The Expanding Role of Aerosols in Systemic Drug Delivery, Gene Therapy, and Vaccination

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Summary

Aerosolized medications have been used for centuries to treat respiratory diseases. Until recently, inhalation therapy focused primarily on the treatment of asthma and chronic obstructive pulmonary disease, and the pressurized metered-dose inhaler was the delivery device of choice. However, the role of aerosol therapy is clearly expanding beyond that initial focus. This expansion has been driven by the Montreal protocol and the need to eliminate chlorofluorocarbons (CFCs) from traditional metered-dose inhalers, by the need for delivery devices and formulations that can efficiently and reproducibly target the systemic circulation for the delivery of proteins and peptides, and by developments in medicine that have made it possible to consider curing lung diseases with aerosolized gene therapy and preventing epidemics of influenza and measles with aerosolized vaccines. Each of these drivers has contributed to a decade or more of unprecedented research and innovation that has altered how we think about aerosol delivery and has expanded the role of aerosol therapy into the fields of systemic drug delivery, gene therapy, and vaccination. During this decade of innovation, we have witnessed the coming of age of dry powder inhalers, the development of new soft mist inhalers, and improved pressurized metered-dose inhaler delivery as a result of the replacement of CFC propellants with hydrofluoroalkane. The continued expansion of the role of aerosol therapy will probably depend on demonstration of the safety of this route of administration for drugs that have their targets outside the lung and are administered long term (eg, insulin aerosol), on the development of new drugs and drug carriers that can efficiently target hard-to-reach cell populations within the lungs of patients with disease (eg, patients with cystic fibrosis or lung cancer), and on the development of devices that improve aerosol delivery to infants, so that early intervention in disease processes with aerosol therapy has a high probability of success. *Key words: aerosol, MDI, DPI, gene therapy, insulin, diabetes, cystic fibrosis, influenza, lung cancer, measles, vaccine, vaccination, bioterrorism, drug delivery.* [Respir Care 2005;50(9):1161–1174.

Introduction

Aerosolized medications have been used for centuries to treat respiratory diseases. However, until recently, inhalation therapy focused primarily on the treatment of asthma and chronic obstructive pulmonary disease (COPD), and the pressurized metered-dose inhaler (pMDI) was the delivery device of choice. By the late 1980s it was becoming apparent that chlorofluorocarbon (CFC), the propellant used to deliver drugs from pMDIs, was an environmental threat, and in 1991 the Montreal protocol was approved by the global community. This protocol called for the planned withdrawal of CFCs in all technologies and led the pharmaceutical industry to begin to search for alternatives to this propellant for aerosolized drug delivery. At about the same time, biotechnology companies were forming with the goal of finding ways to deliver new drugs, such as proteins and peptides, to the lungs, with the target being the systemic circulation and not the lung itself. These drugs were expensive to produce and often had narrow windows of efficacy, which meant that they needed to be delivered efficiently and reproducibly. Delivery devices and drug formulations that were available at the time to deliver drugs to treat asthma and COPD were neither efficient nor reliable in terms of delivering a reproducible dose to a specific lung region. Thus, there was a critical need to develop new devices and drug formulations that could meet these new delivery criteria and, over the next decade, we witnessed the growth of a new industry that has led to dry powder inhalers (DPIs) and important advances in liquid aerosol delivery.

Other developments were occurring in medicine that would further expand the role of aerosol therapy. First, there was a major development in genetics. In 1989 the defective gene that results in cystic fibrosis (CF) was cloned, and the normal product of this gene was identified as the CF transmembrane conductance regulator (CFTR). Then, in 1992, Stribling et al[1] demonstrated that aerosol delivery to mice of cationic liposomes complexed with plasmid deoxyribonucleic acid (DNA) containing a reporter gene resulted in substantial expression in the lungs, and Rosenfeld et al[2] showed that when replication-defective adenoviral vectors, engineered to express the CFTR complementary DNA, were administered to the airways of experimental animals, transgene expression was observed in the respiratory epithelium. Thereafter, laboratories all over the world began to think about the possibility of curing CF by gene therapy. To accomplish this goal, new genetic material had to be delivered to and taken up by ciliated airway epithelial cells in patients with CF. Although it soon became clear that the best route of administration for the new genetic material would be via aerosol, the delivery technology had yet to be established.

Second, in the 1980s, in Mexico, Albert Sabin and his colleagues were vaccinating children against measles with aerosolized measles vaccine.[3,4] By the mid-1990s it was clear that this was an effective method for measles vaccination, and several investigators began to consider the possibility of mass vaccination campaigns with an aerosolized measles vaccine in other developing countries. Again, because the existing delivery systems for treating asthma and COPD were not applicable in such campaigns, new delivery devices were needed.

Each of these developments has contributed to a decade or more of unprecedented research and innovation that has altered how we think about aerosol delivery and has expanded the role of aerosol therapy into the fields of systemic drug delivery, gene therapy, and vaccination.

Systemic Drug Delivery

Advantages

There are a number of advantages to treating systemic diseases with aerosolized medicines. In contrast to oral therapy, this route of administration eliminates the potential for poor absorption and/or high metabolism in the gastrointestinal tract and it eliminates first-pass losses in the liver. In contrast to injection therapy, inhalation therapy is not associated with pain and this should improve patient comfort and compliance, leading to improved treatment outcome.

