Towards a better mucolytic

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MUC-031 improved biophysical properties of CF mucus *ex vivo*, and cleared mucus plugs and decreased airway inflammation in the mouse muco-obstructive lung disease model of airway surface liquid depletion. https://bit.ly/42QBIMz

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Airway mucus provides an important defence against inhaled particles and pathogens [1, 2]. The essential molecules making up mucus are water (98%), salts (1%), and mucin glycoproteins (0.3%), with miscellaneous non-essential molecules comprising the remainder (0.7%). Despite their presence at such a low concentration, mucins impart the physical properties of mucus because their massive sizes and high sugar content (~80% of their mass) results in formation of a water-avid molecular network. Individual mucin monomers are themselves very large (molecular mass ~1 million Da, compared to 66 kDa for albumin), and they polymerise into linear chains of tens of monomers.

The polymeric nature of mucin assembly causes mucus to behave as a polymer gel, such that biophysical properties are exponentially affected by changes in mucin content (3rd to 8th power scale of mucin concentration). Accordingly, there is great interest in determining how mucin polymerisation may be targeted to improve mucus viscoelasticity when mucin concentration or crosslinking are excessive. The enormous mucin glycopolymers are held together by disulfide bonds, and it has been known for many years that reducing agents, such as dithiothreitol and *N*-acetylcysteine (NAC,) can turn thick mucus into a thin liquid. Such chemicals are termed mucolytics, and introducing one that is safe and effective into the airways of patients with muco-obstructive lung diseases (MOLDs) is a longstanding dream of pulmonary physicians [1, 3–5].

Chronic MOLDs are highly prevalent, with asthma and COPD both present in ~8% of North American and European adult populations, and bronchiectasis including cystic fibrosis (CF) present in smaller numbers [6]. In addition, short-term mucus dysfunction is a common and troubling occurrence in viral respiratory infections [7]. The mechanisms of mucus obstruction differ among these diseases, though there are some common principles [1, 2, 6]. Healthy mucus has the thin consistency of uncooked egg whites, and is rapidly cleared by ciliary beating or cough along with entrained particles and pathogens. Pathologic mucus is more abundant, thicker and more adherent, so may not be cleared by cilia or cough, resulting in the formation of plaques and plugs within the airways.

Common mechanisms of the formation of pathologic mucus are a high concentration of mucin polymers, the formation of lateral crosslinks between mucin polymers, and the presence of non-mucin polymers such as DNA and actin from cellular debris [6, 8]. In turn, a high mucin concentration may result from upregulated mucin production and secretion, deficient salt and water secretion, or both [6, 9, 10]; the formation of disulfide mucin crosslinks may result from factors that rearrange existing disulfide bonds *via* production of redox enzymes and/or reactive oxygen species by lung epithelial cells or leukocytes, or high inhaled oxygen concentration [8, 11, 12]; and DNA and actin are released from degenerating leukocytes and lung structural cells during infection and injury. Therapies addressing all these mechanisms are available or in development [1, 3–5, 9], including cytokine-targeting antibodies to reduce mucin production, hydrating agents such as hypertonic saline solution and mannitol, and rhDNase to sever DNA

polymers. However, the reduction of pathologic mucin crosslinks with a mucolytic, while particularly attractive in theory, remains a work in progress.

NAC is the only US Food and Drug Administration-approved inhaled mucolytic. However, it has low potency so does not effectively improve pathologic mucus clearance, improve airflow, or reduce exacerbations in human subjects [13]. As such, NAC must be given at high enough concentrations that it causes bronchospasm, possibly due to its hyperosmolar formulation or generation of sulfite, and it causes epithelial injury and inflammation in mice [13, 14]. In 2019, preclinical studies of an inhaled phosphine reducing agent were reported that did not show toxicity in isolated cells or in mice, improved the biophysical properties of COPD and CF mucus *ex vivo* and of CF mucus by inhalation, and cleared mucus plugs in a mouse model of MOLD based on airway surface liquid depletion [13]. In 2021, the same compound was reported to improve the biophysical properties of asthma mucus *ex vivo*, and cleared mucus plugs, improved airflow, and reduced inflammation in a mouse model of asthma [15]. Now, Addante *et al.* [16] report the effects of a novel thiol-saccharide mucolytic in this issue of the *European Respiratory Journal*. Attractive theoretical features of such compounds are that naturally occurring sugar scaffolds can be used that are non-toxic, and their high water solubility and polar nature facilitates penetration of mucus gels. The compound chosen for study, MUC-031, improved biophysical properties of CF mucus *ex vivo*, and cleared mucus plugs and decreased airway inflammation in the mouse MOLD model of airway surface liquid depletion. Thus, two compounds with different underlying chemistries have now been found to be safe and effective in preclinical studies of mucus pathologies.

Despite the great promise of mucolytics in the therapy of MOLDs, their optimal use will require careful clinical study. A clever experiment has been reported in preliminary form in which cysteines responsible for high order mucin polymerisation were mutated in mice [17]. In homozygous mutant mice, the mucins only form dimers, while in heterozygous mutant mice, the mucins only form shorter multimers ($\leqslant$8-mers) than those in wild-type mice. Both homozygous and heterozygous mutant mice showed intrapulmonary mucus accumulation, and cultured tracheal epithelial cells from mutant mice were unable to transport mucus vertically by ciliary beating. This suggests that very large naturally occurring mucin polymers are required for mucus to have sufficient elasticity for transport against gravity [18], so mucolytics used at high doses for prolonged periods could have adverse effects.

Nonetheless, used for short periods to dissolve existing mucus plugs or at low doses to maintain mucus clearance in the setting of an established MOLD, mucolytics have the potential to offer great benefit. Indeed, efficacious protection may occur through disruption of the many other intramolecular disulfide bonds that are prevalent (>100 disulfides per mucin monomer unit), and whose disruption may weaken mucin strands without depolymerising them. Another issue is whether the two airway polymeric mucins, MUC5AC and MUC5B, can be differentially targeted. MUC5AC is expressed at high levels in allergic asthma and is primarily responsible for mucus obstruction [19], whereas steady production and secretion of MUC5B is required for baseline mucociliary clearance and lung health [20]. Therefore, selective targeting of MUC5AC with a mucolytic could offer added benefit in some MOLDs. Most importantly, major advances are being made in understanding the biology, chemistry and physics of airway mucus, and therapeutic progress is sure to follow.

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