Evaluating the Efficacy of Mucoactive Aerosol Therapy

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Key words: mucus, mucociliary clearance, cough, asthma, cystic fibrosis, bronchitis, mucolytics, mucokinesis, mucoactive, aerosol.

Introduction

Respiratory tract secretions consist of mucus, surfactant, and periciliary fluid. The airway surface fluid is present as a bilayer with a superficial gel or mucus layer and a layer of periciliary fluid interposed between the mucus layer and the epithelium. A thin layer of surfactant separates the mucus and periciliary fluid layers. The mucus layer extends from the intermediate to the upper airway and is approximately 2–10 μm thick in the trachea. Airway mucus is the secretory product of the goblet cells (mucous cells of the pseudostratified columnar epithelium) and the submucosal glands. It is a nonhomogeneous, adhesive, viscoelastic gel composed of water, carbohydrates, proteins, and lipids. In health, the mucus gel is primarily composed of a 3-dimensional tangled polymer network of mucous glycoproteins or mucin. Mucin macromolecules are 70–80% carbohydrate, 20% protein, and 1–2% sulfate bound to oligosaccharide side chains. The protein backbones of mucins are encoded by MUC genes, at least 5 of which are expressed in the respiratory tract, although MUC5ac and MUC5b appear to be the predominant mucins in the lower respiratory tract.

The depth and composition of the airway surface fluid depend on secretion from the mucous cells and submucous glands and active ion transport across the surface epithelium as a mechanism for altering the hydration of secretions. The composition and physical characteristics of airway surface fluid allow for normal ciliary function and airway hygiene, and protect the airway from drying. Lysozyme, immunoglobulins, and antibacterial peptides in airway surface fluid provide a defense mechanism for the airways.

Mucus is transported from the lower respiratory tract into the pharynx by air flow and mucociliary clearance. Expec-
torated sputum is composed of lower respiratory tract secretions along with nasopharyngeal and oropharyngeal secretions, cellular debris, and microorganisms. Disruption of normal secretion or mucociliary clearance results in impaired pulmonary function and lung defense, and increased risk of infection. Abnormal respiratory secretions can cause mucus inspissation, postobstructive atelectasis, and airway and parenchymal lung injury with bronchiectasis and pulmonary fibrosis, leading to severe pulmonary dysfunction.

**Medications for Clearing Secretions**

Medications that enhance the clearance of secretions from the respiratory tract or decrease the volume of respiratory secretions are collectively called mucoactive agents (Table 1).

**Table 1. Mucoactive Agents**

<table>
<thead>
<tr>
<th>Mucoactive Agent</th>
<th>Potential Mechanisms of Action</th>
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<tbody>
<tr>
<td>Classical mucolytics</td>
<td></td>
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<tr>
<td>N-acetylcysteine</td>
<td>Alters the chemical structure of disulfide containing proteins and peptides</td>
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<tr>
<td>Nacystelyn</td>
<td>Increases mucus chloride content (improved hydration of secretions) and severs disulfide bonds</td>
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<td>Peptide mucolytics</td>
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<tr>
<td>Dornase alfa</td>
<td>Hydrolyzes DNA molecules with reduction in DNA length</td>
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<tr>
<td>Gelsolin</td>
<td>Depolymerizes F-actin</td>
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<tr>
<td>Thymosin β4</td>
<td>Depolymerizes F-actin</td>
</tr>
<tr>
<td>Nondestructive mucolytics</td>
<td></td>
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<tr>
<td>Hypertonic saline</td>
<td>Reduces ionic bonds and increases secretion hydration</td>
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<tr>
<td>Dextran</td>
<td>Breaks hydrogen bonds and increases secretion hydration</td>
</tr>
<tr>
<td>Low molecular weight heparin</td>
<td>May break both hydrogen and ionic bonds</td>
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<tr>
<td>Mucoregulatory agents</td>
<td></td>
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<tr>
<td>Anticholinergic agents</td>
<td>Decreases volume of stimulated secretions</td>
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<tr>
<td>Glucocorticoids</td>
<td>Decreases airway inflammation and mucin secretion</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Decreases airway inflammation</td>
</tr>
<tr>
<td>Macrolide antibiotics</td>
<td>Decreases airway inflammation and mucin secretion</td>
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<tr>
<td>Mucokinetic agents</td>
<td></td>
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<tr>
<td>Bronchodilators</td>
<td>Can improve cough clearance by increasing expiratory flow</td>
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<tr>
<td>Surfactants</td>
<td>Decreases sputum adhesiveness</td>
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</tbody>
</table>

