Chapter 11
Nanopulmonology

Introduction

Pulmonology deals with treatment of respiratory diseases, which is a challenging task with rising incidence and limitations of currently available treatments. Application of nanobiotechnology to pulmonology, nanopulmonology, offers NP-based drug and gene delivery for treatment of lung diseases as well as a route for delivery of systemic therapy. Delivery of exogenous genes to the airway epithelium in vivo has been limited by several physiological barriers, resulting in the low success rate of these systems. NP-based drug delivery systems have revolutionized the field of pharmacotherapy by presenting the ability to alter the pharmacokinetics of the conventional drugs to extend the drug retention time, reduce the toxicity, and increase the half-life of the drugs (Swai et al. 2009).

Nanoparticles for Pulmonary Drug Delivery

Pulmonary drug delivery is attractive for both local and systemic drug delivery as a noninvasive route that provides a large surface area, thin epithelial barrier, high blood flow, and the avoidance of first-pass metabolism. Nanoparticles may be used for systemic drug delivery via pulmonary route or for effect on the respiratory system. Nanoparticles can be designed to have several advantages for controlled and targeted drug delivery, including controlled deposition, sustained release, and reduced dosing frequency, as well as an appropriate size for avoiding alveolar macrophage clearance or promoting transepithelial transport (Rytting et al. 2008). The selection of natural or synthetic materials is important in designing particles or nanoparticle clusters with the desired characteristics, such as biocompatibility, size, charge, drug release, and polymer degradation rate.

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Systemic Drug Delivery via Pulmonary Route

Biodegradable polymers can be used for nanocarrier-based strategies for the systemic delivery of drugs, peptides, proteins, genes, siRNA, and vaccines by the pulmonary route. Chemical modifications to PLGA add several benefits in the selection of a suitable material for nanocarriers in the lung. The introduction of positive or negative charges can enhance the encapsulation efficiency and release profile of oppositely charged drugs, proteins, or genetic material.

An issue that remains surrounded by considerable debate is the question whether the lung should be used as an entry port for systemic drug administration. In this context, the safety of the nanocarriers and a lack of inflammatory and immunogenic potential need to be demonstrated under chronic treatment conditions. Such studies have not been presented with drug-loaded nanocarriers but will be necessary during future clinical trials. Such issues are not limited to pulmonary drug delivery but are also important in oral and intravenous administration.

Nanoparticle Drug Delivery for Effects on the Respiratory System

It is generally accepted in the field of pulmonary delivery that particles must be in the range of 1–3 μm to be delivered effectively to the deep lung. Larger particles have too much energy and hit the walls of the branching upper airways. Smaller particles do not have enough energy and tend to drift and adhere to the walls of the upper airways. However, as particle size is reduced further still, into the nanometer range, an increase in deep lung deposition is seen. Efficient particle deposition in the alveoli can be achieved with nanoparticles in the size range of 50–100 nm as compared to 1–5 μm for the lower airways, but this range of nanoparticles has not been exploited in therapeutic setting (Rogueda and Traini 2007). This is partly due to technological limitations of generating stable aerosols on nanoscale. Nebulizers are the most advanced in using the nanoscale, pressurized metered dose inhalers require further development to realize their potential, and dry powder inhalers are specifically in need of a dry solid nanoparticle generation technique to make it a reality.

Fate and Toxicology of Nanoparticles Delivered to the Lungs

Soluble nanoparticles are absorbed in the blood circulation whereas insoluble nanoparticles have local action in the lung or they can be cleared by lymphatic uptake or by macrophages. Other parameters that influence toxicity of inhaled nanoparticles are chemical composition, bioavailability, surface area, and morphology.

The suitability of nanoparticles, synthesized from porcine gelatin, human serum albumin, and polyalkylcyanoacrylate, as drug and gene carriers for pulmonary application was investigated in vitro on primary airway epithelium cells and the cell

line 16HBE14o (Brzoska et al. 2004). Confocal laser scan microscopy and flow cytometry experiments showed that the nanoparticles were incorporated into bronchial epithelial cells provoking little or no cytotoxicity and no inflammation as measured by IL-8 release. Based on their low cytotoxicity and the lack of inflammatory reaction in combination with an efficient uptake in human bronchial epithelial cells, protein-based nanoparticles are suitable drug and gene carriers for pulmonary applications.

Nanoparticle Drug Formulations for Spray Inhalation

Drugs delivered through inhalers are usually either in a suspension (as particles dispersed in liquid) or in a solution (when the drug is dissolved in the liquid). However, there are problems with both methods – a suspension can lead to sediment in the inhaler and less of the drug reaching the target area of the lung, while solutions present problems in dissolving the drug in the inhaler propellant liquid and can make the drug itself less stable. Preparation of the drug in nanoparticle form ensures that the correct dosage reaches the lung and the drug retains its stability, providing the possibility of slowing the release of the drug in the lung for longer therapeutic effect. This could lead to the possibility of more drugs being administered effectively by inhaler, rather than by tablet or injection. Patients suffering from conditions as diverse as asthma and diabetes could benefit from this method of drug delivery.

