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# Pharmacological approaches to discovery and development of new mucolytic agents

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**Abstract**

Airway mucus is the secretory product of the mucous cells; it is a variable mixture of water, mucous glycoproteins, low molecular weight ions, proteins, and lipids, whose physical properties are important for airway defense. The factors that contribute to the physical properties of mucus are complex, and there are a number of pharmacological strategies that can potentially serve to improve the clearability of airway mucus. Novel mucoactive approaches include strategies for mucoregulation – decreasing the abnormal volume of mucus secretion – and medications designed to improve the cough clearability of airway secretions. In vitro results suggest potential benefits from the additive effects of selected combinations of mucoactive medications. Further studies are required to confirm these findings, to perform direct assessments of mucus clearability, and to extend the observations to patients with various types of pulmonary diseases where mucoactive treatments are required.


*Keywords:* Airway mucus; Mucociliary clearance; Mucoactive agents; Mucolytic agents; Mucus secretion

**Contents**



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## 1. Introduction

There are a large number of medications that are meant to change the properties of airway secretions. In theory these approaches facilitate the clearance of surface mucus by improving the function of airway cilia and enhancing the mucociliary interaction, or by decreasing mucus hypersecretion especially in patients with chronic airway inflammation. Collectively these medications are referred to as mucoactive medications [1]. The mucoactive medications are further subclassified into: (a) mucokinetic agents that improve ciliary function; (b) mucolytic medications that reduce secretion viscosity either by adding water to the airway (expectorants and ion channel modifiers), depolymerizing the mucin network (classic mucolytics), severing abnormal polymers in mucus including DNA and filamentous actin (peptide mucolytics) or dispersing polymers by means of charge shielding; (c) mucospissic agents that increase the viscosity of abnormally thin secretions; (d) secretagogues that increase the volume of airway secretions; (e) mucoregulatory medications that decrease the overproduction of mucus; and (f) medications that improve the cough clearability of secretions by increasing airflow or decreasing mucus adhesivity.

It is clear from this list of potential mechanisms of action that many of these medications have opposing effects on airway secretions making it equally clear that none of these therapies are uniformly effective. Although it may be that certain types of therapy would benefit patients with a specific disease or at a specific stage in their disease and that certain combinations of these actions may be even more effective, this has never been demonstrated clinically. The majority of medications used around the world, as 'mucolytics' are expectorants that have never been adequately tested in clinical trials; the best that can be said is that for the most part they are safe when used as directed. Because of lack of demonstrated effectiveness very few of these medications have been approved for use as mucoactive drugs in North America.

The *appropriate* use of mucoactive medications depends on the knowledge of the physiology and pathology of airway mucus secretion and clearance. Understanding the mechanisms of secretion and how secretion and clearance are altered in disease is essential for the development of effective therapies for mucus clearance disorders.

## 2. Physiology of airway mucus secretion and clearance

### *2.1. Mucus secretion*

Mucus is a heterogeneous, adhesive, viscoelastic material (gel) secreted as a product of surface mucous or goblet cells and mucous cells of the submucosal glands. Transepithelial and serous cell secretions are thought to add to this mixture. The viscoelastic properties of mucus are largely due to elongated glycoproteins called mucins. The two principal gel-forming mucins of the airway are MUC5AC, principally a product of goblet cell secretions, and MUC5B thought to be primarily secreted by the submucosal glands [2,3]. The mucin monomers are crosslinked 'end-to-end' by disulfide bonds between amino and carboxy termini. Mucins are glycosylated in the endoplasmic reticulum and mucin oligomers are condensed in secretory granules. Upon exocytosis these rapidly swell into a

tangled network. This swelling is driven by Donnan equilibrium and reptative diffusion [4]. As a charged polyionic gel, the mucin disperses to a balance dictated by free energy, producing an entangled gel of moderate viscosity and elasticity. In cystic fibrosis, the mucins packed inside the secretory granules may have a decreased capacity to swell from the condensed phase inside the secretory granules, to an expanded gel phase due to the lack of fluid secretion by the serous cells, leading to decreased mucus transportability and accumulation in the airway lumen [5].

Viscosity or energy loss is a property of all liquids and an ideal Newtonian liquid is defined only by viscosity. Therefore with applied stress, there is deformation of the liquid until the stress is removed. An ideal or Hookian solid stores all energy of an applied stress and releases this energy as recoil once the stress is removed. As a pseudoplastic gel, mucus exhibits non-Newtonian behavior in that with initial stress, energy is stored but as the stress continues the material deforms and flows like a liquid, only to have partial recovery of stored energy when the stress is removed. Both viscosity and elasticity are essential for mucus secretion and clearance. If mucus were a solid substance, it would be difficult for it to be extruded from submucosal glands and would resist spreading. If mucus had no elasticity, on the other hand, cilia would be unable to transmit kinetic energy to the mucus layer to propel it forward. In general high viscosity and high elasticity both impede mucociliary clearance [6].

Sputum is airway mucus mixed with bacteria and cellular debris often with products of inflammatory cell necrosis including DNA and filamentous actin (F-actin) polymers and inflammatory mediators. Although chronic inflammatory airway diseases such as asthma, chronic bronchitis, and cystic fibrosis (CF) are associated with hyperplasia and hypertrophy of submucosal glands and goblet cells, there are few data suggesting that there are increased proportions of mucins in the airway secretions of patients with these diseases. Indeed in CF, there may even be decreased mucin secretion, with the DNA and F-actin network providing the principal polymer structure [7]. Furthermore, alterations in the polymeric structure of submucosal gland derived MUC5B mucin has been demonstrated in airway casts taken from a patient dying of asthma [8]. This abnormal branching structure of the MUC5B mucin could account for the profound increase in viscosity of asthma secretions compared to sputum of patients with other airway diseases.