**Mucolytics**

Mucociliary clearance is in part dependent on the viscoelasticity of the secretions. Mucolytics involve disruption of the cross-linking or tangled interaction between the macromolecules that form the gel. Normally, reduction in cross-linking and viscoelasticity of the mucus gel improve mucociliary clearance. However, occasionally, the mucus may be too thin for effective clearance. In such an instance, increasing the cross-linking with a mucospissic agent may improve clearance. These agents collectively are called mucotropic agents.

**Classic Mucolytic Agents**

Classic mucolytic agents work by severing disulfide bonds, calcium binding, depolymerizing mucopolysaccharides, and liquefying proteins. Agents containing free sulfhydryl (thiol) groups reduce the disulfide bridges interconnecting cysteine residues on adjacent mucin proteins. These include N-acetyl L-cysteine (NAC). NAC reduces the disulfide bond, thus reducing the elasticity and viscosity of the mucus gel. In vitro studies have demonstrated a dose-dependent effect of NAC on the viscoelastic properties of secretions. In vivo, it is ineffective when given orally.

**Peptide Mucolytics**

Sputum contains products of inflammation, including cellular debris and neutrophil-derived DNA and filamentous actin (F-actin). DNA and F-actin copolymerize to form a rigid polymer network entangled with the mucin gel. Peptide mucolytics degrade these abnormal filaments and leave the glycoprotein network relatively intact. Dornase alfa has been approved for the treatment of cystic fibrosis. It cleaves DNA polymers, reducing both sputum viscosity and adhesivity. Other peptide mucolytics under investigation include the F-actin depolymerizing agents gelsolin and thymosin β4. In vitro studies have demonstrated that F-actin depolymerizing agents in conjunction with dornase alfa result in a greater reduction in sputum viscoelasticity and cohesivity. The F-actin depolymerizing agents both destabilize the actin-DNA filament network and increase the depolymerizing activity of dornase alfa on the DNA filaments.

**Nondestructive Mucolytics**

Although agents that affect ionic charge interactions and hydrogen bonds are not true mucolytic agents, they can alter polymer interactions, reducing both viscosity and elasticity without reducing the polymer chain length. These agents include both ionic agents such as sodium chloride and nonionic agents such as dextran.
Ionic agents are thought to shield the fixed charges along the macromolecular core of the mucin polymer, making it less stiff and less extended, thus reducing the entanglements with neighboring macromolecules. Hypersomolar saline breaks ionic bonds within the mucus gel, reducing the degree of cross-linking and the viscoelasticity of sputum. It may also tonically increase the water content of mucus. In the presence of infected mucus, hypertonic saline can separate the DNA molecules from the mucoprotein component of the mucus, making the mucoprotein susceptible to proteolytic enzyme digestion.

Agents that alter hydrogen bonding within mucus may improve mucus clearance. Although each individual hydrogen bond is weak, the total potential of these bonds is substantial, since the oligosaccharide side chains make up about 80% of the mucin structure. The lower-molecular-weight fractions are primarily responsible for the mucoactive effects of dextran. In addition to the breakdown of hydrogen bonds, an osmotic effect with increased hydration of the mucus could also result in improved clearance of secretions.

**Mucokinesis**

For an effective cough, there must be adequate air flow to detach secretions from the epithelium and mobilize sputum so that it can be expectorated. Mucokinetic agents improve the cough clearance of secretions by increasing air flow or decreasing the interaction between secretions and epithelium.