Nanobiotechnology for Improving Insulin Delivery in Diabetes

One of the important areas of application of nanotechnology-based pulmonary drug delivery is diabetes. Some of the techniques are described in the following paragraphs.

Inhalation of Glucose-Sensitive NP for Regulated Release of Insulin

In an attempt to achieve inhalable self-regulated insulin release, a microparticle agglomerate of nano-sized liposomal particles consisting of a blood sugar–sensing protein named concanavalin A (Con A) has been constructed, which is loaded with insulin, and cross-linkages are capable of cleavage by glucose (Karathanasis et al. 2007). Con A releases the particles to bind independently to the sugars, which then release their insulin. The particles exhibited a small aerodynamic diameter within the human respirable range, but a large geometric diameter that prevents macrophage uptake and clearance. Upon intratracheal instillation of the glucose-sensitive nanoparticle into the lungs of rats, hyperglycemic events triggered an acceleration of the release of insulin achieving normoglycemia shortly after sensing

the elevated systemic glucose. This work is a demonstration of an inhalable particle with long residence times in the lungs capable of modulating insulin release based on systemic glucose levels and thus mimic the functions of the pancreas. This approach has the potential of improving management of diabetes by regulated insulin delivery.

Pulmonary Delivery of Insulin by Surface Acoustic Wave Technology

There has been growing interest in the potential for the systematic delivery of drugs and therapeutic agents (e.g., peptides and proteins) via pulmonary (inhalation) means. Surface acoustic wave technology (SAW) enables well-controlled generation of fine nanoparticles and is ideal for this pulmonary drug delivery, particularly for a number of drugs that require frequent dosing. An important application of this technology will be for the development of a device producing insulin nanoparticles delivered across the pulmonary alveoli. There is already evidence for increased efficacy of inhaled insulin compared to injected insulin, due to faster uptake and clearance. An economically viable microdevice for portable pulmonary drug delivery for human use based on SAW has significant commercial potential. Another advantage of using noninvasive techniques as alternatives to frequent injections would be the profound impact on a child's willingness to comply with diabetic treatment.

Nanotechnology-Based Treatment of Pulmonary Disorders

Management of Cystic Fibrosis

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians. CF affects approximately 1 in 2,000–2,500 live births with a carrier rate in white Americans of 1 in 25. There are about 30,000 CF patients in the USA, and 2,000 babies with CF are born every year. An estimated eight million are carriers. Parents of a child with CF, who are carriers but do not have the condition, are at a one-in-four risk of having a child with CF with each pregnancy. CF is a multisystem disorder of children and adults characterized by an abnormality of exocrine gland function, which manifests as chronic respiratory tract infections and malabsorption. Cystic fibrosis transmembrane conductance regulator (CFTR) gene has been isolated. Determining the sweat chloride concentration is the standard screening test (high level of chloride ion means a positive test). It is not suitable for prenatal diagnosis and cannot detect carriers. The method of choice is identification of DF508 (the most common mutation) by PCR, which detects 85–95 % of CF carriers.

CF is a potentially lethal disease although the current life expectancy has improved to >30 years with advances in the medical treatment. CF also affects the digestive system, resulting in progressive disability and early death. Thick mucous

production in the airways leads to distorted mucociliary clearance and weakening of the immune system leading to frequent lung infections. Currently used methods for the treatment of pulmonary complications of CF include physiotherapy, bronchodilator therapy, mucolytic agents, corticosteroids, and lung transplant. These methods are directed at the management of manifestations and none of these addresses the cause of the disease. Because of the devastating clinical sequelae and the lack of definitive therapy, CF is prime candidate for gene therapy.

The goal of gene therapy is correction of the mutant CFTR gene with wild-type (wt) DNA sequences to restore normal CFTR protein and function. Experiments with wtCFTR cDNA expression vectors have shown that Cl ion transport phenotype associated with CF can be corrected to resemble that in normal cells. Several methods of gene transfer are used including those involving nanobiotechnology.

Nanobiotechnology-Based Gene Transfer in CF

Nonviral DNA Nanoparticle–Mediated CFTR Gene Transfer

Nanoparticles have been used for CFTR gene delivery in the nose of CF patients in clinical trials and led to partial correction of the chloride transport defect in nasal epithelium (Griesenbach et al. 2004).

Nanoparticles consisting of single molecules of DNA condensed with PEG-substituted lysine 30-mers have been shown to efficiently transfect lung epithelium following intrapulmonary administration (Fink et al. 2006). Nanoparticles formulated with lysine polymers having different counterions at the time of DNA mixing have distinct geometric shapes: trifluoroacetate or acetate counterions produce ellipsoids or rods, respectively. Based on intracytoplasmic microinjection studies, nanoparticle ellipsoids having a minimum diameter less than the 25-nm nuclear membrane pore efficiently transfect nondividing cells. This 25-nm size restriction corresponds to a 5.8-kbp plasmid when compacted into spheroids, whereas the 8- to 11-nm diameter of rod-like particles is smaller than the nuclear pore diameter. In mice, up to 50 % of lung cells are transfected after dosing with a rod-like compacted expression plasmid, and correction of the CFTR chloride channel was observed in humans following intranasal administration. To further investigate the potential size and shape limitations of DNA nanoparticles for in vivo lung delivery, reporter gene activity of ellipsoidal and rod-like compacted luciferase plasmids ranging in size between 5.3 and 20.2 kbp was investigated. Equivalent molar reporter gene activities were observed for each formulation, indicating that microinjection size limitations do not apply to the in vivo gene transfer setting.