There are complex and interrelated mechanisms controlling mucus secretion in the airway. There is presumably a baseline level of mucus secretion and clearance necessary for airway hygiene, although the exact volume of these secretions and regulation of mucus homeostasis in health is less clearly defined than in disease. Mucus secretion can be induced not only by a variety of externally administered secretagogues but also by products of inflammation. Serine proteases such as neutrophil elastase are potent secretagogues and reactive oxygen species, secretory phospholipases, and arachidonic acid metabolites, particularly prostaglandin F2$\alpha$ and the cysteinal leukotrienes are potent secretagogues [9].

Mucus is also under neurogenic regulation with muscarinic stimulation increasing mucus secretion primarily by the M3 pathway [10]. Products of the nonadrenergic, noncholinergic (peptidergic) nervous system are also potent secretagogues. In particular, substance P and neurokinin A can rapidly induce mucus secretion. Many of these secretory pathways involve the epidermal growth factor receptor (EGFR) [11] or ion channel signaling. The purinergic agonists ATP and UTP are capable of stimulating mucin secretion via the P2Y2 pathway [12] and activation of the CRCX2 chloride pathway may also be a significant factor in hypersecretion in patients with bronchitis or asthma [13].

The overexpression of sialyl-Lewis X determinant in the mucins of severely CF infected patients suggests that differences in glycosylation process may occur in CF and that bacterial infection influences the expression of sialyl and fucosyltransferases in the human airway mucosa [14]. Moreover, mucin-sulfatase activity of *Pseudomonas aeruginosa* may also contribute to its association with CF airway infection. Among the bacterial exoproducts, *P. aeruginosa* lipopolysaccharide is able to upregulate mucin gene transcription via the activation of NF-$\kappa$B [15].

### *2.2. Mucociliary clearance*

Airway mucus is cleared by two major mechanisms—mucociliary clearance and airflow interac-

tion; the latter assumes increasing importance as lung disease develops [16]. According to model studies mucociliary clearance is critically dependent on maintaining the depth of periciliary fluid [17,18]. The normal daily volume of respiratory secretion arriving at the larynx has been estimated to be about 10 ml/day. The total production of airway surface fluid throughout the airway system is not known, but is probably much higher than the volume actually reaching the larynx; this apparent reduction in airway fluid volume is believed to be due to absorption of water through the lower bronchi via active ion transport mechanisms [5].

Effective mucociliary clearance requires transmitting kinetic energy from beating cilia to the mucous layer. This requires that there be a discrete interface between mucus and cilia, that the cilia have appropriate power (defined by the force and length of the stroke contacting the mucus), adequate ciliary beat frequency, and mucus with biophysical properties allowing it to deform and store energy and then release that potential energy as kinetic energy for transport. Cilia beat in a low viscosity periciliary fluid layer that appears to be regulated in depth and composition by transepithelial and pericellular ion and water transport. Evidence strongly suggests that this periciliary fluid layer is separated from the mucus layer by surfactant phospholipids serving to prevent mucus infiltration of the periciliary fluid and to allow effective transmission of energy from beating cilia to the mucus without entanglement [19].

With inflammation there is cellular loss, loss of ciliary function, destruction of the surfactant layer by airway phospholipases, and alteration of the biophysical properties of secretions. When this is severe and prolonged, particularly in association with mucus hypersecretion, cough or airflow clearance mechanisms become more important and with severe disease, these can become the primary means of airway secretion clearance.

### *2.3. Cough clearance*

Cough or airflow dependent clearance is most effective with high expiratory cough flow (the product of volume and time), especially when there is high peak cough flow to detach (shear) secretions from the epithelium and when the secretions are not too adherent the airway epithelium. Theoretically, when expiratory cough flow and secretion adhesivity are held constant, secretions will be better cleared if they have lower viscosity [20]. This assumes that cough flow is sufficient to detach secretions in bulk from the epithelium and move them in bulk up the airway. In many cases, sputum viscosity and adhesivity appear to be linked. Potentially mucolytics can serve to free adherent secretions from the epithelium. In general, however, therapeutic interventions that increase expiratory airflow including bronchodilators, chest physical therapy, and improved nutrition will all increase cough clearance.

The depth of mucus and the airflow linear velocity are critical determinants of cough clearance [21]. Mucus physical properties that are important to cough clearance are the viscosity of the mucus, the elastic component, which impedes forward motion and results in recoil after the cough event, cohesivity which allows mucus to hold together, and the surface properties, both on the air–mucus interface, as well as at the interface with the periciliary layer [22]. Mucus that is elastic may be efficient in mucociliary clearance, but it is inefficient in cough clearance [20], and thus a dynamic balance between mucus viscosity and elasticity may be determined by nature.

### *2.4. Mucus viscoelasticity*

There are many factors that contribute to the viscoelasticity of mucus. Among these are the type of mucus glycoprotein, the hydration of the secretions, and the degree of mucus crosslinking and entanglement. The latter, in turn, is influenced by the pH and ion content of the secretions, as well as the presence of inflammatory mediators and enzymes. Mucolytics reduce viscosity by disrupting polymer networks in the secretion (Fig. 1). Classical mucolytic agents work through the severing of disulfide bonds, binding of calcium, depolymerizing mucopolysaccharides and liquefying proteins. Newer, peptide mucolytics degrade pathologic filaments of DNA and actin.