Drugs Under Development

The most visible drug that is under development for systemic delivery via inhalation is inhaled insulin to treat diabetes. Nevertheless, the same rationale of improving patient comfort and compliance through needle-free delivery has led to research into inhalation therapy for other proteins and peptides that are currently administered only via injection. In addition, because the lung offers rapid absorption kinetics, small molecules can also benefit from

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pulmonary delivery, and a number of these are under development as well. Peptides and small molecules that are being investigated for pulmonary delivery to the systemic circulation include: morphine and fentanyl to treat pain; dihydroergotamine for migraine; interferon b to treat multiple sclerosis; leuprolide acetate to treat prostate cancer, infertility, and post-menopausal breast cancer; calcitonin to treat Paget disease and osteoporosis; parathyroid hormone to treat osteoporosis; heparin to inhibit thrombosis; and growth hormone releasing factor to treat pituitary dwarfism.

Optimal Target

The optimal target within the lungs for delivery of drugs to the systemic circulation is the alveolar region. This is because the alveolar region composes a resorptive surface of 75 m^2, mucociliary clearance is minimal, and the cell barrier to absorption is extremely thin (0.1 mm). These advantages lead to increased residence time for the drug and a large absorptive surface, all of which enhance the absorption efficiency, compared to delivery of drug to more proximal lung regions.[5]

Inhaled Insulin to Treat Diabetes

The best example of the expanding role of aerosol therapy in terms of systemic drug delivery is the development of insulin as an aerosol to treat diabetes. In the United States, an estimated 16 million people have diabetes mellitus. The majority of these have non-insulin-dependent diabetes mellitus, or type 2 diabetes. To treat their diabetes, type 2 diabetics can change their diet, exercise, and/or take oral medications. But these treatments will eventually fail, and, when they do, these patients will need to inject insulin before meals and at bedtime (approximately 4 times per day). Type 1 diabetics (approximately 1 million in the United States) must inject insulin from the time of diagnosis.

During the 1990s it became clear that inhaled insulin was efficacious in controlling blood glucose levels in patients with diabetes.[6–9] A good example of this control is shown in Figure 1, which is based on data that were presented in a previous review.[10] In this patient, fasting blood glucose levels reached normal levels approximately 3.5 hours after inhalation of 1.0 U/kg body weight of insulin, and this level of control was similar to that achieved after subcutaneous injection of 0.1 U/kg body weight of insulin (left half of figure). Five minutes before the ingestion of a test meal, this same patient inhaled 1.5 U/kg body weight of insulin. Over the next 3 hours (right half of figure), postprandial blood glucose levels were well controlled and remained within normal limits. This was in contrast to the lack of control with the placebo aerosol.

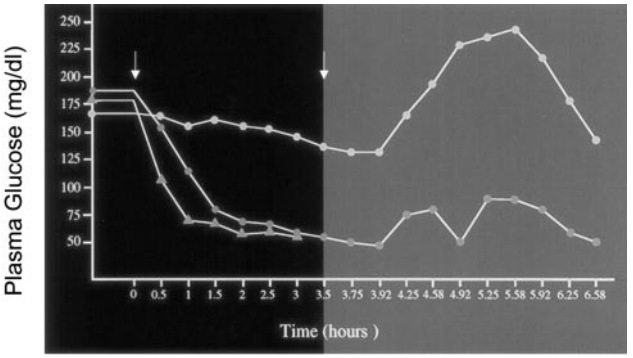

Fig. 1. Control of plasma glucose levels in a volunteer with type 2 diabetes during fasting (left half of the figure) and postprandial states (right half of the figure) after insulin inhalation. During the fasting state, an inhaled dose of approximately 1 U/kg body weight aerosolized insulin lowered the plasma glucose level into the normal range, and a dose of 1.5 U/kg body weight of aerosolized insulin controlled plasma glucose levels following the ingestion of a test meal. This is in contrast to the lack of control with placebo aerosol, as shown in the top curve in the figure. Arrow at 0 hours indicates time of initial insulin and placebo inhalation and insulin injection (subcutaneous). Arrow at 3.5 hours indicates inhalation of second dose of insulin and placebo. (Based on data in Reference 10.)

Devices and Formulations. Although it was known that inhalation of insulin aerosol was efficacious, a number of challenges to systemic delivery of insulin via inhalation remained unresolved for several years. First, there was the need to deliver a substantially higher dose of insulin to the lung than was needed via subcutaneous delivery to achieve the same systemic effects. Aerosol devices that were available in the early 1990s were too inefficient to deliver these large doses, since a large proportion of the drug was either retained in the device or was never delivered past the oropharyngeal region. In addition, conventional devices that were available at the time required compressors and electricity to generate the aerosol particles. Clearly, a portable device that didn't require electricity and delivered a high percentage of the drug to the lung was needed to increase patient acceptance and compliance. The solution to this challenge led to the development of portable, more efficient inhalation devices and new drug formulations.

