Inhaled protein/peptide-based therapies for respiratory disease

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Abstract

Asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF) are all chronic pulmonary diseases, albeit with different etiologies, that are characterized by airflow limitation, chronic inflammation, and abnormal mucus production/rheology. Small synthetic molecule-based therapies are commonly prescribed for all three diseases. However, there has been increased interest in "biologics" to treat these diseases. Biologics typically constitute protein- or peptide-based therapies and are often more potent than small molecule-based drugs. In this review, we shall describe the pros and cons of several different biological-based therapies for respiratory disease, including dornase alfa, a recombinant DNAase that reduces mucus viscosity and short palate lung and nasal epithelial clone 1 (SPLUNC1)-derived peptides that treat Na⁺ hyperabsorption and rebalance CF airway surface liquid homeostasis.

Keywords: CFTR, Biotherapies, Neutrophil elastase (NE), Inflammation, Goblet cell metaplasia, Nebulization, Aerosolization, Omalizumab, Alpha-1-antitrypsin (AAT), Pulmozyme, Mucociliary clearance, PLUNC, ENaC, BPIFA1

Introduction

For hundreds of years, the pulmonary system has been used to deliver pharmacologically active compounds to the body [47]. The lungs allow for efficient drug delivery as they have a large surface area and are well vascularized [35]. For example, inhaled nicotine is readily absorbed across the pulmonary epithelia into the bloodstream where it can exert its psychotropic effects on the brain [5]. Conversely, for many peptides/proteins, an inability to cross the respiratory epithelium after inhaled delivery may actually be advantageous as it would result in a high ratio of lung to systemic bioavailability and thus would reduce off-target effects [25]. As a case in point, inhaled antibiotics achieve far higher concentrations with far fewer side effects than orally delivered antibiotics [55, 62]. The majority of drugs in use today are classed as “small molecules.” That is, organic chemicals typically bind to their receptor to elicit a response [41, 57]. Since these molecules are often extremely durable, until metabolized by the liver and/or cleared by the kidney, they can have side effects in other organs [22]. In contrast, biological therapeutics, including proteins (e.g., antibodies, enzymes) and peptides, show considerable promise and are emerging as alternatives to small molecule-based drugs [19]. Some protein-based therapies have failed in the clinic, since they are more labile than small molecules and are prone to proteolytic degradation in the blood [32, 39]. However, protein-based therapies show great promise for many types of respiratory disease since they can be delivered to the target organ directly by inhalation. Additionally, whilst small molecules typically have nanomolar potency, biologicals often have picomolar to femtomolar potency due to their increased ability to bind to their protein target with high affinity. This increased binding is achieved due to the ability of proteins and peptides to change their conformation during binding to better fit the binding pocket in their receptor [2]. This review concentrates on asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD), three respiratory diseases typified by airflow limitations and poor alveolar gas exchange.

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Review

Characteristics of asthma, CF, and COPD

Asthma is typified by chronic airway inflammation caused by a combination of environmental and genetic factors [44]. Symptoms include airway hyperreactivity, airway narrowing, goblet cell metaplasia/mucus hyperproduction, and eosinophilia [13, 16]. Asthma is typically treated by a combination of β-agonists and corticosteroids to relax smooth muscle and reduce inflammation, with a subset of patients being non-responsive to these medications, suggesting an unmet need for new asthma therapies [31].

CF is a multi-organ inherited disease, caused by mutations in the CF gene product, the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated anion channel [53]. The lack of functional CFTR and subsequent epithelial sodium channel (ENaC) hyperactivation result in Cl− hyposecretion and Na+ hyperabsorption, respectively, that combine to dehydrate airway surfaces [3, 12]. CF lung disease is characterized by the accumulation of dehydrated/viscous mucus, leading to chronic infection/inflammation goblet cell metaplasia, neutrophilia, and bronchiectasis [26, 38]. The positive effects from nebulization of hypertonic saline or mannitol by CF patients indicate that rehydration therapy is a viable therapeutic mechanism for the treatment of CF lung disease [14, 46].