Breakage or reduction of the bonds within the mucous gel can be achieved through disruption of the gel network—the process known as mucolysis. Mucolysis can be achieved either through physical intervention, such as high-frequency oscillation [23],

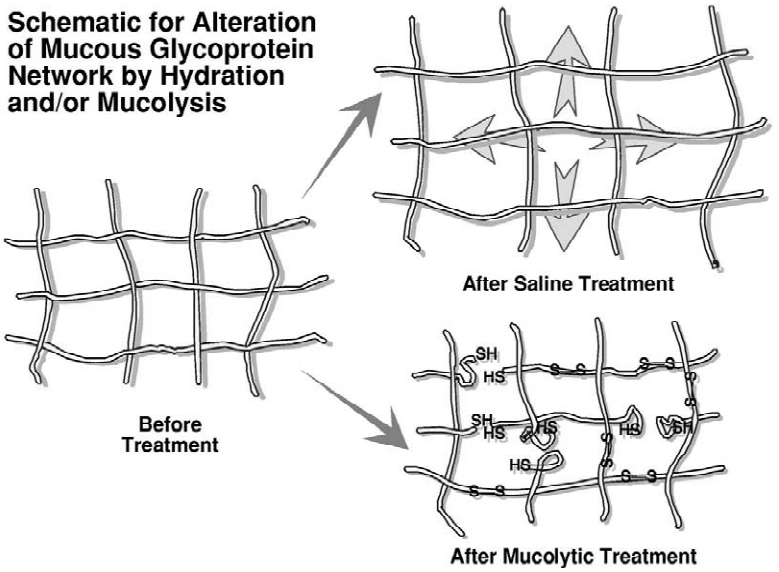


Fig. 1. Two approaches to mucolysis: Direct acting mucolytics work by disrupting network bonds; indirect mucolytic action involves rearrangement of the crosslink network.

or by biochemical or pharmacological agents (also referred to as mucotropic agents), such as *N*-acetylcysteine or rhDNase. Thus, by breaking the macromolecular bonds, mucolysis will result in reducing the viscoelasticity of the mucous gel. If this process is carried out to the right extent, it will facilitate mucus clearance for the patient.

Alternative strategies for reducing the concentration of crosslinks in the mucous gel (see Fig. 1) include hydration or swelling, an attractive goal in theory, but difficult to achieve, and rearrangement of the macromolecules to reduce their size and degree of interpenetration, as with hypertonic saline or oligosaccharide treatments (see below).

### *2.5. A molecular basis for mucolytic therapy*

Mucus consists primarily of water and is thought to have only 5–7% solid material consisting principally of mucins but also containing secreted antimicrobial proteins and peptides, phospholipids, and particulate and cellular debris. Mucins are large glycoproteins that are expressed in two forms; the secreted gel-forming mucins that form the mucous gel layer, and membrane tethered mucins present on the epithelial surface that may act as cell surface receptors. Mucin glycoproteins range in size from several hundred to several thousand kDa. Most of the molecular mass of the glycoprotein consists of the oligosaccharide sugars which link to the serine and threonine rich protein core called apomucin. Although more than 20 mucin genes have been identified with at least nine of these expressed in the airway, the principal gel-forming mucins that comprise the airway mucous gel are MUC5AC and MUC5B—the former being primarily a product of the surface goblet cells [24] and the later mostly secreted from the submucosal glands. The glycosylated mucin proteins form a gel by linearly polymerizing as mucin oligomers resulting in very long and extended molecules which then form a tangled network. This tangled network produces a gel with fairly low viscosity and elasticity permitting it to be easily secreted and cleared by cilia [23,25] (Fig. 2).

As indicated in the diagram, the three-dimensional structure that forms the mucous gel is dependent upon a number of forms of bonding. The main elements include the following: (1) disulfide bonds—these covalent links join glycoprotein subunits into extended mucin oligomers. (2) Because of their extended size, these mucin polymers readily form entanglements with neighboring macromole-

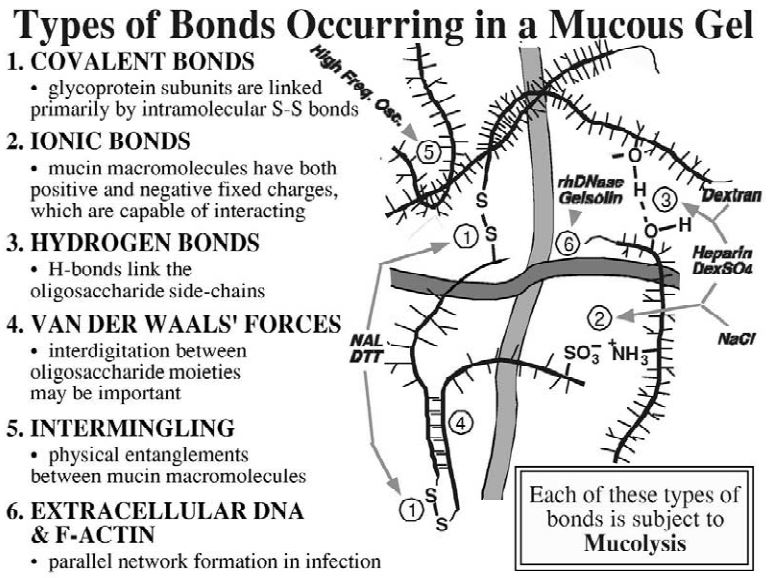


Fig. 2. Types of bonds occurring in a mucous gel, illustrating potential targets for mucolytic therapy.

