Effects of Mucokinetic Drugs on Rheological Properties of Reconstituted Human Nasal Mucus

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Objective: To investigate the effects of mucokinetic drugs on the rheological properties of human nasal mucus in patients with chronic sinusitis.

Design: We reconstituted human nasal mucus obtained from 74 patients with chronic sinusitis and determined the effects of 4 mucokinetic drugs, including acetylcysteine, deoxynuclease I, 2% sodium bicarbonate, and a combination product containing tyloxapol (Alevaire), on rheological properties of reconstituted human nasal mucus (RHNM). We used 5% RHNM dissolved in phosphate-buffered solution as the optimal buffer and concentration of RHNM for the study because it showed a viscoelastic response similar to that of freshly collected nasal mucus from patients with chronic sinusitis.

Methods: Four experiments were performed to determine the influence of each drug on dynamic viscosity and elasticity of 5% RHNM. Distilled water was used as a control.

Results: Acetylcysteine and deoxynuclease I significantly decreased both dynamic viscosity and elastic modulus, while distilled water had no effect on rheological properties of 5% RHNM in vitro. Alevaire significantly reduced both dynamic viscosity and elastic modulus. Sodium bicarbonate significantly reduced elastic modulus but not dynamic viscosity. Reduction of elastic modulus by Alevaire was significantly greater than that by sodium bicarbonate, while there was no difference in reduction of dynamic viscosity between them.

Conclusion: Our results indicate that RHNM may be useful for studying the topical effects of various drugs on nasal mucus from patients with chronic sinusitis.


Nasal mucus collected from diseased nose and paranasal sinuses is more elastic and viscous than normal, and its transportability by cilia becomes impaired. This usually happens in prolonged inflammatory states, such as chronic sinusitis (CS). Such mucus contains many inflammatory mediators from host inflammatory cells and pathogens, and may contribute to the amplification of pathological conditions leading to further deterioration of mucociliary clearance. In nasal and/or sinus surgery, hypersecretion of viscous mucus and marked crust formation may impair both mucociliary clearance and ventilation of the nose and paranasal sinuses, and may prolong wound healing. Mucolytic therapy in such instances could improve abnormal rheological properties of mucus and may improve mucociliary clearance, thus helping to break the vicious cycle.

However, most rheological studies of mucolytic agents have been limited to sputum or tracheobronchial secretions. Few studies have been conducted on the in vitro effects of mucolytic drugs on the rheological properties of human nasal mucus. This is because the nasal mucus is a heterogeneous material and the amount of mucus obtained is limited even in patients with diseases of the nose and sinuses. Therefore, it is essential to prepare enough homogeneous nasal mucus for studying the topical effects of mucolytic drugs.

The present study consists of 2 series of experiments. In the first series, we reconstituted an arbitrary amount of human nasal mucus that was homogeneous and exhibited rheological properties similar to those of fresh nasal mucus from patients with CS. In the second series, we determined the in vitro effects of 4 mucolytic drugs on rheological properties of reconstituted human nasal mucus (RHNM). One of the drugs chosen for this study was acetylcysteine as a disulfide bond–reducing agent. Acetylcysteine is one of the most
MATERIALS AND METHODS

PREPARATION OF RHNM

Nasal mucus from 74 patients with CS was collected by means of a sterile disposable middle ear fluid collector (Juhn Tym Tip; Xomed, Jacksonville, Fla). The patients ranged in age from 8 to 64 years, with a mean of 32.2 years. Thirty-five were female and 39 were male. Mucus samples were dialedyzed against deionized distilled water for 24 hours to remove as much of the electrolyte and low-molecular-weight substances (<14 000 kd) as possible by using a dialysis membrane (Viskase Corp, Chicago, Ill) and lyophylized by means of a freeze drier (Eyela Freeze Drier FD-5; Tokyo Rikakikai, Tokyo, Japan). To provide sufficient material for the study, nondialyzable solids of nasal mucus obtained by lyophilization were pooled and ground to powder. The resulting lyophylized dry powder was stored at −80°C for later use. Lyophylized dry powder was dissolved in phosphate-buffered solution (PBS; pH 7.5) or Tris chloride–buffered solution (TBS; 0.1-mol/L Tris, 0.05-mol/L sodium chloride, pH 7.0) to prepare 6 concentrations of RHNM, consisting of 0%, 2%, 3%, 4%, 5%, 6%, and 7% (weight per volume). The reconstituted samples were stirred gently with a stick twice a day. Reconstituted mucus was kept at 4°C for 48 hours. After 48 hours, the mucus gelled as a homogeneous solution. The homogeneity was proved by measuring the viscoelasticity of RHNM in several different parts of mucus.