COPD is the third leading cause of death world-wide and can have a number of different causes, with tobacco exposure being the most common [10]. COPD is typified by alveolar destruction, coughing/chronic mucus production, chronic inflammation, and protease imbalance which lead to irreversible airflow limitation and a progressive loss of lung function [30]. COPD treatments include inhaled bronchodilators and steroids [23]. In severe cases, long-term oxygen therapy is required but to date, there are no effective therapies to reverse the disease, even after smoking cessation.

Antibody therapies

Monoclonal antibodies (mAbs) are now a well-established class of FDA-approved drugs used to treat asthma (e.g., omalizumab/Xolair) [25]. Therapeutic mAbs are typically full-length IgGs that have a molecular weight of ~150 kDa [42, 52]. Unlike previous generations of mAbs, most mAbs currently used in clinical trials are fully human in origin and are produced using either transgenic animals or phage display technology, which helps to reduce immunogenicity, increase effector function, and prolong their serum half-life [9, 52]. Whilst we only highlight what we feel are the advantages and disadvantages regarding this type of therapeutic, we direct the readers to several other excellent reviews that cover this area in more detail [4, 11, 43, 58].

mAbs offer several advantages over small molecules. First, they bind with high affinity and specificity to a wide variety of proteins. Second, they are relatively stable, allowing them to remain active for long periods of time. Third, since their breakdown products are amino acids, they are not converted into toxic metabolites [8, 52]. Whilst inhalation offers an attractive route for delivery of mAbs, perhaps surprisingly, mAbs are delivered parenterally for respiratory disorders, with the inhalation route yet to make it into the clinic. However, mAbs retain their physical and immunological properties after aerosolization, suggesting that it is only a matter of time before mAb inhalation is utilized therapeutically [25, 37, 42].

When considering the route of administration for mAbs, matching the delivery route to the therapeutic target’s location is paramount. This was highlighted by studies using the mAb omalizumab to treat allergic asthma. Omalizumab is a chimeric mAb that specifically binds to and neutralizes IgE, thereby preventing its interaction with mast cells and the subsequent release of histamine and other inflammatory mediators [1]. Unlike intravenous administration, pulmonary delivery of omalizumab failed to attenuate the response to inhaled allergens in asthmatic subjects [15, 17]. The observed lack of efficacy in the aerosolization trial was likely a failure of the pulmonary route to deliver sufficient systemic omalizumab to neutralize free systemic IgE [25]. Another possible disadvantage of mAbs compared to small molecules is their molecular weight. Most small molecules are hundreds of daltons to a few kilodaltons whereas mAbs are in excess of 100 kDa, which makes inhaled delivery less efficient, but would be less of a problem for systemic delivery [51].

A final consideration regarding the use of mAbs as required therapeutics that was also illustrated by the aerosolized omalizumab trial is immunogenicity. Although inhaled omalizumab was generally well-tolerated, one test subject developed serum IgG and IgA antibodies against omalizumab. This finding led the authors to speculate that inhaling full-length mAbs may be more immunogenic than administering them parenterally [15]. However, the degree of aggregation of aerosolized omalizumab after nebulization was not evaluated, and since aggregated proteins are known to be highly immunogenic, this may have been the cause [52]. Regardless, we agree that the development of drug-specific IgG and IgA antibodies are a concern that need to be monitored and that further studies are needed to better understand the immunogenicity of inhaled mAbs.

Peptides and proteins

Short palate lung and nasal epithelium clone 1 (SPLUNC1) is a ~25-kDa protein that contains an ENaC
inhibitory domain, which for historical reasons was called the S18 region [29]. Unlike traditional ion channel antagonists which block ENaC’s pore, SPLUNC1 inhibits ENaC by inducing endocytosis [54] (Fig. 1a). Since SPLUNC1 fails to regulate ENaC in the CF lung (Fig. 1b) [21], Spyryx Biosciences is currently developing a SPLUNC1-derived peptide, which functions in CF airways as an ENaC inhibitor (Fig. 1c) [18, 59]. This restoration of CF airway surface liquid (ASL) hydration is predicted to (i) improve mucociliary clearance and (ii) decrease infection/inflammation [7, 34]. Additionally, these peptides are intrinsically disordered so they are heat stable. Another advantage of intrinsically disordered proteins/peptides is that they achieve a greater contact area with their target protein, thus maximizing binding efficiency [6]. S18-derived peptides are protease resistant, do not freely cross the respiratory epithelium, and do not reach the kidney to induce the hyperkalemia, as seen with small molecule ENaC antagonists like amiloride [27, 28]. Chronic inhalation therapy using these peptides could produce local immunogenicity and irritation, but given that SPLUNC1-derived peptides are naturally occurring in normal but not CF lungs, immunogenicity would seem unlikely [29]. A limitation of this type of therapeutic is that it would only ameliorate CF lung disease and would not treat other CF-affected organs.

