurf) (Burroughs Wellcome Co, Research Triangle Park, NC), was first successfully used to treat prematurely delivered rabbit pups and is now being used in prematurely born neonates for the treatment of RDS. The studies reported herein were conducted in babies at the Royal Alexandra Hospital in Edmonton, Alberta, who were enrolled in an Exosurf rescue trial designed by the manufacturer.

Materials and Methods

We collected respiratory mucus from eight premature neonates with RDS who received airway treatment with Exosurf, and six neonates with RDS who did not receive Exosurf. All babies were intubated for 48 h or less (range, 6 to 48 h) at the time of mucus sampling. No baby received any medication other than parenteral antibiotics. Surfactant treatment group was determined by random allocation. Neonates were excluded from the study if they did not have RDS or if they had a secondary pulmonary diagnosis as well as RDS such as pneumonia. RDS was diagnosed using standard criteria, that is, progressive respiratory insufficiency and hypoxemia noted during the first hours of life in a prematurely born infant with roentgenographic changes of bilateral interstitial pulmonary infiltrates and air bronchograms.

Exosurf was reconstituted with sterile water immediately before use so that each 1 ml of solution contained 13.5 mg of dipalmityloylphosphatidylcholine, 1.5 mg of cetyl alcohol, 1 mg of tyloxapol, and 5.85 mg of sodium chloride. In a modified Wilhelmy balance, an Exosurf film has a minimal surface tension of less than 10 dyne/cm when its surface is compressed, and regains nearly all of the film

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Surfactant was administered according to the protocol of the Exosurf rescue trial, briefly summarized herein. Informed consent was obtained from the parents of an infant between 2 and 10 h of age with RDS who required endotracheal intubation. Either 2.5 mL/kg of Exosurf or a similar bolus of air was slowly instilled through the endotracheal tube over 1 to 2 min (30 to 50 mechanical breaths). After repositioning the infant, and without interrupting mechanical ventilation, a second dose of 2.5 mL/kg of surfactant or air was administered. No baby was treated before being allowed to breathe spontaneously. Another treatment was given 12 h after the initial therapy if the baby was still intubated. Approval was obtained from the University of Alberta Committee on Human Experimentation and the Royal Alexandra Hospital Human Ethics Committee to collect mucus samples from the endotracheal tubes of these intubated neonates.

As a comparison group, mucus was collected from eight full-term neonates without RDS who required nonpulmonary surgery and endotracheal intubation for the administration of a general anesthetic. These term babies were given atropine as surgical premedication while the neonates intubated for the treatment of RDS were not. We also collected mucus from the outside of the endotracheal tubes (ETTs) of 11 children between the ages of 1 and 7 years who received atropine premedication and from 23 nonsmoking young adults with no pulmonary abnormalities who were not given atropine before intubation for surgery. We have continued to collect a library of mucus samples from a variety of patients using the ETT technique, so the mucus analysis of some of the young adults had been included in our initial report of this technique.  

Mucus Collection

Mucus was collected from the outside of suction catheters inserted for airway hygiene or the outside of the ETT at the time the baby was extubated. Mucus was not collected by endotracheal suctioning because aspiration of mucus under pressure can shear the glycoprotein structure and change the mucus properties. After the ETT or catheter was removed from the airway, the distal 5 cm was cut into a test tube containing light paraffin oil (No. 0-121 Fisher Scientific, Fair Lawn, NJ) to prevent mucus dehydration. The mucus coating the tube was removed by scraping the ETT and it was analyzed within 1 h of collection by investigators (B.K.R. and O.R.) who were blinded to the treatment status of the infant with respect to surfactant instillation.

Measuring Mucus Properties

Viscoelasticity: Viscosity is the absorption of energy from an object moving through a liquid. Elasticity is the ability of a solid or non-Newtonian liquid to transmit energy back to a moving object. The viscoelastic properties of the mucus samples were measured by magnetic rheometry.  