MEASUREMENT OF VISCOELASTICITY

Viscoelastic properties were measured by means of a controlled-shear-rate rheometer (Nippon Rheology Equipment Inc, Chiba, Japan). This rheometer was generally based on deformation in the cone-and-plate geometry. The angle between the cone and the plate was 1°. Approximately 0.3 mL of RHNM was placed on the plate. The cone was vibrated, with the top of the cone contacting the plate, by angular frequency (radian per second; 1 radian/s = 0.16 Hz) and rotation torque (newton meter) of the plate was measured. The dynamic viscosity (η′) and elastic modulus (G′) of the mucus were calculated by means of the following formulas: dynamic viscosity η′ = 3Mf/2πR²ν, and elastic modulus G′ = 2F/rπR, where f is the angle between cone and plate (radian), R is the radius (in centimeters) of the cone, and F is the thrust load force (in pascals). Elastic modulus is indicative of the elastic behavior of the specimen, and η′ is a measure of viscous behavior. Both G′ and η′ were determined at a constant temperature of 25°C at the angular frequency of 0.1, 0.2, 0.3, 0.5, 1, 2, and 5 Hz. Measurements for at least 3 runs on each sample were averaged to give the value of viscoelasticity.

DETERMINATION OF OPTIMAL BUFFER AND CONCENTRATION OF RHNM

The optimal concentrations of RHNM for the study were determined. Each RHNM sample dissolved in PBS or TBS was divided into 6 concentrations, consisting of 2%, 3%, 4%, 5%, 6%, and 7% RHNM. Dynamic viscoelasticity was measured on freshly collected nasal mucus from patients with CS or on RHNM by means of a controlled-shear-rate rheometer.

EFFECT OF DRUGS ON RHEOLOGICAL PROPERTIES OF RHNM WITH TIME

Mucokinetic drugs used in this study were acetylcysteine (Sigma Chemical Co, Tokyo), DNase (Sigma), sodium bicarbonate (Sigma), and Alevaire (Nipponshoji, Osaka, Japan). Distilled water was used as a control. The concentrations of mucokinetic agents used in this study were determined according to the clinically used dose. The optimal concentration of RHNM measured previously was divided into 6 aliquots. They were treated with the 4 drugs and distilled water. The concentrations of the drugs examined were as follows: 10% acetylcysteine, 50 000 U of DNase, and 2% sodium bicarbonate. Distilled water was used as a solvent for acetylcysteine and DNase. The RHNM and drugs were mixed in a volume ratio of 1:5 and incubated at 37°C for 30 minutes and 1, 2, and 4 hours without stirring. Four experiments were performed to determine the influence of each drug on rheological properties of RHNM.

STATISTICAL ANALYSIS

Repeated-measures analysis of variance was used to determine the effect of each drug with time on rheological properties of RHNM. Wilcoxon rank sum test was used to compare each drug and control at the same incubation time. The value of P<.05 was accepted as statistically significant. The values are given within the text as the mean ± SD.

RESULTS

MEASUREMENT OF OPTIMAL BUFFER AND CONCENTRATIONS OF RHNM

The volumes of individual samples ranged from a few microliters to 5.5 mL. The amount of dry powder obtained by freeze drying was about 30% of nondialyzable solids (weight per volume). When the mucus was reconstituted at high concentrations, RHNM hydrated slowly. With the aid of gentle intermittent stirring during a period of 48 hours, viscoelastic gels developed. The gross appearance of RHNM resembled that of mucopurulent nasal mucus. Dynamic viscoelastic spectra were...
measured for 2%, 3%, 4%, 5%, 6%, and 7% RHNM dissolved in PBS or TBS at 1 Hz, 25°C. A rapid decrease in \( \eta' \) accompanied by a moderate increase in \( G' \) throughout the angular frequency range (0.1 to 5 Hz) was the typical result (Figure 1). Viscoelasticity of RHNM dissolved in PBS increased as the concentration increased from 2% to 7%, while it showed a plateau from 5% to 7% RHNM in TBS (Figure 2). Since viscoelastic responses of 5% RHNM in PBS were indistinguishable from those of freshly collected nasal mucus from patients with CS when measured at 1 Hz, 25°C, the concentration of RHNM used in the following study was determined as 5% in PBS. As a measure of homogeneity, in 5% RHNM in PBS, \( G' \) was 2.019 ± 0.091 Pa·s and \( \eta' \) was 0.081 ± 0.002 Pa·s.