Mucus adheres to the cilia and the epithelium. A layer of surfactant between the periciliary fluid and the mucus gel improves spreading of the mucus and promotes efficient energy transfer from the beating cilia to the mucus, thus preventing entanglement of cilia in mucus and promoting cough clearance of secretions. Phase 2 clinical trials using a surfactant aerosol demonstrated a significant increase in pulmonary function associated with improved in vitro mucus clearability in patients with cystic fibrosis and chronic bronchitis.

Bronchodilators can be considered mucokinetic agents in those patients who demonstrate an improvement in air flow with bronchodilator therapy. By virtue of their ability to increase expiratory flow in intermediate and large airways, they can substantially improve cough clearance. Since β agonists are mucus secretagogues, it is important to demonstrate improvement in pulmonary function before these agents are prescribed. Although β agonists improve ciliary beat frequency, this does not appear to improve mucus clearance in patients with lung disease.

**Mucoregulatory Agents**

Agents that inhibit mucus production or secretion are called mucoregulatory agents. These include anticholinergic agents (decrease the volume of stimulated secretions without increasing viscosity), glucocorticosteroids, indomethacin, and some macrolide antibiotics. Reduction of mucus secretion by macrolide antibiotics (erythromycin and clarithromycin) is unrelated to their antibacterial activity but may be related, in part, to their anti-inflammatory properties.

**Aerosol Delivery of Mucoactive Agents**

Most mucoactive agents have been developed as oral preparations. Oral medications are usually easy to administer and dosing is relatively precise, although absorption may be variable. Some medications, such as the macrolide antibiotics, appear to be effective only when given orally. Other drugs, such as guaifenesin, may best target effector cells if given orally. Ideally, oral mucoactive agents should be easily ingested and adequately absorbed from the gastrointestinal tract and should be effective in decreasing secretions or enhancing clearance. Often, systemically administered mucoactive agents may be only partially effective because of a poor therapeutic ratio. Therefore, in recent years, there has been an increasing trend toward targeting the airways directly by means of aerosol therapy. The potential advantages of inhaling mucoactive agents include localized action in the airways, more rapid onset of action, and decreased adverse effects. Peptide and protein compounds, which would otherwise be broken down in the stomach, can be administered via inhalation and often will retain activity.

Various mucoactive agents are being investigated for possible delivery via aerosol. These include NAC, dornase alfa, osmolar agents, surfactant, and anti-inflammatory agents. In patients with secretion retention, aerosolized drug delivery is affected by decreased drug transport across infected secretions, drug losses due to binding to glycoproteins, inactivation by various mucus components, and inefficient drug delivery to peripheral airways because of mucus plugging.

**N-acetyl L-cysteine**

In vitro studies have shown a dose-dependent effect of NAC on the viscoelasticity of sputum, such that the greater the concentration of NAC, the greater the reduction in secretion viscosity. NAC also is an antioxidant by increasing glutathione. The use of NAC via aerosol is limited, since it can induce bronchospasm in some patients. It has an unpleasant taste and odor, making it unpopular with many patients. A systematic review of the use of nebulized NAC in patients with cystic fibrosis found only 3 randomized clinical trials, none of which showed a clinically relevant beneficial effect of therapy. A lysine derivative of NAC, nacystelyn (NAL), can reduce mucus viscoelasticity in dogs and in cystic fibrosis patients. NAL im-
proves tracheal mucus velocity in anesthetized dogs, and this improvement is accompanied by an increase in mucus chloride content consistent with increased secretion hydration. NAL may be an attractive alternative to NAC, as it can be administered via pressurized metered dose inhaler and has a pseudoneutral pH, compared to that of nebulized NAC (pH 2.2). European clinical trials of aerosolized NAL are in progress.

**Dornase Alfa**

Recombinant human deoxyribonuclease I (rhDNase or dornase alfa) cleaves DNA molecules, resulting in decreased sputum viscosity and tenacity. Administration of dornase alfa via aerosol to patients with stable cystic fibrosis lung disease has been shown to improve forced expiratory volume in the first second (FEV₁), reduce the frequency of pulmonary exacerbations, and improve the measured quality of life. Ultrasonic devices denature peptides such as dornase alfa. Clearly, the choice of a nebulizer system for delivery of any drug depends on the physicochemical properties of the agent, dose of the drug, and patient compliance issues.