cules; these act as time-dependent crosslinks, which are susceptible to mechanical degradation. (3) The sugar units that make up the oligosaccharide side-chains (about 80% of the mucin weight), form hydrogen bonds with complementary units on neighboring mucins. Although each bond is weak and readily dissociates, the numbers of bond sites make this type of bonding potentially very important. (4) Much stronger bonds due to van der Waals' attractive forces can potentially occur between complementary saccharide moieties on neighboring chains. However, the diversity of mucin oligosaccharides [26] may make such strong interactions uncommon. (5) Mucins are also ionized, containing both positively charged amino acid residues as well as negatively charged sugar units, principally sialic acid and sulfated residues. These increase in airway disease in general; in CF the proportion of sulfated residues is particularly elevated [27]. The ionic interactions between fixed negative charges result in a stiffer, more extended macromolecular conformation, effectively increasing the polymer size and adding to the numbers of entanglements. (6) Added to this in airway diseases characterized by infection and inflammation, especially CF, are the extra networks of high molecular weight DNA and actin filaments released by dying leukocytes, exopolysaccharides secreted by bacteria, and glycosaminoglycans such as chrondroitin sulfate [28].

## 3. Mucoactive medications

### *3.1. General approaches to mucolysis*

To change the physical properties of the viscous and rigid mucous gel, the direct strategy is mucolysis. Mucolysis refers to the disruption of the mucous gel, generally by altering the degree of crosslinking or the interactions between the macromolecules that form the gel. Normally it is desirable to reduce the crosslinking and viscoelasticity in the mucous gel in order to improve clearance, but occasionally, the mucus will be too thin for effective transport [29,30]; hence, increasing the crosslinking of the mucus by a mucospissic agent could be appropriate. Mucolytics and mucospissics are known collectively as mucotropic agents [1].

### *3.2. Direct-acting mucolytics*

The most important form of crosslinking in the mucous gel is due to the physical entanglements between neighboring mucin macromolecules as their

broadly coiled spheres interpenetrate at the usual mucin concentrations (ca. 1% by weight). A typical mucin molecule (molecular weight 2–3 million Daltons) in aqueous isotonic medium is a random coil, extended, fuzzy sphere about 400–600 nm in diameter (radius of gyration ca. 250 nm) [31], and at 1% concentration by weight ($4.3 \times 10^{-6}$ M), the center of each molecule lies about 70–75 nm from its nearest neighbors. Hence mucins at physiological concentration exist as highly interpenetrating polymer coils, and the main form of crosslinking is through intermolecular entanglements that act as time-dependent crosslinks (Fig. 3).

The basic mucin gene-products consist of long, heavily glycosylated peptide units with shorter segments of largely nonglycosylated units containing cysteine residues [2,31]. Mucins are generally secreted as oligomers, held together by –S–S– bridges derived from unpaired cysteine units near the nonglycosylated C-terminus end of the mucin peptide [32,33]. Classical mucolytics, such as *N*-acetylcysteine [34] and other thiol reducing agents, degrade the three-dimensional network that forms the mucous gel by breaking macromolecular backbone units that hold the polypeptide core together. For example, by reducing the S–S bridging unit that polymerizes a mucin dimer, the average length of the coiled mucin polymer is reduced by half, reducing its sphere of influence by perhaps a similar amount, and greatly decreasing the degree of entanglement with its neighboring macromolecules.

### *3.3. Classic mucolytics: N-acetylcysteine and related compounds*

*N*-acetylcysteine (NAC), a derivative of cysteine, is a thiol reducing agent. NAC has been widely used as a mucolytic agent in many countries, although not in the US or Canada. It has been reported to reduce the viscosity of purulent sputum in both cystic fibrosis and chronic bronchitis patients [35], thus

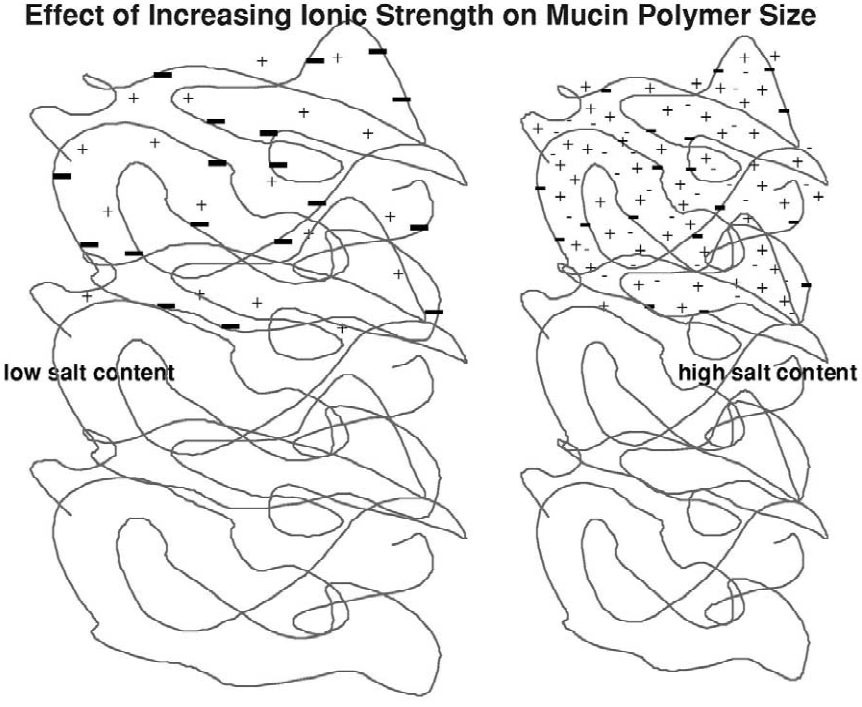


Fig. 3. A model for intermolecular penetration in mucin gels: In the example shown, the mean size of each of the three polymer coils is decreased by the addition of salt, which shields the fixed charges along the polymer backbone. This decreases the degree of penetration into neighboring molecules, thereby reducing the number of gel-forming crosslinks.

enhancing the removal of pulmonary secretions by ciliary action or cough.