**EFFECTS OF EACH DRUG ON RHEOLOGICAL PROPERTIES OF RHNM WITH TIME**

No changes in \( \eta' \) and \( G' \) of 5% RHNM were observed until 4 hours at room temperature. The effects of acetylcysteine and DNase on \( \eta' \) and \( G' \) of 5% RHNM with time are plotted in Figure 3 and Figure 4. When compared with distilled water, acetylcysteine and DNase reduced both \( \eta' \) and \( G' \). Dynamic viscosity began to decrease significantly 1 hour after addition of acetylcysteine or DNase, while \( G' \) decreased significantly 30 minutes after addition of drugs. However, there was no additional reduction in either \( \eta' \) or \( G' \) from 1 hour through 4 hours after addition of drugs. Reduction of \( \eta' \) by acetylcysteine and DNase was 40% to 45% and 45% to 50% compared with untreated RHNM, respectively. The addition of acetylcysteine and DNase also reduced \( G' \) by 25% to 30% and 45% to 55% compared with untreated RHNM, respectively. Alevaire significantly reduced \( \eta' \) and \( G' \) of RHNM with increasing incubation time when compared with distilled water (Figures 3 and 4). The 2% sodium bicarbonate significantly reduced \( G' \) but not \( \eta' \) of RHNM. Reduction of \( G' \) by Alevaire was significantly greater than that by sodium bicarbonate.

**COMMENT**

A large amount of uniform mucus is a prerequisite for detailed rheological studies, because the untreated nasal mucus has heterogeneous structure and the amount of mucus collected from a patient is usually not sufficient for viscoelastic measurement by an ordinary rheometer. In the present study, nasal mucus was collected from 74 patients with CS, and a large amount of homogeneous mucus was reconstructed. The values of pH were 7.5 and 7.0 in RHNM by PBS and by TBS, respectively. Since a previous study by our group showed that pH of nasal mucus from patients with CS was 7.2 ± 0.6, the pH of RHNM remains in the range of CS mucus. There was a difference in viscoelasticity between reconstructed nasal mucus by PBS and by TBS. Although exact mechanisms are unknown, difference in pH and ionic strength may contribute to this difference. Five percent reconstituted mucus in PBS was found to be equivalent in viscoelastic properties to nasal mucus from patients with CS. The present study also showed a rapid decrease in viscosity accompanied by a moderate increase in elasticity with increasing angular frequency (Figure 1). The shapes of these curves are a reflection of the gel-like nature of mucus and are observed both in mucoid and mucopurulent nasal mucus from patients with CS. Our results indicate that rheological properties of 5% RHNM in PBS are consistent with those of human nasal mucus from patients with CS. Although this study did not include the biochemical analysis of RHNM, extensive previous work with tracheobronchial mucus has shown that the reconstituted mucus shows no significant difference in chemical composition compared with freshly collected mucus. Furthermore, transportability of mucus by frog palate cilia is no different between reconstituted and fresh tracheobronchial mucus. Therefore, we are confident that the reconstituted nasal mucus from patients with CS used in this study represents a satisfactory model for rheological study of nasal mucus.

Viscoelasticity was measured at 25°C, although the surface temperature of the nose ranges between 32°C and 37°C. This is because previous studies by our group and the results obtained in this study were compared with those of our previous study.

The viscoelasticity of airway mucus is correlated with the presence of disulfide bonds between long molecules of mucus glycoproteins. Breakage of disulfide bonds results in a marked decrease in the viscoelasticity of mucus. Acetylcysteine is one of the sulfhydryl mucolytics that disrupt disulfide bonds of mucus. In our present study, changes in the viscoelasticity of nasal mucus with added acetylcysteine were characterized by a rapid fall within 1 hour, followed by a maintenance of low viscoelasticity for 4 hours. Acetylcysteine was the most effective of the 4 agents used in this study in lowering the viscoelasticity. Although acetylcysteine possesses a sulfuric taste and odor, topical administration of 10% acetylcysteine may be of value in a variety of disorders characterized by production of highly viscoelastic mucus.
There is an optimal value of the viscoelasticity of mucus for effective mucociliary transport. A significant negative correlation exists between mucociliary transport and a higher-than-optimal viscosity or elasticity. The mean value of viscoelasticity of nasal mucus in patients with CS is much higher than the optimal viscoelasticity. Thus, acetylcysteine could have benefits in terms of improving the rheological properties and the clearance of nasal mucus in CS. However, our preliminary study showed that a high concentration of acetylcysteine transformed RHNM from a gel into flowing liquid (data not shown). Therefore, high-dose mucolytics may cause overliquifying and lead to suboptimal clearance.