**Osmolar Agents**

Isotonic saline aerosol has been shown to induce expectoration (sputum induction) even in healthy subjects. Significant improvement in FEV₁ has been demonstrated after the use of hypertonic saline aerosol therapy by cystic fibrosis patients. The response to hypertonic saline inhalation is dose-dependent, with better results seen with more concentrated saline solutions. Patients with hyperreactive airways may have bronchospasm following administration of hypertonic saline. Hypernatremia can result following administration of hypertonic saline in patients with renal insufficiency.

Secretion clearance can be stimulated by monosaccharides or disaccharides such as mannitol and lactose. A dry powder of mannitol has been used as an osmotic stimulus to increase mucociliary clearance in healthy subjects and in patients with asthma and bronchiectasis. Mannitol inhalation increases mucociliary clearance, and the response is comparable to that obtained by inhalation of hypertonic saline. The increase in secretion clearance was not related to induced cough. Dextran administration via aerosol has also been shown to increase tracheal mucus velocity in dogs.

Low-molecular-weight heparin, a charged oligosaccharide, may have greater mucolytic and mucokinetic properties than dextran. It may disrupt hydrogen bonds similar to low-molecular-weight dextran, and improve ionic interactions like hypertonic saline. Aerosolized low-molecular-weight heparin also shows promise in the treatment of asthma, presumably by interfering with antigen-receptor binding.

**Surfactant**

Pulmonary surfactants help maintain bronchiolar and alveolar stability, particularly at low lung volumes. In the airway, surfactant can provide lubrication, increase cilia beat frequency, and improve interaction between cilia and secretions, resulting in improved cough and mucociliary clearance. Airway surfactant facilitates the formation of an interfacial bilayer that helps transfer kinetic energy between the cilia and the mucus layer.

Direct tracheal instillation of surfactant is impractical for most patients with reduced mucociliary clearance. This mode of surfactant delivery is also expensive and inefficient because of nonuniform distribution of surfactant, necessitating administration of large volumes of the drug. An appealing alternative is administration of surfactant via aerosol. Whereas surfactant instillation did not improve severe ventilation-perfusion mismatch in an isolated lung model of acute lung injury, nebulized surfactant markedly reversed it. Surfactant aerosol therapy has also been shown to improve both in vitro mucociliary clearability of sputum and pulmonary function in patients with chronic bronchitis or cystic fibrosis.

Meconium aspiration syndrome is a disease characterized by mechanical obstruction of the airways, inflammation and chemical pneumonitis, and inactivation of surfactant function, and this can lead to pulmonary hypertension. These infants are often treated with surfactant therapy. Those infants not sick enough to require intubation but requiring supplemental oxygen may benefit from inhaled surfactant therapy. Surfactant inhalation appears to be a promising new approach for providing surfactant therapy.

**Anti-inflammatory Agents**

Anti-inflammatory agents reduce airway mucosal inflammation, and may decrease secretion production and retention. In inflammatory airway disease, epithelial cell desquamation results in increased secretions. The increase in proteases, DNA, and F-actin increase mucus viscosity and decrease mucociliary clearance. Although low-dose oral corticosteroids improve the clinical course of selected cystic fibrosis patients, clinical trials of inhaled corticosteroids have shown conflicting results. Another anti-inflammatory agent, indomethacin, administered via nebulizer, effectively reduced mucus hypersecretion in diffuse panbronchiolitis patients. Airway targeted antioxidant and antiprotease therapies, if effective, could decrease airway secretion volume and purulence.
Combination Therapy