Both CF and COPD airways exhibit increased neutrophil elastase (NE) activity, which has the potential to damage the lung and also to cleave and activate ENaC, exacerbating mucus dehydration and further reducing mucociliary clearance [24, 40, 45, 50]. Alpha-1-antitrypsin (AAT) is an endogenous NE inhibitor which is predicted to improve pulmonary function by blocking NE. Kamada Inc. has an inhaled biological based on human AAT, which is in phase 2 clinical trials for treatment of CF [20, 33]. A potential limitation of AAT is that in addition to NE, several other proteases including cathepsins and metalloproteases are also upregulated in CF/COPD which may also contribute to the lung damage but would not be blocked by AAT.

CF and COPD airways are characterized by high levels of DNA [49] and actin [60] in the lung lumen, which are released by necrotic neutrophils [36]. Excess DNA and actin adversely alter mucus rheology and increase viscosity, leading to decreased mucociliary clearance [48]. Therefore, another approach to increase mucociliary clearance in CF and COPD lungs is to decrease mucus viscosity by cleaving extracellular DNA. Dornase alfa is a recombinant version of human Dnase1 protein that is used as a therapeutic for CF [61]. Dnase1 cleaves extracellular DNA in the lung lumen leading to reduced DNA length/concentration and, therefore, reduced sputum viscosity. Pulmozyme is a recombinant version of human Dnase1 marketed by Genentech for the treatment of CF. Pulmozyme is administered via nebulization and has been shown to reduce the incidence of CF infections [56].

Conclusions

Biotherapies constitute the fastest growing sector of approved drugs, but their delivery via the lung remains a nascent field. It is increasingly clear, however, that inhaled biological therapeutics can offer some strong advantages over traditional therapeutics including

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**Fig. 1** Rationale for SPLUNC1-derived peptide therapy for CF lung disease. a In normal airways, bicarbonate secretion through CFTR maintains ASL pH at ~7.0. At this pH, secreted SPLUNC1 can bind to ENaC, leading to internalization and inhibition of the channel. This helps maintain airways hydration and mucus clearance. b In CF airways, the acidic ASL, caused by a lack of bicarbonate secretion through dysfunctional CFTR, causes SPLUNC1 to adopt an inappropriate conformation, where the ENaC inhibitory domain (also known as the S18 region) cannot bind to ENaC, leading to Na\(^+\) hyperabsorption and ASL dehydration. c S18-derived peptides are pH-independent and can inhibit ENaC to reduce Na\(^+\) absorption and help normalize airway hydration/mucus clearance in acidic CF airways.
increased potency, reduced systemic availability, and potentially, a longer duration of action. There are several biological drugs that are either approved or in the development pipeline, and here, we have highlighted some of which we feel are showing promise to succeed where traditional small molecules and the parenteral delivery route have failed. These examples make it clear that this is an exciting field that warrants future investigation.

Abbreviations
AAT: alpha-1-antitrypsin; ASL: airway surface liquid; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; COPD: chronic obstructive pulmonary disease; ENaC: epithelial sodium channel; mAb: monoclonal antibody; NE: neutrophil elastase; SPLUNC1: short palate lung and nasal epithelium clone 1.

Competing interests
All three contributing authors have a financial interest in Spyryx Biosciences.

Authors’ contributions
RF, ST, and RT all co-wrote and edited the review. All authors read and approved the final manuscript.

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