Physical changes in the bronchial glycoprotein with NAC are the result of thiol reduction; they are associated with reduction in molecular size, sedimentation coefficient, and viscosity [36]. NAC reduces the disulfide bond (S–S) to a sulfhydryl bond (–SH), which no longer participates in crosslinking, thus reducing the elasticity and viscosity of mucus [37,38]. NAC demonstrates similar effects on both purulent and nonpurulent sputum. Other in vitro studies reported increasing mucolytic activity of NAC in solutions over the pH range of 5.5 to 8.0 [34]. These mucolytic properties of NAC were tested and confirmed in in vitro experiments, where treatment with NAC resulted a reduction in the elastic shear modulus of canine tracheal mucus [39].

Nevertheless, NAC, with a p$K_a$ of 2.2, has disadvantages, particularly in the area of topical or aerosol administration. It is difficult for the drug to reach more peripheral airways or poorly ventilated areas in therapeutic concentrations; also there is the risk of bronchospasm in hyperreactive patients. NAC can be too mucolytic in its action: it can overliquefy the mucus in central airways yet underliquefy the mucus in the periphery, both instances leading to sub-optimal clearability [40].

Recently, the pH-neutral lysine derivative of NAC, nacystelyn (NAL), has been shown to reduce mucus viscoelasticity, both in dogs [41] and in patients with cystic fibrosis [42,43]. In dogs, nacystelyn MDI administration led to a decrease in mucus viscoelasticity and an increase in tracheal mucus velocity, and was accompanied by an increase in mucus chloride content, consistent with improved hydration of the airway surface fluid [41], presumably due to its lysine component [44].

### *3.4. Dornase alfa, gelsolin*

Sputum contains products of inflammation including cellular debris, neutrophil derived DNA, and filamentous actin (F-actin). DNA and F-actin are colocalized in sputum to form a rigid network entangled with the mucin gel [7]. Peptide mucolytics degrade these abnormal filaments, leaving the parallel glycoprotein network relatively intact. This is illustrated schematically in Fig. 4.

#### *3.4.1. Dornase alfa*

High concentrations of undegraded DNA (up to 15 mg/ml) have been shown to be the leading cause of the tenacious and viscous nature of the sputum [45]. Although there is no direct relationship between the concentration of DNA in sputum and sputum viscosity, the addition of exogenous DNA to sputum

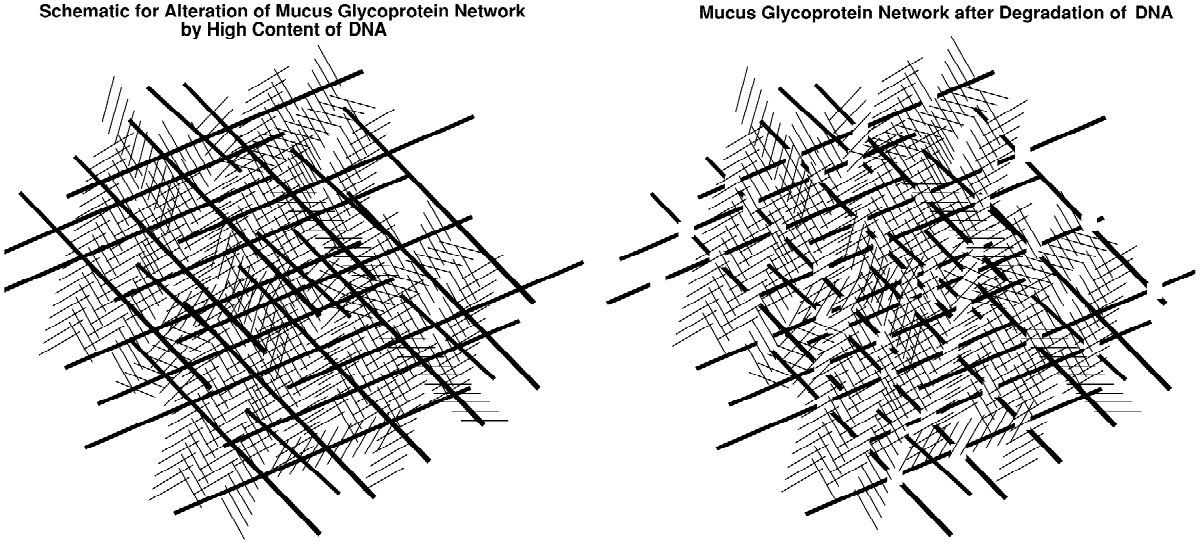


Fig. 4. A model to describe the action of DNase at reducing the excess viscoelasticity in cystic fibrosis mucus.

increases both viscosity and elasticity [46]. Dornase alfa (Pulmozyme) is the recombinant human enzyme DNase I. Dornase alfa reduces sputum viscosity [47] and tenacity [48]. This reduction in viscosity of sputum is associated with a decrease in the size of the DNA molecules located within the sputum. In theory, dornase reduces only the excess viscoelasticity, leaving the glycoprotein gel intact (quasinormal), as illustrated in Fig. 4. Hence, it is believed not to overliquefy mucus, although this could happen in some circumstances [49].

Aerosol administration of dornase alfa to patients with stable CF lung disease and preserved airflow (FEV1 > 30% predicted) improved FEV1 by 5–7%, reduced the frequency of pulmonary exacerbations, and improved measured quality of life [50]. At present, dornase alfa is approved for the treatment of cystic fibrosis and is the only FDA-approved mucolytic medication available for prescription in the US.