Devices and formulations that are furthest along in development of inhaled insulin include the Nektar, Aradigm, and Aerogen products (Fig. 2). Each of these products delivers aerosol containing a high percentage of 1–3 μm particles, which are considered optimal for targeting the alveolar lung region, and incorporate methods for controlling breathing parameters that are known to influence aerosol deposition in the lung (eg, inspiratory flow rate and lung volume at the time of inhalation). The Nektar device (Nektar Therapeutics, San Carlos, California) uses compressed air to disperse dry powder insulin into a spacer before inhalation. The patient then inhales insulin from the

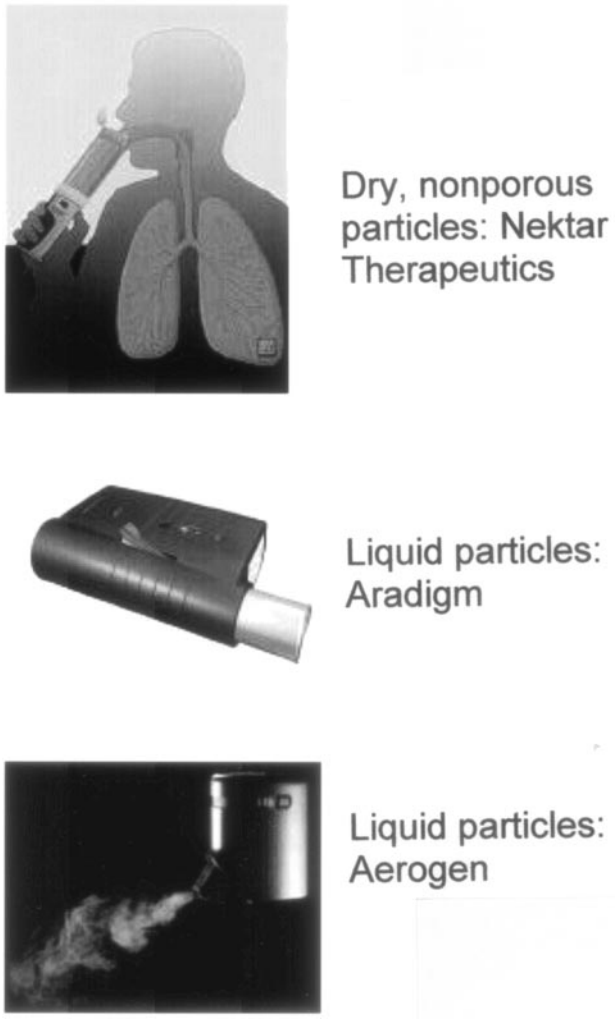

Fig. 2. First-generation devices developed for the delivery of dry powder and liquid aerosol formulations of insulin.

spacer during a slow, deep breath. The Aradigm device (Aradigm, Hayward, California) is a breath-actuated, aqueous mist inhaler. Liquid insulin aerosol is delivered electronically by means of mechanical extrusion when the patient's inspiratory flow rate and lung volume are appropriate. The Aerogen device (Aerogen Inc, Mountain View, California) is a breath-actuated, liquid aerosol inhaler that delivers insulin to the patient during inspiration by means of vibrating mesh technology. Nektar has completed phase III testing and has applied for Food and Drug Administration approval. Aradigm is in early phase III testing. Aerogen is in phase II development.

Bioavailability. A current challenge to systemic delivery of insulin is the need to maximize its bioavailability. Although we now have very efficient devices that can deliver high concentrations of dry powder and liquid aerosolized insulin to the alveoli, the biopotency (defined as the area under the glucose infusion curve) and the bioavailability (defined as the area under the insulin plasma curve) of inhaled insulin remain low for either formulation. For example, the biopotency of liquid insulin, expressed as a percentage relative to subcutaneous administration, is 10–16% for the Aradigm product[11,12] and 18–22% for the Aerogen product.[13] Similarly, the bioavailability of dry powder insulin is 10–15% for the Nektar product.[9] This relatively low bioavailability of insulin is probably due to a combination of factors, including insulin's relatively large molecular weight (5,000 Daltons), degradation by native airway peptidases and proteases, and phagocytosis by alveolar macrophages. Other drugs being developed for systemic delivery via inhalation have higher bioavailabilities because they have lower molecular weights and are lipid soluble. The reduced bioavailability for aerosolized insulin relative to subcutaneous delivery necessitates very high doses of insulin to the lung to achieve systemic effects similar to subcutaneous delivery, and this could increase the cost of aerosolized insulin relative to subcutaneous delivery.

The need to increase the bioavailability of insulin aerosol has led to the development of second-generation delivery devices. These devices deliver insulin formulations that have been engineered to increase their bioavailability; they include products from Alliance Pharmaceutical (San Diego, California), Elan ([previously Dura Pharmaceutical], San Diego, California), Alkermes (Cambridge, Massachusetts), and MannKind (Valencia, California) (Fig. 3). The Alliance Pharmaceutical product consists of a dry, porous particle (PulmoSpheres) in a suspension that is delivered via pMDI. These particles are lipid based, have extremely low densities, and have been engineered to be uniformly in the 1–3 μm range. The Elan product delivers micronized insulin crystals in the nanometer particle range (approximately 0.1 μm), which theoretically could enhance drug delivery to the alveolar region. Drug is delivered via a handheld, battery-powered, multi-dose system. The electromechanical components of this device have led to delivery problems, and testing of the device is currently on hold. The Alkermes device is a breath-actuated DPI that delivers large, porous particles of insulin. Like PulmoSpheres, these particles are lipid based and of extremely low density. The large geometric size of the particles (approximately 10–15 μm) reduces the tendency to aggregate, and this increases dispersion of the powder during delivery. At the same time, these particles behave aerodynamically like nonporous particles in the 1–3 μm range, facilitating alveolar delivery. Once deposited in the alveolar region, the large size of these particles may make them less susceptible to degradation by phagocytosis. The MannKind device (Medtone DPI) delivers a formulation (Technosphere) that assembles in an ordered lattice array at low pH and captures the insulin within it, which protects

