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Pulmonary delivery of sirna for the treatment of cystic fibrosis and pulmonary delivery platforms

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Abstract

Cystic fibrosis (CF) is one of the most deadly diseases of lungs that involves symptoms such as breathing difficulties, coughing and lung infection. Despite important therapeutic advances, the definitive treatment for CF remains elusive. CF is a good candidate for gene therapy because it is relatively common, lethal and monogenic and it does not have adequate treatment options. In this review article, we have reviewed gene therapy as a potential treatment option for CF. Various platforms and strategies for pulmonary gene delivery have also been discussed in detail.

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Introduction

Cystic fibrosis (CF) is the most common, life-shortening genetic disease in Caucasians. It affects the transport of salt and water across cells and affects different organs such as lungs, intestines, pancreas and liver. However, lung disease dominates the burden of care and clinical picture. The clinical symptoms involve difficulty in breathing, coughing mucus and frequent lung infections and loss of life.

Pathogenesis

CF is caused by mutation in a gene. The genetic defect was unidentified until 1989 [1]. However, with the advent of positional cloning, mutations were localized to 250000-bp gene on chromosome 7 [2]. The gene product of 1480-amino acids was called as cystic fibrosis transmembrane conductance regulator (CFTR). There are more than 1000 mutations reported to be responsible for CF which are grouped according to the structural and functional effects on CFTR. The most common mutation accountable for 70% of CF alleles is a 3-bp deletion in axon 10 resulting in deletion of phenylalanine at position 508 in CFTR protein (Δ508) [3], [4].

The CFTR is a chloride channel regulated by cAMP dependent protein kinase and adenosine triphosphate. The channel is expressed in apical membrane of epithelial cells [5]. All the mutations hamper chloride secretion through defect in protein production, protein processing, regulation and conduction. In addition, they also interact with sodium channel to control the water- and salt-content of the liquid protecting the airways called as airway surface liquid (ASL). CFTR exchanges chloride ions between the cytoplasm and the airway lumen [6]. The water- and salt-content of airway secretion is also based on epithelial sodium channel (ENaC). The defective CFTR causes an increased ENaC-mediated sodium uptake from the luminal secretions of the airways, depleting the ASL and making it thick and viscous which leads to a defective mucociliary clearance characterizing the pathophysiologic complications of CF [7], [8].

CF is a multisystem disease affecting one or more organs such as the pancreas, lungs, liver and reproductive organs. However, lungs are clinically most inflicted organs, accounting for 90% of deaths due to lung disease. In normal lungs, mucocilliary clearance is responsible for removing particulates, inorganic debris, airborne bacteria, and viruses [9]. The inefficient clearing of particulates and inflammatory reactions result in mucus plugging and pulmonary obstruction. The hyperinflation and airway obstruction lead to bronchiectasis. As a consequence, the airways are progressively colonized by specific pathogenic bacteria such as *P aeruginosa*, followed by *Staphylococcus aureus, Haemophilus influenzae,* and *Stenotrophomonas maltophilia.* Ultimately, chronic bronchopulmonary infection occurs which damages lungs and leads to death [10].

Treatment

The advent of technology has led to many new ideas for causative treatment, but at present treatment of CF is largely symptomatic.

Airway clearance techniques

The CF patient require to be managed by multidisciplinary group of experienced healthcare professionals in specialist center. Airway clearance techniques are important aspect of CF treatment [11],[12]. Activated cycle of breathing technique and autogenic drainage are also among these techniques. The techniques are supported by a number of devices to create positive expiratory pressure with or without airway oscillation.

High-frequency chest wall oscillation vests is similar kind of technique [13].

Chronic pulmonary drugs

In order to reduce the viscoelasticity of sputum mucolytic agents can be used. Recombinant human DNase (rhDNase) is the only licensed mucolytic. The reports suggest that rhDNase could prolong life in CF [14, 15]. Osmotic agents such as hypertonic saline, 7% twice daily, have been reported to reduce exacerbation rate by drawing water to the cell surface [16] Inhaled mannitol also benefits mucociliary clearance and has been licensed in Europe in 2011 [17]. Long-term azithromycin, is a well-established treatment for CF proven for reducing lung function decline and exacerbation rate [18].