#### *3.4.2. F-actin depolymerizing agents*

Actin is the most prevalent cellular protein in the body, playing a vital role in maintaining the structural integrity of cells. Under proper conditions, actin polymerizes to form filamentous actin (F-actin). Extracellular F-actin has been shown to contribute to the viscoelasticity of expectorated CF sputum [51]. Concentrations of F-actin in CF sputum as high as 0.15 mg/ml have been reported, and there is an increased ratio of filamentous (F-) to globular (G-) actin in CF sputum when compared to normal secretions.

Gelsolin is an 85 kDa (577 nucleotide) actin-severing peptide that has been demonstrated to significantly reduce the viscosity of CF sputum at low shear rate [51]. Thymosin β4 is a small (4.8 kDa, 43 nucleotide) peptide that binds G-actin both inhibiting the formation F-actin and shifting the polymerization kinetics to promote the rapid depolymerization of F-actin [52]. Dornase alfa also binds G-actin and in the process, its DNA enzymatic activity is blocked. In vitro studies with both gelsolin and thymosin β4 demonstrate that these agents are synergistic with DNase such that a greater reduction in sputum viscoelasticity and cohesiveness is seen with smaller amounts of these agents [51,53]. Thus F-actin depolymerizing agents both destabilize the actin-DNA filament network as well as increase the depolymerizing activity of dornase on the DNA filaments.

DNase and gelsolin in CF sputum act in principle in a similar fashion to NAC. The mucus gel is strengthened by the addition of undegraded DNA and F-actin, acting as a reinforcing filler. The contribution of such units to viscoelasticity is highly molecular weight dependent, varying by a factor as high as $M^{3.4}$ [54,55]. Hence a reduction in molecular weight of just 20% (one break per four molecules on average) could result in a decrease in elasticity by 50% (factor of 2), which would be meaningful in terms of clearability [6].

### *3.5. Non-destructive mucolysis: ionic and hydrogen bonds*

The other target areas illustrated in Fig. 2—the ionic interaction and the hydrogen bonds—have received less attention, but are perhaps no less promising. DNase, gelsolin and acetylcysteine derivatives are all similar in action in that they degrade the three-dimensional network by mucolysis, or macromolecular size disruption, as shown schematically in Fig. 1. This tends to preferentially affect the elasticity components of the network (as opposed to viscosity), which in model studies improve cough or airflow clearance more than clearance by ciliary action [20]. Agents that affect ionic charge interactions and hydrogen bonds, on the other hand, are not true mucolytic agents because they alter the crosslink density without reducing polymer chain length, the result of which is a common reduction in both elasticity and viscosity, and a preferential improvement in ciliary clearance according to model studies [56]. Indeed, recent laboratory studies support the 'non-mucolytic' approach to improving mucus rheology for ciliary clearance, both with ionic agents such as sodium chloride [55,57], or nonionic agents such as dextran [58].

#### *3.5.1. Hypertonic saline/dry-powder mannitol*

Hypertonic saline, HS, is not a true mucolytic treatment, but may act in part by effectively reducing the entanglements in the mucus gel. As suggested by Fig. 3, hypertonic saline breaks up the ionic bonds within the mucin gel, thereby reducing the effective

degree of crosslinking and lowering the viscosity and elasticity [55,57]. Hypertonic saline is also believed to promote mucus clearance by extracting fluid from the respiratory tract epithelium, thereby increasing the volume and water content of mucus [59,60]. Upon administration of hypertonic saline by means of a nebulizer, water from the tissues may be shifted across the epithelial membrane to dilute the saline. The diluted fluid is then eliminated from the respiratory tree by the mucociliary escalator or by coughing. Dry-powder mannitol, an osmolyte discussed below, may act by a similar mechanism.

In infected mucus, hypertonic saline separates the DNA molecules from the mucoprotein, making the mucoprotein susceptible to proteolytic enzyme digestion. Lieberman [61] reported that the cleavage of DNA by hypertonic saline increased as the ionic strength of the solution increased beyond 0.15 M. In 1978, Pavia et al. [62] investigated the effect of hypertonic saline on chronic bronchitis patients. They found that, in comparison to normal saline, hypertonic saline (1.21 M) doubled the rate of mucociliary clearance with an increase in the weight of sputum expectorated. Recently, hypertonic saline has been found to enhance the removal of lung secretions in patients with cystic fibrosis [63,64]. This stimulation of mucociliary transport by saline solutions occurs in both normal individuals and chronic bronchitis patients.

HS may also function to stimulate mucociliary and cough clearance by an osmotic effect, drawing water into the mucous gel and the periciliary layer. This is clearly a short-term effect, since the epithelium has the capacity to quickly restore the salt balance. Even the co-administration of amiloride to inhibit Na-linked water resorption, resulted in no additional short-term clearance benefit for CF patients [64]. Perhaps longer-acting Na-channel blockers might prolong the effects of inhaled osmolytes, and prove beneficial.

### *3.5.2. Oligosaccharide mucolytic agents*

Agents that are capable of altering the hydrogen bonding of the mucous gel can produce potentially beneficial effects on mucus clearability. Although each bond is weak, the total potential for hydrogen bonds is immense, since the oligosaccharide side-chains make up about 80% of the mucin structure. This was found to be the case with dextran [58], a compound that can also block bacterial adhesion [65]. Other laboratories have reported stimulation of clearance with simple mono- or disaccharides such as mannitol and lactose [66,67], and in the case of mannitol, administration by dry-powder aerosol resulted in an improvement in mucociliary clearance rate [66,68]. In the study by Feng et al. [58], low molecular weight dextran treatment in vitro significantly reduced the viscoelasticity and spinnability of both CF sputum and healthy dog mucus, and increased the predicted mucociliary and cough clearability in a dose-dependent fashion. In dogs, dextran administered by aerosol at 65 mg/ml increased tracheal mucus velocity, but this increase was not sustained for higher concentrations [69].