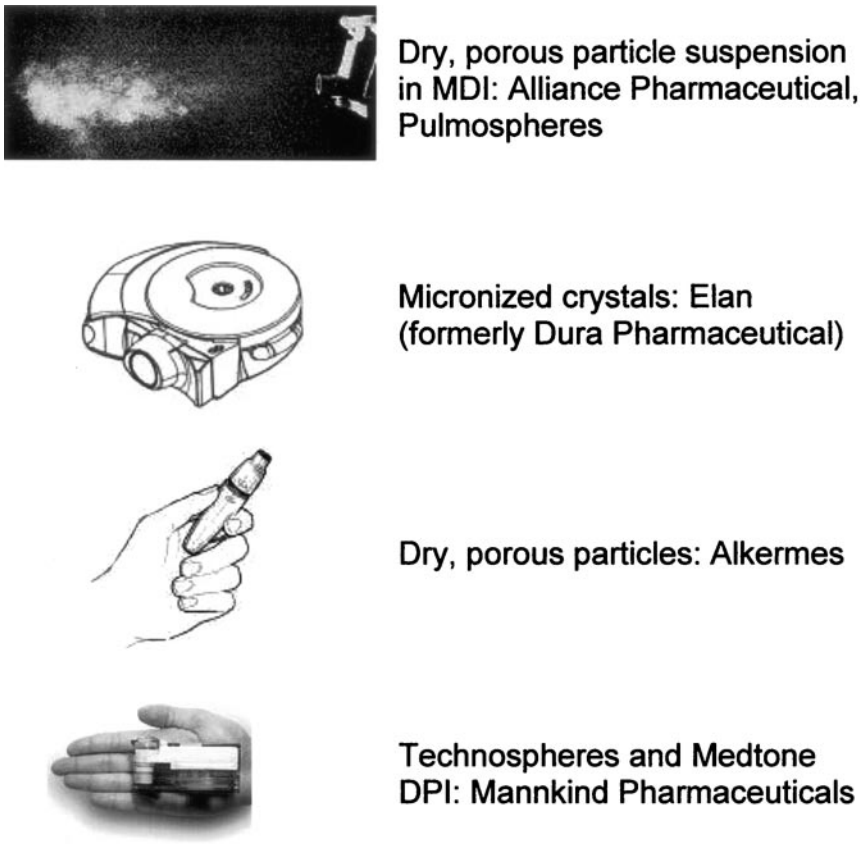

Fig. 3. Second-generation devices developed for the delivery of dry powder and liquid aerosol formulations of insulin.

the insulin from proteases. Once delivered to the alveolar region, the array dissolves at the neutral pH, releasing the insulin for absorption. Drug is delivered by means of a capsule-based, high impedance inhaler.

Only the Alkermes and MannKind products have been tested for insulin bioavailability. The Alkermes product has been reported to have a bioavailability of 16% relative to subcutaneous administration,[14] which is similar to the bioavailability reported for the Nektar, Aradigm, and Aerogen products. In contrast, the MannKind product appears to demonstrate a higher biopotency (26%) relative to subcutaneous administration.[15]

Another approach to increasing bioavailability is by "PEGylation" technology. PEG stands for "polyethylene glycol," which is a neutral, water-soluble, nontoxic polymer that protects the insulin during delivery into the lung. It is being tested by the Nektar group. In a recent study with dogs, PEGylation resulted in prolonged systemic activity of insulin.[16]

Others are exploring the possibility that the pharmacologic availability of insulin might be increased by adding absorption enhancers and/or protease inhibitors to the formulation. For example, we know from animal studies that the addition of the absorption enhancers sodium glycocholate, mixed micelles, and lauryl-b-D-maltopyranoside to an insulin formulation increases the pharmacologic availability by 50.0%, 29.9%, and 85.0%, respectively, compared to 11.9% for a control formulation of insulin.[17] Similarly, the pharmacologic availability of insulin can be increased by the addition of protease inhibitors such as aprotinin, soya bean trypsin inhibitor, and bacitracin to the formula; increases of 23.6%, 30.1%, and 81.2%, respectively, have been reported.[17] Although promising, the safety profile of long-term lung exposure to these formulation additives is unknown. We do know that short-term exposures to absorption enhancers similar to those described above led to patient complaints of nasal irritation and nasal congestion, when they were added to liquid solutions that were used in the intranasal administration of insulin.[18,19]

Safety. The third challenge to systemic drug delivery of aerosolized insulin is safety. This is important because the insulin will be inhaled several times a day for many years, so the long-term safety profile must be examined and established. Of the many devices and formulations being developed for systemic delivery of insulin, only the Nektar and Aradigm products have been studied for long periods (months to years) with lung function being evaluated. For the Nektar product, diabetes type 1 and type 2 patients showed no significant changes in pulmonary function or

diffusing capacity after 3 months of treatment.[9] However, in a trial that lasted 6 months, type 1 and type 2 patients showed increased insulin binding antibodies, increased coughing, and reduced diffusing capacity for carbon monoxide, compared to injected insulin.[20,21]

Since those data were reported, several longer-term studies have been performed to address efficacy and long-term safety.[22,23] In one study, several hundred patients with type 2 diabetes were treated with either inhaled insulin or the oral agent metformin and were evaluated over 52 weeks and 104 weeks. Quoting from one of the abstracts that summarized the data, the authors reported that, "Changes from baseline FEV_1 [forced expiratory volume in the first second] were slightly larger for the INH [inhaled insulin] group, compared with the oral agent group, at week 24, but this difference did not increase further at weeks 36 or 52. There were no differences for D_{LCO} [diffusing capacity for carbon monoxide] between groups."[23] Another study examined the sustained long-term efficacy and safety of continuous therapy with inhaled insulin for up to 4 years in 89 type 1 and 2 patients. Another group of 23 patients were treated with oral agents or subcutaneous insulin for up to 2 years. Annualized changes in FEV_1 and D_{LCO} were similar for the 2 treatment groups.[24] Results from these recent long-term studies suggest that any changes in pulmonary function following treatment with the Nektar product are small and similar to changes observed in patients who are treated with noninhaled insulin.