Briefly, a microscope is used to position a steel microsphere approximately 100 μm in diameter in a 1- to 5-μl sample of mucus. This is then placed in the field of an electromagnet where the sphere is oscillated at driving frequencies of 1 and 100 rad/s. The image of the sphere is magnified and projected onto photocells where the magnitude of displacement of the phase lag with respect to the driving force are displayed on an oscilloscope screen and the loops so generated can be used to calculate the viscoelasticity of the mucus. These measurements are reported as the mechanical impedance, G* and the loss tangent, tan δ. tan δ is the ratio of viscosity to elasticity and is inversely related to mucus recoil. G* is the vector sum of viscosity and elasticity and is a measure of mucus rigidity.

Collection Volume and Mucus Hydration (Percentage of Solids):
The mucus sample was completely dried in a microwave oven on a previously weighed glass slide. The ratio of the dry weight to wet weight of the mucus is used to calculate mucus hydration, which is reported as percent solid composition (%SC).

Mucociliary Transportability: The frog palate is a mucus-secreting ciliated epithelium. A leopard frog (Rana pipiens) is pithed and killed and the excised palate is allowed to be depleated of endogenous mucus at 4°C over 12 to 18 h. A 1- to 5-μl sample of mucus is placed on the palate and the average transport rate of this sample is normalized to the transport rate for the collected endogenous frog mucus.

Statistical Analysis

A statistics package (StatView II, Abacus Concepts Inc, Berkeley, Calif) was used for data analysis. Comparisons between surfactant-treated neonates and neonates with RDS who did not receive surfactant were made using two-tailed, unpaired, unweighted t tests. Results are expressed as mean ± standard errors. P values of less than 0.05 are considered significant.

Figure 1. Normalized mucociliary clearance on the mucus depleted frog palate (NFPTR). The transport rate for the surfactant treated babies was 2.2 times faster than that of the control babies with RDS who did not receive surfactant. Mucus from the term babies, children aged 1 to 6 years, and healthy, nonsmoking adults was obtained from the outside of the endotracheal tube used during a nonpulmonary surgical procedure.
 RESULTS

The babies who received Exosurf were similar to those who did not in birth weight (1671 ± 219 g with Exosurf vs 1671 ± 219 g; p = 0.35) and gestational age (30.4 ± 1.4 weeks with Exosurf vs 29.4 ± 1.4; p = 0.65). The postnatal age at the time of sampling was similar for both groups. The total volume of mucus collected from the surfactant-treated and untreated neonates with RDS was similar (7.55 vs 7.17 g; p = 0.93).

Neonates who were treated with surfactant had significantly faster mucociliary transportability (normalized frog palate transport rate or NFPTP) compared with the untreated babies (Fig 1). The NFPTP was 21 percent slower in untreated babies compared with term neonates and in turn, the term babies had mucus that was transported 26 percent slower than those treated with surfactant.

Mucus from babies treated with Exosurf had significantly lower rigidity log G* (p = 0.0001) than either the untreated babies with RDS or from any of the healthy subjects whose mucus was collected from the end of the ETT after elective surgery (Fig 2). In fact, this group had the lowest G* of any patients or experimental animals that we have studied. Tangent δ was not significantly different between Exosurf treated and untreated neonates (tangent δ 1 rad/s in the treated babies 0.32 ± 0.06 vs untreated 0.24 ± 0.01; p = 0.26). This suggests that the lower mucus rigidity with surfactant administration is due to a decrease in both elasticity and viscosity.

Surfactant-treated babies had mucus that was significantly better hydrated than the babies with RDS who did not have surfactant (Fig 3). Mucus hydration in the treated babies was comparable to that of nonsmoking adults without pulmonary disease.

Figure 2. Mucus rigidity factor (log G* at 1 rad/s). The transport rate for the surfactant-treated babies was 2.2 times faster than that of the control babies with RDS who did not receive surfactant. The children and healthy term babies received atropine as a preoperative medication while the other groups did not. Atropine is known to increase mucus rigidity.

Figure 3. Mucus hydration reported as the percent solids composition of the mucus (%SC).

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DISCUSSION

Alterations in the physical properties of mucus can result in pulmonary disease by a variety of mechanisms, including loss of barrier protection, airway obstruction, and failure of particle or microbial clearance from the airway. Because mucociliary coupling, mucus spreading, and the adhesive properties of mucus are all determined at the surface of the mucus (at the interface between the mucus and the air, the periciliary serous fluid layer, or the epithelial surface), it is important to investigate the role of surfactants in the physical and transport properties of mucus.