Gene therapy

Despite important therapeutic advances, the definitive treatment for CF remains elusive. CF is a good candidate for gene therapy because it is relatively common, lethal and monogenic and it does not have adequate treatment options. Further, the heterozygotes appear to be phenotypically normal, expression of CFTR is low and lungs are accessible through non-invasive techniques [19], [20].

CF Gene Therapy

This basic strategy for gene therapy involves complementation or augmentation of mutant alleles with wild-type CFTR. One of the most straight forward approach involves delivery of wild-type CFTR gene to lung epithelium. Such attempts in transgenic animals have shown correction of chloride transport. As lung is the center of CF pathophysiology, gene can be administered ex vivo – where cells are harvested first, genetically modified, and then returned to the body (used for adenosine deaminase deficiency). However, ease of accessibility of lung allows direct lung gene therapy through use of gene therapy vectors applied directly to the epithelial cells leading to expression of wild-type CFTR.

Preclinical studies of gene therapy using both viral and non-viral gene delivery vectors have been shown to correct chloride ion transport in transgenic mice. The vector can be classed into: adenovirus, adenovirus associated virus and cationic lipids or polymers. These trial have used lung as target for gene delivery due to its direct relevance to afflicted organs, as well as upper respiratory system including nasal and maxillary sinus epithelium [21], [22, 23]. The virus mediated gene transfer involves administration of the virus to the lung epithelium. However, the results reported are not always consistent and only few show some changes in chloride transport. They also report dose dependent mild local inflammation and progressive lack of expression following repeated administration [24]. Thus, none of these defective gene correction have been found very promising from clinical point of view.

Pulmonary delivery platforms

Inhalation route

This is most popular due to non-invasive nature of administration. There are three major aerosolization systems; nebulizers, meter dose inhaler (MDI), and dry powder inhaler (DPI). With suitable modifications these devices can be made compatible for siRNA delivery.

Nebulizers

These are the oldest aerosol devices and still have the importance for generating continuous stream of liquid droplets for easy penetrability in size range of 1-5 um. They offer ease of use due to no need of synchronization of actuation and inhalation which also eliminates any training for users [25]. It is most preferred method for administering high dose antibiotics [26] [27]. However, during nebulization high shear stress is generated repeatedly as 99% of the generated aerosol droplets are recirculated back into the reservoir, which can induce degradation of nucleic acid. This combined with lesser stability of biomolecules in liquid form than dry form makes it unsuitable for siRNA delivery otherwise the vector chosen should be capable of protecting it from high shears.

Metered dose inhalers

These are designed to delivery discrete doses to respiratory tract in the form of aerosol. It uses an actuator to dispense a metered dose of 25 - 100 µL of liquid containing suitable amount of active ingredient [28]. The propellant undergoes flash evaporation from discharged liquid droplets to produce drug having desired aerodynamic size [29]. These are considered to be “the most intricate dosage form used in medicine today” as their performance is result of combination of formulation, container, metering valve and actuator performance [30], [31]. However, the compatibility of propellant with formulation is a potential concern. The formulation is generally presented in the form of suspension or solutions. The suspensions are the preferred one, as propellants are non-polar liquids in which most drugs have poor solubility. Similar to nebulizers, MDIs also present high shear to the formulation, and therefore, may not be the best direction for developing inhalable siRNA.

Dry Powder Inhaler

DPIs presents drugs for inhalation in the form of clouds of dry particles in air stream which is drawn through the device by inspiratory action of patient. In contrast to MDIs they are devoid of dependence of coordination between drug aerosolization and inspiration. This method has been successfully used to deliver therapeutic macromolecules such as insulin [32], parathyroid hormone [33].