The Aradigm product has been evaluated with patients with type 2 diabetes over a 3-month period.[25] In that study, patients received either inhaled or subcutaneously injected insulin. Results indicated that the number of treatment emergency adverse events and the number of patients experiencing these events were similar in the 2 treatment groups. Also, there were no significant differences between the 2 groups for any of the pulmonary function tests in terms of change from baseline. Median total insulin antibody level increased in the inhaled insulin group but remained unchanged in the subcutaneously injected group. No clinical signs or symptoms were reported with these increases in antibodies. The Aradigm product has also been evaluated with asthmatic patients and with smokers. Those studies showed that asthma patients absorb less insulin than nonasthmatics[26] and smokers absorb more insulin than nonsmokers.[27] The latter studies suggest that the inhaled insulin dose may need to be adjusted if the diabetic patient has asthma or smokes.

Gene Therapy

Advantages

Another good example of the expanding role of aerosol therapy is the treatment of lung diseases with aerosolized gene therapy. There are a number of advantages to this form of therapy. First, aerosolized gene therapy provides a direct, noninvasive means for targeted delivery to different regions of the lung. Second, this route of administration delivers a high dose to the target site. Third, aerosolized gene therapy causes fewer adverse effects than intravenous administration.

Inhaled Complementary DNA to Treat Cystic Fibrosis

The most visible gene therapy drug under development is inhaled complementary DNA to treat CF. CF is an autosomal recessive disease and is the most common lethal genetic disease among whites. There are 30,000 cases in the United States, 3,000 cases in Canada, and 27,000 cases in Europe. It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene located on chromosome 7 and is associated with defective chloride transport in airway epithelial cells. Lung pathology in CF includes abnormal chloride transport, increased mucus viscosity, decreased mucociliary clearance, recurrent infection, chronic inflammation, and airway destruction.

Gene Transfer Agents. The goal of aerosolized gene therapy in treating CF is to reconstitute CFTR function and normal chloride channel function in the lungs. Progress to date includes 20 clinical trials that have been carried out since the cloning of the CFTR gene in 1989, the development of 3 gene-transfer agents: adenovirus, adeno-associated virus 2, and cationic liposomes.[28] All 3 vectors have demonstrated proof of principle for gene transfer to the airway. Nevertheless, gene transfer efficiency with each of these vectors has been too low to achieve clinical benefit.

Challenges. Low gene transfer efficiency can be attributed to many factors. First there is the challenge of delivering an adequate dose to the target cells. In this case, the target cells are the ciliated airway epithelial cells, and it is thought that only a 5–10% correction is required to overcome the chloride ion transport defect in cultured CF airway epithelium.[29] Nevertheless, the explosion of dry powder formulations and delivery devices that has led to substantial progress in treating systemic disorders such as diabetes with aerosolized medications has not been applicable to the delivery of gene vectors to treat CF. This lack of applicability has resulted in a reliance on conventional aerosol delivery systems (ie, nebulizers), which typically deliver 2–30% of the nominal dose to the airways.[30] Such inefficient delivery systems could account for the low gene transfer figures reported in most clinical trials with inhaled CFTR vector. Recently, more efficient liquid aerosol delivery systems (ie, soft mist inhalers) have been developed and are becoming commercially available. However, none