We postulated that babies with surfactant deficiency might have abnormal mucus properties that could promote airway obstruction, and that therapy with an exogenously administered surfactant preparation might ameliorate these mucus abnormalities. Furthermore, if exogenously administered surfactant facilitates airway opening, such mucus-modifying properties might be important for the surfactant to spread distally to the acinus.

The decreased viscoelasticity, increased hydration, and improved mucociliary clearance for the mucus from babies with RDS who received surfactant are compatible with a decrease in mucus surface tension. It has been demonstrated that a surfactant layer reduces surface tension at the mucus-air interface and promotes particle displacement into the mucus layer, perhaps by increasing the wettability of particles. Surfactant might also work as a lubricant to facilitate the sliding of the mucus gel layer on the periciliary fluid. If surface tension at the air-mucus interface reflects the interaction at the mucus-epithelium interface, high surface tension could inhibit ciliary penetration into the mucus layer, increase the adhesion of mucus to the cilia and epithelium, and reduce the transfer of water (e.g., mucus hydration) from the serous fluid layer. These findings are consistent with in vitro studies of frog mucus clearance after surfactant application. Allegra and colleagues sprayed porcine surfactant onto a frog palate and found that this increased the transport rate of endogenous frog mucus by 16 percent while saline solution spray decreased mucociliary transport by 37 percent.

To our knowledge, these are the first reported data on the properties of airway mucus in either healthy neonates or in premature babies with RDS. The mucus from untreated neonates with RDS was similar to that of neonates without pulmonary disease; these mucus samples had significantly increased rigidity and solids content and depressed mucociliary clearability when compared with mucus from adults without pulmonary disease. Compared with mucus from these adults, babies treated with exogenous surfactant had mucus with normal ciliary transportability that was hyperhydrated and with low viscoelasticity. Theoretically, the best control group for this study would have been an intubated and ventilated group of premature neonates without RDS or other lung disease, but such patients are extremely uncommon and none was cared for in our neonatal nursery over the 16 months that this study was conducted.

There are limitations to the interpretation of these data. Although we presume that most of the observed mucus changes are due to the alteration of mucus surface tension by the surfactant preparation, we did not directly measure the surface tension of the collected mucus. As well, mucus samples were immersed in paraffin oil and cleansed of the oil by petroleum ether before measurements began. This has been shown to have no effect on the bulk viscoelastic properties of the mucus, but it could well change the surface properties of mucus.

Although mucus removed from ETTs might be expected to come from babies who were able to be extubated and thus clinically healthier than those in whom suction catheter samples were taken, all of the samples from the untreated babies with RDS were taken from the ETTs while three of eight samples from Exosurf treated babies were from an ETT. In these treated babies there was a lower log C* for the ETT specimens and mucociliary clearability was higher in the ETT specimens when compared with the catheter specimens (although these data did not reach statistical significance). If anything, this would indicate the mucus rigidity and mucociliary clearability differences between the treated and untreated groups could have been underestimated by sampling bias. The most direct way of eliminating this bias would be to conduct a series of experiments in an animal model of RDS and to make serial measurements of mucus properties in the same animal before and after initiating surfactant therapy.

It is also possible that ventilator settings or inspired oxygen concentrations might change the properties of the secreted mucus. These data were held as part of the Exosurf trial and were not available to us. Although we are not aware of any data relating inspired oxygen to changes in the properties of respiratory mucus, because high concentrations of inspired oxygen can damage the airway epithelium, we are presently engaged in studies of pulmonary oxygen toxicity and mucus secretion in an animal model. There is less evidence to suggest that ventilator settings will acutely alter airway mucus properties per se, particularly during short surgical procedures and presumably for short courses of intensive care unit ventilation when there is no evidence of airway barotrauma.