However, formulating as DPI for siRNA presents considerable challenges as it demands not only flowability and dispersibility of the powders but also the retention of biochemical efficacy of the conformationally sensitive macromolecules. The problem can be addressed by formulating macromolecules using lyophilization or spray drying and subsequently processing them into flowable and dispersible powder as reported in literature [34], [35-38]. Spray drying is economically more feasible process than lyophilisation. However, for formulating siRNA in DPI a need for suitable vector for protecting it from shear of spray drying is required. The size of lyophilized or spray dried product can be carefully adjusted to improve deposition in respiratory tract. Improved stability and sterility of macromolecules is the key benefit offered by DPIs liquid aerosols. The delivery performance of PDIs also varies with different device designs. However, for final efficacy of the formulation the patient inspiratory flow rate also needs to be taken into consideration. In addition, the problems of de-aggregation and agglomeration of dry powders should be addressed.

Although inhalation becomes the most preferred way to deliver siRNA to the lungs; however, none of the clinical study on siRNA therapy is administered by inhalation. Intratracheal or intranasal route has been used in most of the *in vivo* studies, which could be due to difficulty in developing effective inhalable siRNA, retaining bioactivity during processing and storage [39].

Intratracheal route

This is most commonly used for administering to respiratory tract of animals. However, the method is described as non-physiological and surgery based uncomfortable makes it unsuitable for human administration. In case of animals, they have to be anaesthetized and trachea is exposed through which an endotracheal tube is or needle is inserted projecting its tip at a defined position just before tracheal bifurcation. Using a microsyring the drug solution can be instilled into the airways [40], [41].

A non-invasive method has been described using a microsprayer inserted endotracheally to deliver the aerosol into lungs under anaesthesia. Otherwise, animal intubation through mouth and trachea using a catheter or needle can be used to instil solution or suspension form of the medicine. As these procedures are done through mouth these are called as oro-tracheal administration [42]. Many studies have reported intra-tracheal route for administration of siRNA [43-45]. The intratracheal route results in minimal loss of drug and provides high delivery efficiency. This is a good advantage for any proof-of-concept study. However, since this route is an artificial way to deliver drugs and it results in no-uniform deposition of drug compared to inhalation [46]. It also eliminates oropharynx deposition and concomitant drug loss. All these factor obscure the effect of aerosol size, the critical factor in DPI development, on lung deposition making it difficult to compare and evaluate the delivery efficiency of particulate formulation.

Intranasal route

This is another non-invasive route of administration to lungs and it has been reported in number of studies [47-50]. To the deeply anaesthetized animal the formulations are administered drop-wise to the naris to be breathed. Zang et al. used this route to administer naked siRNA to inhibit the expression of HO-1 gene in injured lung of mouse [49]. However, as there is significant difference in the anatomy of physiology of mice and human lungs the efficacies observed in mice cannot be extrapolated to human use. Since mice are obligate nasal breathers, a high proportion of nasal dose is deposited in lung. Further, the anaesthetics have been reported to impair the mucociliary clearance in these animals which might overestimate the *in vivo* efficacy of formulations [51]. Heyder et al. tried the feasibility of this route in humans and found that majority of particles deposited in nose while only 3% of 1 –5 µm particles deposited in bronchial airways through nose breathing [52]. However, this route has been used in clinical trial to deliver siRNA in treatment of diseases of upper respiratory tract such RSV infection. In addition the large surface area offered by this route has long remained an incentive to explore for systemic delivery of siRNA.

Conclusion

CF is the most common, life-shortening genetic disease and gene therapy has shown promising outcome for the treatment of the same. Preclinical studies of gene therapy using both viral and non-viral gene delivery vectors have been shown to correct chloride ion transport in transgenic mice. The vector can be classed into: adenovirus, adenovirus associated virus and cationic lipids or polymers. There are three major aerosolization systems; nebulizers, meter dose inhaler (MDI), and dry powder inhaler (DPI) as non-invasive nature of administration. With suitable modifications these devices can be made compatible for siRNA delivery.

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