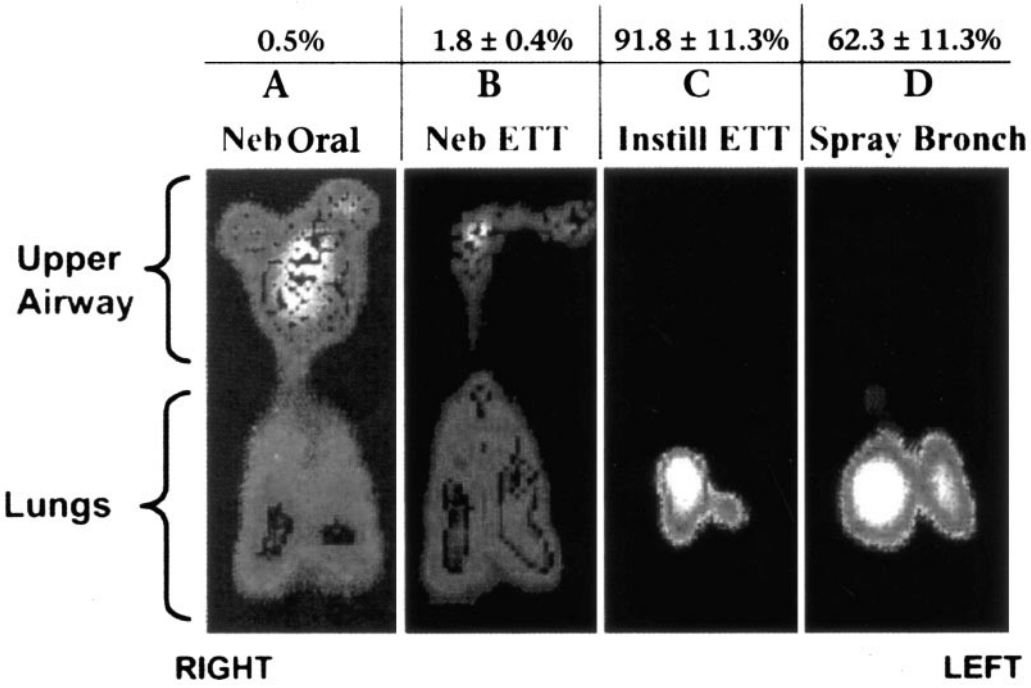

Fig. 4. Deposition efficiencies within the lungs of a rhesus macaque with an oral nebulizer (Neb Oral), nebulization through an endotracheal tube (Neb ETT), instillation through an endotracheal tube (Instill ETT), and microspraying through a bronchoscope (Spray Bronch). Microspraying resulted in a higher lung dose than Neb Oral or Neb ETT and a more reproducible dose than Instill ETT. (From Reference 30, with permission.)

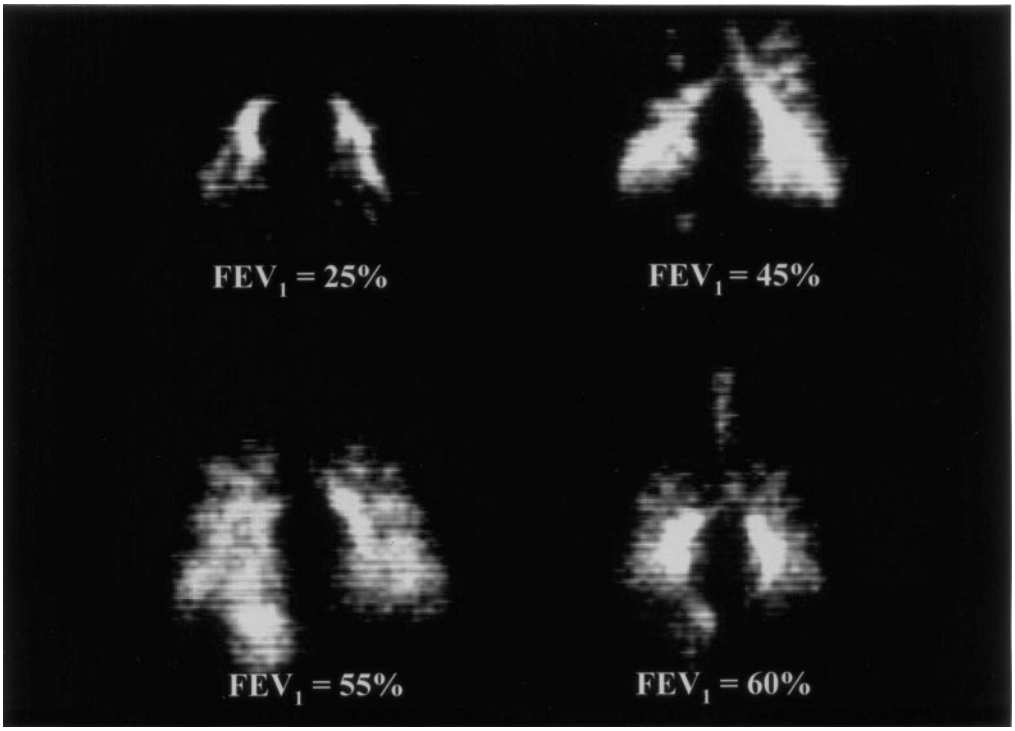

Fig. 5. Gamma-camera images of the lungs of 4 adult patients with cystic fibrosis, showing the effects of airway obstruction on aerosol deposition.

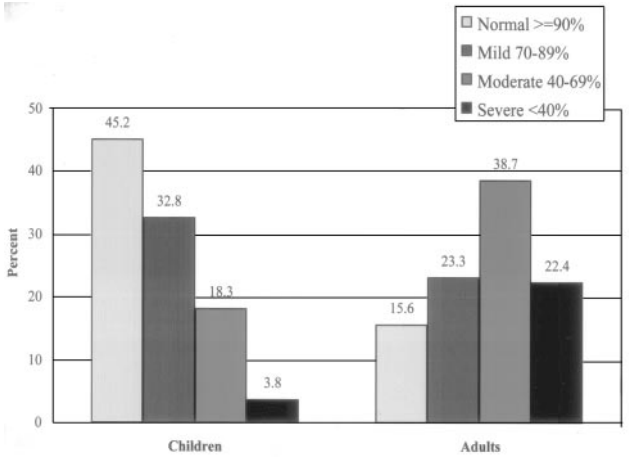

Fig. 6. Percent of predicted forced expiratory volume in the first second (FEV_1) in children versus adults with cystic fibrosis. The majority of children with cystic fibrosis have either normal values or values indicating mild disease. In contrast, the majority of adults with cystic fibrosis have values indicating either moderate or severe disease. (From Reference 31, with permission.)

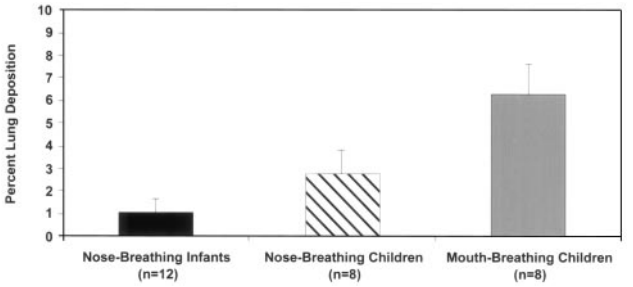

Fig. 7. Percent deposition of aerosol in the lungs of infants and older children with cystic fibrosis. Deposition in the lungs of nose-breathing infants is significantly lower than deposition in the lungs of older nose-breathing children, or the same children breathing with a mouthpiece. (Adapted from Reference 32.)

of these newer devices has been tested in clinical trials with CFTR vector.