These changes in mucus rheology and transportability have been attributed to disruption of network crosslinks due to hydrogen bonds between neighboring mucin macromolecules. It has been observed that the fluidifying or mucoactive effect of dextran on mucus is primarily confined to the lower molecular weight fractions of dextran [70], consistent with the concept that dextran does not involve true mucolysis (mucin degradation), but rather the creation of mucin-dextran hydrogen bonds that are structurally and rheologically ineffective. There are potentially two mechanisms to account for the mucokinetic effects of saccharide compounds. An osmotic mechanism, attracting fluid into the airway milieu similar to that proposed for ionic treatments [60], could account for the effects on mucus clearance. This would presumably be maximal for monosaccharides, and decrease in importance with increasing molecular size. On the other hand, the H-bond breaking mechanism is probably optimal for saccharide units matching the length of mucin oligosaccharides, i.e. 3–15 units [71]. The reduced crosslinking of the mucous glycoprotein network should make the mucus more easily clearable by both ciliary and cough mechanisms.

Recently, we have looked at agents that might alter both the hydrogen bonds and the ionic interactions. Our initial studies found that one such agent—the charged oligosaccharide, low molecular weight heparin—had a greater mucolytic and mucokinetic capacity than the neutral saccharide polymer, dextran. This was seen in in vitro rheological testing

[72] and in excised frog palate clearance measurements [73]. Subsequently, similar mucolytic effects were seen with aerosolized low molecular weight dextran sulfate, which significantly reduced tracheal mucus viscoelasticity in anesthetized dogs [74]. Heparin and dextran sulfate, as charged oligosaccharides, may combine the features of hydrogen bond disruption, as seen with low molecular weight, neutral dextrans, and improving ionic interactions, as with hypertonic saline [75]. Aerosolized low molecular weight heparin also shows promise as an antiasthmatic medication, presumably by interfering with antigen-receptor binding [76], and as an antiinflammatory therapy in CF, reducing the content of neutrophil elastase in sputum [77] and decreasing serum IL-8 [78].

Charged oligosaccharides are believed to decrease mucus viscoelasticity by: (1) interaction of their negative charges with the amino groups of the mucin molecule, thereby reducing their association with neighboring mucin sulfate or sialic acid moieties; and/or (2) interfering with intermolecular hydrogen bonding due to their oligosaccharide moieties (similar to dextran); and/or (3) ionic shielding effects of mobile counterions (principally sodium) on the polyionic moieties of the mucin molecule. Charged oligosaccharides may also stimulate the movement of ions across the epithelium, thereby increasing hydration of the airway surface fluid [60], similar to the action of chloride secretagogues [41].

### *3.6. Interaction between mucoactive agents*

Each of the elements illustrated in Fig. 2 is a potential target for mucoactive therapy. The most successful therapy in CF, and the only mucoactive agent with proven efficacy, is dornase alfa, which has been found to improve lung function in a broad spectrum of patients [50]. Its target, of course, is the excess DNA characteristic of CF sputum, and its attractiveness as a therapy is that it does not affect the normal glycoprotein network, meaning that it cannot overliquefy the mucus, at least in theory. However, even with such advances in mucolytic therapy, there is plenty of room for improvement. One area of great potential is the development of combination therapies, since no single mucoactive therapy may be appropriate for all patients or even for individual patients during different stages of their disease. Also, with combination approaches, there is the potential for synergism of action.

Laboratory studies have shown that dornase alfa and mechanical oscillation have additive effects on sputum rheology [79], and clinical studies have been designed to examine the interaction in vivo. Since oscillations may serve to both rearrange crosslinks, and reduce polymer size, the interaction between the methods may be synergistic. In other laboratory studies, dornase alfa has also been combined with gelsolin to break up the actin network colocalized with the DNA [51], and with nacystelyn to degrade disulfide bonds [80,81], in both cases with additivity or synergy of effect. Actin also inhibits the DNA-hydrolyzing activity of rhDNase [82]; therefore by binding actin, the action of dornase alfa in CF sputum may be enhanced. As further mucolytic treatments are developed, their interaction and their potential for synergy need to be examined. Ultimately, the goal is to tailor mucolytic prescriptions to suit the needs of individual patients, and to complement their other therapy.

## 4. Novel therapeutic approaches

The therapeutic approaches discussed in this section are based on a broad understanding of mucus secretions and clearance. While some of these approaches are theoretical, others are practical extensions of existing therapeutic approaches and some are being actively developed as potential therapies.

### *4.1. Mucoregulation—decreasing abnormal volume of mucus secretion*

Chronic inflammation is a potent stimulus for hyperplasia and hypertrophy of secretory cells and mucus hypersecretion. In many cases this can be managed by using broadly effective antiinflammatory agents such as corticosteroids to decrease the inflammatory response and antibiotics to decrease the infectious burden in the airway.

There are several pathways responsible for mucus secretion and hypersecretion and in many cases these pathways are redundant. In allergic disorders and

asthma there is thought to be activation of TH2 lymphocytes leading to production of a specific spectrum of cytokines including IL4, IL5, IL9, and IL13. Of these TH2 cytokines, IL13 appears to be a potent mucus secretagogue that may be involved in the secretion of the abnormal submucosal gland (MUC5B) secretions associated with acute severe asthma [83]. Thus this would be an attractive therapeutic target in patients with asthma or allergies.