There is another possible explanation for some of the differences in mucus viscoelasticity that were observed. The babies treated with Exosurf received 5 ml/kg of medication with vehicle while the control
babies with RDS received 5 ml/kg of air. Air was given to these control babies as part of the Exosurf clinical protocol because of the possibility that a bolus of vehicle without medication might produce a clinical deterioration. This opens the possibility of the vehicle causing a dilutional effect in the mucus from the babies with RDS. There are two reasons why we believe that this is not the reason for the viscoelastic and hydration changes in the mucus from Exosurf treated babies. The volume of mucus collected from the Exosurf treated babies was nearly identical to that collected from those who did not have surfactant therapy, suggesting that there was not a large dilution effect. Furthermore the hydration of the mucus from the treated babies was similar to that of mucus from the children, as shown in Figure 3, and from healthy adults, as previously reported.6,11

There are several lines of evidence supporting both the existence and importance of bronchial surfactant in the normal lung. Alveolar surfactant is probably cleared from the air sacs into the airways. Also, there is evidence that the larger airways can secrete their own surfactant lipids.12-14 In biopsy and autopsy material, osmiophilic membranes containing surfactant have been demonstrated in airway mucus, especially along the border between sol and gel phases of bronchial mucus layers.9,15 It has been proposed that airway surfactant separates the sol and gel phases of the mucus and might promote the sliding of one layer on the other.9,16 Schürch and colleagues9 have shown that in the excised trachea and major bronchi of healthy hamsters, the surface tension at the mucus-air interface was $32 \pm 2$ dynes cm$^{-1}$. This low value strongly supports the presence of a surfactant layer in the proximal airways of the healthy lung.

In sensitized dogs the inhalation of Ascaris antigen in low dose releases a large volume of watery mucus. When sufficient antigen is inhaled to cause bronchoconstriction, a rigid and poorly cleared mucus is released with properties similar to those seen in the control babies in this study.17 It is tempting to speculate on the possible role of surfactant in reversing airway obstruction by mucus plugging in asthma, especially when considering that β-adrenergic agents and glucocorticosteroids are two of the most widely used medications for the treatment of asthma and each has been demonstrated to stimulate the synthesis and release of surfactant phospholipids from alveolar type 2 cells.12

The properties of the low viscosity periciliary lining fluid that separates the mucus layer from the epithelium are largely determined by ion transport. The periciliary fluid has been shown to be critical for mucus cough clearability18,19 and the periciliary fluid may be the major determinant of mucus surface properties both by direct contact with and by fluid diffusion into the mucus layer. Cystic fibrosis is presumed to be caused by abnormalities of chloride transport. The mechanism of action of aerosolized amiloride, a sodium channel blocking diuretic recently demonstrated to ameliorate cystic fibrosis lung disease, could be to compensate for the effects of chloride blockade on the regulation of the hydration in the periciliary fluid. Amiloride has been demonstrated to reduce cystic fibrosis sputum rigidity and increase both cough and ciliary clearability.20,21 These changes are similar to those described herein for mucus after surfactant therapy for RDS lending credence to the hypothesis that cystic fibrosis mucus is abnormal secondary to electrolyte abnormalities in the periciliary fluid.

It has recently been reported that the lipid and presumably surfactant composition of expectorated cystic fibrosis sputum is related to sputum viscoelasticity, although the mechanism for this is unclear.22 The results of the study reported herein lend further credence to these observations and would tend to support the potential use of a surfactant aerosol as a mucokinetic agent for the treatment of cystic fibrosis lung disease.

In conclusion, we have demonstrated that babies with RDS who are treated by the airway administration of synthetic surfactant have mucus that is better hydrated, less rigid, and better transported by mucociliary clearance than mucus from neonates with RDS who do not receive surfactant. These data suggest that surfactant deficiency may promote airway obstruction by mucus in RDS. Surfactant therapy may also be useful in the treatment of other lung diseases with a significant component of airway mucous obstruction.

ACKNOWLEDGMENTS: The writers thank Dr. Neil Finer and Ms. Barb Hayes of the Royal Alexandra Hospital, Edmonton for providing the mucus specimens for analysis and for helping to plan this study. We would also like to thank Dr. Samuel Schürch of the University of Calgary for suggestions regarding the interpretation of these data.

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7th World Congress for Bronchology
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Esophagological Association. For information, contact Dr. Udaya Prakash, Secretary General and Director, East-18, Mayo Clinic, Rochester, Minnesota 55905.