One possible alternative to the conventional delivery systems could be microspraying technology. Studies with animals have shown this technology to be superior to delivery via oral nebulization or through an endotracheal tube (Fig. 4).[30] This type of delivery also appears to produce a more uniform deposition pattern, compared to intratracheal instillation, which is less reliable in terms of targeting both lungs reproducibly (see Fig. 4). Microspraying has the disadvantage of being an invasive procedure that requires the placement of a bronchoscope in the lungs prior to delivery. Nevertheless, microspraying holds the promise of delivering a substantially higher percentage of vector to both lungs (approximately 60% of the nominal dose), compared to other delivery systems, and this could lead to efficient gene transfer in future studies.

Another challenge to targeting airway epithelial cells with inhaled CFTR vector is overcoming the effect of airway obstruction on aerosol deposition in the lungs. Figure 5 shows aerosol deposition in 4 adult patients with CF. These images were obtained by scanning the lungs with a gamma camera following the patient's inhalation of an aerosol containing the radioisotope ^{99m}technetium. Note that the deposition of the radioisotope in all 4 patients is uneven and highly concentrated in lung regions that are assumed to represent the larger central airways. In addition, deposition in the lung periphery (typically indicated by a well-defined lung border) is less visible in patients with greater airway obstruction (ie, patients with reduced FEV_1). Given these deposition patterns, it should not be surprising that aerosolized gene transfer to the airway epithelial cells in these adult patients with CF is inefficient.

A better population to treat with aerosolized gene vector might be toddlers and infants with CF, because a majority of these individuals have milder airway disease, compared to older children and adults with CF (Fig. 6).[31] Milder airway disease should favor a more even aerosol deposition pattern and target epithelial cells in both the large and smaller airways. Delivering an adequate dose of aerosolized gene vector to the lungs of infants with CF poses another challenge, since it appears that deposition in infant lungs by means of a conventional nebulizer is significantly less than in older children (Fig. 7).[32]

Other challenges to improving the efficiency of CFTR gene transfer via inhalation include penetrating the thick mucus barrier that lies above the epithelial cell layer; developing vectors that recognize receptors on the apical surface of airway epithelial cells (rather than the basolateral surfaces, which are difficult to reach); delivering the DNA to the cell nucleus, which requires evading cytoplasmic proteases and penetrating the nuclear membrane; and establishing safety with repeat dosing, since epithelial cells that are transfected will be sloughed and replaced with nontransfected cells approximately every 40 days.

Inhaled DNA to Treat Lung Cancer

The other disease that shows promise for treatment via aerosolized gene therapy is lung cancer. In vivo and in vitro studies have demonstrated that binding of DNA with cationic polypeptides such as polylysine, polyethyleneimine, protamine, and histones may be useful for gene delivery.[33–37] These are nonviral vectors. Among these polypeptides, polyethyleneimine has received the most attention as a carrier for gene delivery because of its stability during nebulization. Studies have demonstrated that aerosol delivery of polyethyleneimine DNA complexes results in substantial gene expression in the lungs of mice,[38] and aerosol polyethylenimine-p53 therapy and aerosol polyethyleneimine interleukin-12 therapy significantly reduce

the number and size of osteosarcoma lung metastases in mice.[39] Studies are underway to clarify the response to long-term repeated exposures of these aerosol gene therapies in lung cancer models.

Vaccination

Advantages

A third example of the expanding role of aerosol therapy is vaccination via aerosol. The rationale for aerosolized vaccination is based on the following advantages over injection therapy. First, vaccination via inhalation avoids the need for disposal strategies for the large number of needles that would be used in mass vaccination campaigns in developing countries. Second, it prevents the spread of blood-borne diseases such as human immunodeficiency virus (HIV), which can be transmitted by improper use and handling of used sharps. Third, it induces protection by exposing the airway mucosa to virus, which is the natural route of infection for many diseases. Finally, it may work better with young children, in whom the persistence of maternal antibodies does not appear to interfere with mucosal immunization but does interfere with subcutaneous immunization.

Candidate Diseases for Aerosolized Vaccination

Several diseases are candidates for vaccination via inhalation, including measles, influenza, and rubella plus measles with a combination vaccine. The possibility of aerosol vaccinations against bioterrorism agents is also being explored.

Vaccination via aerosol is known to be effective. Thousands of people were successfully vaccinated with aerosols of live, attenuated strains of anthrax, plague, tularemia, and smallpox in the former Soviet Union, using tent-exposure systems.[40] Nevertheless, there are at least 3 considerations that need to be addressed when developing inhaled vaccines as commercial products: safety, choice of drug formulation, and choice of delivery device.

Safety of Inhaled Vaccines

It is likely that vaccination via inhalation will involve delivery of the vaccinating agent into the nasal cavity with some patients, especially nose-breathing infants. With nasal delivery there is the possibility of exposing the central nervous system to the vaccinating agent, via the cribiform plate, which is located within the olfactory region on the superior turbinate. Virus deposited on the olfactory mucosa could be absorbed into the neuron cells by endocytosic or pinocytotic mechanisms and could then be transported via intracellular axonal transport to the olfactory bulb, the brain, and possibly the cerebral spinal fluid.[41] This possibility was recently studied with rats that inhaled solid, ultrafine particles intranasally for 7 days, at 6 h/d. After 7 days of exposure the rats showed increased particle uptake in the olfactory bulb, compared to day zero.[42] However, the olfactory region in rats is known to have a substantially greater surface area than that of humans, which could lead to greater exposure and uptake, so it is not known if these findings can be extrapolated to humans.