Deoxyribonucleic acid is a high-molecular-weight polymer mainly released from host neutrophils and is supposed to contribute to the viscoelasticity of purulent sputum. Deoxynuclease I is a proteolytic enzyme that de-polymerizes DNA and may result in a reduction of viscoelasticity of mucus containing a large amount of DNA. The DNase used in this study reduced the viscoelasticity of RHNM in vitro. The reduction in viscosity was greater than that in elasticity (Figures 3 and 4). These results are in fair agreement with the results reported by Marriott. He applied a proteolytic enzyme to human bronchial mucus and found that the reduction in elasticity was not as great as that in viscosity. It has been reported that the ratio of the viscosity and the elasticity is an important determinant of mucociliary transport. Since an inverse correlation between the ratio of viscosity to elasticity and mucociliary transport rate was

Figure 2. Dynamic viscosity and elasticity at 1 Hz, 25°C, according to various concentrations of reconstituted human nasal mucus (RHNM). Closed and open circles indicate RHNM dissolved in phosphate-buffered solution and Tris chloride–buffered solution, respectively. Bars indicate 1 SD. Both viscosity and elasticity of RHNM dissolved in phosphate-buffered solution increased with increasing concentrations from 2% to 7%, while those of RHNM in Tris chloride–buffered solution showed a plateau from 5% to 7%. Horizontal solid lines represent mean viscosity or elasticity of freshly collected nasal mucus from patients with chronic sinusitis. Shaded area represents 1 SD of mean value of viscosity or elasticity of nasal mucus from patients with chronic sinusitis. Viscoelasticity of 5% RHNM in phosphate-buffered solution was similar to that of freshly collected nasal mucus from patients with chronic sinusitis.

Figure 3. Effects of acetylcysteine (NAC), deoxynuclease I (DN), Alevaire (AL), and 2% sodium bicarbonate (HC) on dynamic viscosity of reconstituted human nasal mucus (RHNM). Values are expressed as percentage of untreated RHNM (NT). Acetylcysteine, DN, and AL decreased dynamic viscosity of RHNM with increasing incubation time, while no effect on viscosity was observed with HC. There was a difference in reduction of viscosity between AL and HC. Distilled water (dH) was used as a control. Asterisk indicates significantly different from dH (P < .05) (n = 4); dagger, significantly different from HC (P < .05) (n = 4).

Figure 4. Effects of acetylcysteine (NAC), deoxynuclease I (DN), Alevaire (AL), and 2% sodium bicarbonate (HC) on elasticity of reconstituted human nasal mucus (RHNM). Values are expressed as percentage of untreated RHNM (NT). Acetylcysteine, DN, AL, and HC decreased elasticity of RHNM with increasing incubation time. Reduction of elasticity by AL was significantly greater than that by HC. Distilled water (dH) was used as a control. Asterisk indicates significantly different from dH (P < .05) (n = 4); dagger, significantly different from HC (P < .05) (n = 4).
noted, greater reduction in the viscosity than in the elasticity by DNase may contribute to an improvement of nasal mucociliary clearance in CS.

The DNase used in this study was purified from bovine pancreas. Although bovine pancreas DNase does not digest living tissues, prolonged use of this agent may produce antibodies against beef protein and may result in an occasional but severe bronchospasm. Recently, recombinant human DNase was synthesized, and its clinical effects were reported in patients with cystic fibrosis when it was administered into the lower respiratory tract in an aerosol form. Thus, recombinant human DNase rather than bovine pancreatic DNase could be a useful mucolytic agent in patients with CS.

Sodium bicarbonate has long been used as a topical solution for cleansing wounds, and it also has been used empirically as an irrigating solution of the trachea in patients who have had tracheotomies. There is evidence that 2% sodium bicarbonate reduces the viscoelasticity of sputum. In our present study, 2% sodium bicarbonate significantly reduced the elasticity of RHNM. The reduction in the elasticity might be caused by the alkaline environment provided by this agent.

In the present study, 2% sodium bicarbonate was used as an active control for Alevaire. This is because Alevaire contains the same concentration of sodium bicarbonate, and there has been considerable debate as to whether Alevaire is more effective than sodium bicarbonate alone in treating lower respiratory tract mucus. As shown in our study, Alevaire significantly decreased both viscosity and elasticity of RHNM compared with distilled water. Moreover, the decrease in elasticity was greater with Alevaire than with 2% sodium bicarbonate. The effect of Alevaire on rheological properties of nasal mucus might be caused by tyloxapol, a complex polymer capable of lowering surface tensions, and 2% sodium bicarbonate. It is known that Alevaire or 2% sodium bicarbonate administered repeatedly has no significant adverse effects. Since sodium bicarbonate is far less expensive than other mucolytic drugs, it could be useful in nasal and/or antral washing in patients with CS and/or those who have undergone functional sinus surgery.

As shown in the present study, the significant effects of the drugs were observed within the study period (from 30 or 60 minutes to 4 hours). In patients with CS, nasal mucociliary clearance was nil in about 50% of patients. In such conditions, topically administered drugs may stay in the nose for a long time and could decrease the high viscoelasticity of nasal mucus.

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