Chitosan-DNA-FAP-B nanoparticles are good candidates for targeted gene delivery to fibronectin molecules (FAP-B receptors) of lung epithelial cell membrane. In a study, aerosol delivery of chitosan-DNA-FAP-B nanoparticles resulted in 16-fold increase of gene expression in the mice lungs compared with chitosan-DNA nanoparticles, suggesting that chitosan-DNA-FAP-B nanoparticle can be a promising carrier for targeted gene delivery to the lung (Mohammadi et al. 2011).

An hCFTR expression plasmid was optimized as a payload for compacted DNA nanoparticles formulated with PEG-substituted 30-mer lysine peptides. Compared to hCFTR cDNA, the codon-optimized version (CO-CFTR) produced a ninefold increased level of hCFTR protein in CF mice, when compacted as DNA nanoparticles (Padegimas et al. 2012).

Liposome-Mediated CFTR Gene Transfer

Lipofection of cells in vitro with CFTR cDNA constructs can, like virally transduced cells, elicit the electrophysiological responses characteristic of the CFTR ion channel. The advantages of liposome-mediated gene transfer are the potential for standardized production of large amount of vector, freedom from risk of viruses, and the possibility of readministration with minimal host reaction. The disadvantages are the lack of sustained expression using current strategies. The safety and efficacy of this technique was demonstrated in rodents, and clinical trials have been conducted in CF patients.

Magnetofection for Enhancing Nonviral Gene Transfer to the Airways

Superparamagnetic nanoparticles with either the therapeutic CF gene or a reported gene attached to them were inhaled and targeted to the airway epithelium via positioning of a strong magnet over the target site, which functions to pull the particles into contact with the cells (Dobson 2006). To improve the in vivo transfection efficiency of DNA delivery of this system, an oscillating magnet array system (TransMAG) is being developed, which will introduce energy and a lateral component to advance the movement and interaction of the particles coupled with Lipofectamine 2000 to form a plasmid DNA (pDNA) liposome complexes, to enhance interaction with the epithelial cells (Xenariou et al. 2006).

NP-Based Delivery of Antibiotics for Treatment of Pulmonary Infections in CF

Pulmonary infections are common in CF and are currently treated with antibiotics that are prescribed on the basis of the infectious agent, but many of these bacteria are resistant to multiple antibiotics and require prolonged treatment with intravenous antibiotics such as tobramycin, ciprofloxacin, and piperacillin. Inhaled therapy with other antibiotics is also followed in some cases to improve lung function by impeding the growth of colonized bacteria. These antibiotics may produce side effects such as hearing loss and kidney failure. To address these shortfalls, a liposomal formulation of ciprofloxacin powder manufactured using a sprayfreeze-drying process with the required mass mean aerodynamic diameter and fine particle fraction has been used (Sweeney et al. 2005). This is administered by inhalation and thus increases the bioavailability of the drug.

Respiratory tract infections are the primary cause of death in persons with CF, and there are no effective therapies for patients infected with bacterial species that are resistant to all known antibiotics. A surfactant-stabilized oil-in-water nanoemulsion, NB-402 (NanoBio Corporation), was found to be bactericidal against all but two of 150 bacterial strains, regardless of their levels of resistance (LiPuma et al. 2009). NB-402 has been shown to be highly efficacious in vitro against *Pseudomonas aeruginosa*, *Burkholderia, Acinetobacter, Stenotrophomonas,* and other multidrug-resistant bacterial strains from CF patients. In addition, the nanoemulsion retains activity when organisms are growing in biofilms and mucus. Resistance to the nanoemulsion is not anticipated based on its unique mechanism of action of interacting with the bacterial membrane and causing lysis. These results support NB-402's potential role as a novel antimicrobial agent for the treatment of infection due to CF-related opportunistic pathogens. There are plans for this product to enter clinical trials.

Nanotechnology-Based Treatment of Chronic Obstructive Pulmonary Disease

Chronic airway inflammation and mucous hypersecretion are features of chronic obstructive pulmonary disease (COPD), asthma, and CF. One of the major challenges in drug delivery and therapeutic efficacy are airway defense, severe inflammation, and mucous hypersecretion, which are further aggravated by infection. Treatments such as corticosteroids and antibiotics aim to controlling chronic inflammation.

Few of the numerous available nano-based drug delivery systems have been tested for COPD. Targeted nanoparticle-mediated sustained drug delivery is required to control inflammatory cell chemotaxis, fibrosis, protease-mediated chronic emphysema, and/or chronic lung obstruction in COPD. Design and development of nano-based targeted vehicles with integrated therapeutic, imaging and airway-defense penetrating capability are currently being evaluated to treat the underlying cause of CF and COPD lung disease (Vij 2011).