Combination therapy may consist of different medications aerosolized together or in tandem. The most commonly used combination therapy in cystic fibrosis patients is the combination of antibiotics and bronchodilators. Another possible combination is NAL with dornase alfa. Combination therapy at half the concentration of each drug significantly decreased in vitro cystic fibrosis sputum cohesivity, indicating an additive effect of the two drugs. Similar effects were observed when dornase alfa was used in conjunction with either hypertonic saline or gelolin. The use of combination therapy may decrease the number of doses of medications taken through the day, with possible improvement in patient compliance. The clinical efficacy of combination therapy needs to be further evaluated in clinical trials. Where feasible, therapeutic agents to be delivered via inhalation should be formulated for delivery from a pressurized metered-dose inhaler or a dry powder inhaler, since these are more patient-friendly, easier to use, and usually more cost effective.

Clinical Use of Aerosol Mucoactive Agents

The principal indication for the use of mucoactive agents is to reduce airway obstruction by abnormal secretions. Decreasing the volume of airway secretions can reduce gas trapping and improve the performance of the muscles of respiration. The use of mucoactive therapy to decrease secretion volume or to increase mucus clearance should be considered only after initiation of therapy directed at decreasing infection and inflammation and after minimizing exposure to irritants, including tobacco smoke.

Patients most likely to benefit from mucoactive therapy usually have preserved airflow and a history of increased airway secretions. In general, with an FEV₁ of < 25% of predicted, it becomes difficult to expectorate secretions. When airflow is severely compromised, mucolytic agents could theoretically reduce sputum clearance because of retrograde flow. During episodes of acute mucus retention (e.g., acute bronchitis or acute exacerbation of cystic fibrosis), mucoactive agents may be less likely to be effective. The decreased responsiveness of patients to mucoactive therapy may be related to reduced airflow with infection and muscular weakness that can further compromise air flow-dependent clearance. Patients who are unable to protect their airways may be unable to handle increased airway secretions.

The effectiveness of mucoactive therapy can be difficult to assess. When there is an improvement in symptoms and air flow or reduced gas trapping, the benefit of therapy is clear. However, changes in FEV₁ poorly reflect clinical improvement with mucoactive agents. Expectorated sputum volume is not a good measure of the effectiveness of mucoactive therapy. This is partly related to the limitations in accurately measuring the volume of expectorated sputum because of patient reluctance or inability to expectorate, swallowing of secretions, and salivary contamination. Sputum volume also varies from day to day, and at different times of the day. Finally, increased sputum could reflect either increased clearance or increased production of secretions.

The scientific evaluation of secretion properties and the clinical response to therapy will facilitate the development of effective mucoactive therapy and help us to better determine which patients are most likely to benefit from specific therapies.

Future Development

With improved understanding of the role of mucoactive agents in various inflammatory conditions, it will be possible to develop more effective therapies. Because of targeted action in the airways with the possibility of fewer adverse effects, there is increasing interest in delivering therapies at the site of disease in the airways. Improvements in aerosol delivery systems will result in improved aerosol efficiency. Appropriate drug formulations need to be developed that can be easily dispersed using our current delivery systems and systems that will soon be available. Another treatment modality of the future is likely to be gene transfection to treat diseases such as cystic fibrosis. Both cystic fibrosis gene cDNA liposomes and modified adenoviral vectors have been evaluated in patients with cystic fibrosis.

Summary

The aerosol route is attractive for the delivery of mucoactive medications. Mucoactive medications include mucolytics, which depolymerize polymers of mucin (classic mucolytics) or DNA/actin (peptide mucolytics), mucokinetic agents, which increase cough clearance, mucoregulatory medications, which decrease abnormal mucus secretion, and expectorants and ion channel modifiers.

Despite the widespread use of these medications, there are few well conducted studies and thus few data clearly supporting (or failing to support) their use. This will change as our understanding of mucociliary physiology and pharmacology increases and as well designed and well powered clinical trials are conducted with appropriate outcome measurements.

Effective mucoactive therapy should make a profound impact on the care of patients with chronic bronchitis, asthma, cystic fibrosis, and inflammatory airways disease, and will be essential for the effective delivery of gene therapy vectors and bioactive peptides to the airway epithelium.
REFERENCES