Many of the mucus secretory pathways appear to be activated through the epidermal growth factor receptor (EGFR), which can be inhibited by protein kinase inhibitors such as Iressa. Other inflammatory pathways implicated include the M3 muscarinic pathway which can be potentially blocked by M3 specific anticolonergic agents such as darifenacin, neurogenic pathways such as capsaicin which can be inhibited by opioids, the calcium activated chloride channel (CaACC) pathway and the serine protease inhibitors such as anti neutrophil elastase. CXCR2 antagonists, P38MAP kinase inhibitors, and EGFR antagonists can also inhibit neutrophil dominated inflammation.

Although it is intuitively expected that hypertrophy and hyperplasia of the secretory glands and cells would lead to hypersecretion of mucin, there are few data that have conclusively demonstrated increased mucin in airway secretions and there are some data that suggest that there may be decreased mucin secretion particularly in CF [84]. Therefore efforts to decrease the amount of mucin may not have a great effect on disease progress. Nevertheless the volume of airway secretions is markedly increased in the CF airway and it is thought that this is largely due to highly polymerized DNA and F-actin resulting from inflammatory cell necrosis. Efforts to decrease DNA and actin polymers using depolymerizing enzymes such as dornase alfa have been successful at reducing the frequency of exacerbations and improving pulmonary function in persons with CF. Although DNA and F-actin are not actively secreted, the amount of these polymers in the airway can be decreased by regulating cell death. Usually neutrophils are recruited to the site of inflammation and undergo programmed cell death (apoptosis) with resolution of inflammation. Part of the apoptotic process is degradation of intracellular DNA and actin, and if the cells undergo a necrotic death, their polymers are released into the external milieu. Thus a potential target for reducing DNA and actin within the airway would be to induce the apoptotic death of neutrophils as a means of resolving the chronic inflammatory state.

There is a great deal of interest in the use of the 14- and 15-member macrolide antibiotics as biologic response modifiers for the treatment of chronic inflammatory airway disease [85,86]. First documented to be highly effective in reducing airway inflammation, mucus hypersecretion, and morbidity in patients in Japan with diffuse panbronchiolitis, subsequent studies have shown similar effectiveness in the treatment of chronic sinusitis and chronic bronchitis in Japan and several small studies in Europe and North America have shown similar efficacy for the treatment of CF. Macrolide antibiotics appear to have a variety of effects reducing mucus hypersecretion, decreasing airway inflammation, and preventing cell membrane damage [87]. Many of these effects appear to be mediated through inhibition of the nuclear transcription factors NFkB and AP1. Although the 14- and 15-member macrolide and azilide antibiotics demonstrate this property, the 16-member macrolide antibiotics appear to lack immunomodulatory properties.

### *4.2. Medications that improve the cough clearability of secretions (mucokinetic agents)*

With chronic airway inflammation and mucus hypersecretion cough clearance becomes and important means for airway hygiene. Cough clearance is dependent on the power of expiratory airflow, cohesivity of secretions, and a lack of adhesion between the airway epithelium and the secretions. Thus cough clearance can be improved by increasing expiratory airflow and decreasing the stickiness of secretions. It is thought that with many chronic airway diseases there is destruction of the surfactant layer that normally allows secretions to move freely up the airway and there are data that suggest that surfactant delivered as an aerosol can significantly improve pulmonary function in patients with chronic bronchitis [88] and perhaps in patients with CF.

*4.2.1. Agents that depolymerize mucin (classic mucolytics) or DNA and F-actin polymers (peptide mucolytics)*

The molecular basis for mucolytic theory and presently used mucolytic agents has been discussed earlier and what follows are considerations for further development. Efforts to depolymerize the mucin network may be of limited benefit in patients with CF where the predominant airway polymers are DNA and F-actin. At worst this may be contraproductive. With increased mucus sulfation and decreased pH in the CF airway, there are potentially greater charge interactions and condensation of the mucin network such that although the *volume* of airway mucin is no greater, the coiling of the tangled network is tighter, leading to increased viscoelasticity perhaps beyond the optimal for transport. Therefore agents that can increase the hydration of secretions, increase the pH of secretions, or 'open up' the mucin network by charge shielding may have potential for improving the properties of CF airway secretions. Agents currently under investigation include heparin sulfate and low molecular weight dextran as well as agents that improve airway hydration such as P2Y2 agonists (e.g. UTP, INS365), and osmotic agents that may draw water into the airway secretions such as hyperosmolar saline or dry powder manitol inhalation.

DNA polymers are effectively degraded by the inhalation of dornase alfa as discussed earlier; this effect can be potentated by the addition of actin depolymerizing agents such as gelsolin or thymosin beta4. Just as the DNA and F-actin appear to copolymerize in the airway, drugs that attack both DNA and actin polymers appear to act synergistically in the depolymerization process. Stabilization of these peptides to prevent airway degradation could potentially prolong their useful life in the airway and increase their effectiveness. Similarly the ability to penetrate deeper into the airway is potentially of great advantage to patients with severe bronchiectasis and mucus inspissation provided that there is sufficiently preserved expiratory airflow to clear secretions as they are liberated from the airway.

An exciting avenue for further research is the interaction and the potential for synergy between the various mucoactive agents, as well as potential interactions between medications and physical means of airway secretion evacuation, as provided by chest physical therapy maneuvers and airway clearance devices. Just as it is unlikely that a single therapeutic agent will be most effective for all patients with a specific disease it is probable that combination therapy can be tailored to an individual patient's disease and disease severity.

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