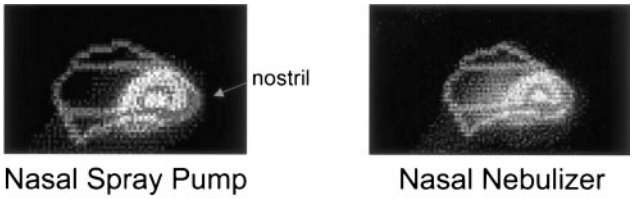

Fig. 8. Gamma-camera images of aerosol delivery within the nasal cavity of a volunteer after inhalation with a nasal spray pump (left) versus a nasal nebulizer (right). The light areas indicate deposition. Note that most of the upper third of the nasal cavity in the nasal-pump image is dark, compared to the same region in the nasal-nebulizer image, indicating better access to the vault of the nasal cavity with the nasal nebulizer. (Adapted from Reference 43.)

Another study looked at this possibility in humans. Delivery of aerosol to the upper region of the nasal cavity was quantified in a small group of adults who inhaled aerosol that was administered via nasal spray pump and via nasal nebulizer. Results from that study indicate that the vault of the nasal cavity is inaccessible with spray-pump delivery, but is more accessible with delivery of small aerosol particles from a nasal nebulizer (Fig. 8).[43] These data suggest that delivery of a vaccinating agent in the form of small aerosol particles from a nasal nebulizer could theoretically lead to deposition near, or on, the olfactory region mucosa. However, it is not known if the deposited material would be absorbed into the brain or central nervous system with this administration route.

Nevertheless, following the introduction of a new intranasal inactivated influenza vaccine in Switzerland in the 2000–2001 influenza season, an increased risk of Bell palsy was reported among recipients of the vaccine.[44] Bell palsy is the sudden onset of unilateral temporary paralysis of facial muscles from dysfunction of the seventh cranial nerve. Although it is unknown if the increase in incidence was due to exposure of the central nervous system to the vaccinating agent via the cribiform plate, the possibility of brain infection via olfactory-region uptake should be monitored in pre-clinical studies and clinical trials with inhaled vaccinating agents.

When developing a vaccinating agent for inhalation, it is important to consider the safety of HIV-infected persons and asthmatics who may be present in the population to be treated or who may be vaccinators. The risk of severe adverse events following administration of an aerosol vac-

cine to HIV-infected persons is unknown. Nevertheless, the potential may be greater in persons without pre-existing immunity to the virus and in those who are severely immunosuppressed. Fatal giant cell pneumonitis and measles inclusion body encephalitis due to measles vaccine virus have been reported in immunosuppressed persons following subcutaneous administration of measles vaccine.[45]

Considerations of safety in asthmatics arise for several reasons. First, excipients in the aerosol formulation may be allergenic and irritating. Second, virus infections may exacerbate asthma. Third, the severity of respiratory viral illness is higher in asthmatics, and asthmatics may be more susceptible to viral infections.

HIV-infected persons and asthmatics could be exposed to vaccine virus that is shed in the respiratory secretions of vaccinated individuals. In addition, vaccinators who have HIV, or are asthmatics, could inhale vaccine if aerosolized vaccine escapes around the mouth and nose of the vaccinee during face-mask breathing. Aerosolized vaccine that escapes during aerosol-blow-by technique could also be inhaled by vaccinators.

The safety of inhaled live influenza virus vaccine has been studied in patients with asthma. In those studies, FluMist (MedImmune Vaccines, Gaithersburg, Maryland) vaccine was administered via nasal spray. Inhalation did not exacerbate asthma in children between 9 and 17 years of age who had moderate-to-severe asthma.[46] However, a study of 9,689 children 1–17 years of age found a higher rate of asthma diagnoses within 42 days of vaccination in children $<$ 5 years of age. All asthma events occurred in children 18–35 months old. No one was hospitalized, and the asthma was resolved by treatment with β_2 agonist, antibiotics, systemic corticosteroids, or inhaled steroids.[47]

Another safety issue is the potential for contamination of the delivery device with respiratory pathogens from the vaccinee's saliva. Saliva could contaminate the face mask or mouth piece and could contaminate the nebulizer during exhalation. This could lead to the spread of pathogens to other vaccinees who use the same delivery device. One solution to this potential for contamination is to use a disposable face mask or mouth piece to eliminate direct exposure between vaccinees, and to use a one-way valve so that the vaccinee cannot exhale into the delivery device.

Choice of Formulation

Experience With Influenza Vaccine Aerosol. The second challenge to the development of inhaled vaccines is the choice of formulation. MedImmune Vaccines developed and received Food and Drug Administration approval for FluMist, a live, attenuated influenza vaccine that is a liquid and is administered via nasal spray. It contains attenuated strains of influenza A (H1N1), A (H3N2), and influenza B viruses. Attenuated viruses produce mild or no symptoms related to influenza virus infection. This aerosol formulation is temperature-sensitive (ie, replication is limited at 37°C for influenza type B strains, and at 39°C for type A strains) and cold-adapted (ie, replicates efficiently at 25°C).[48,49] These properties allow for viral replication only in nasopharyngeal epithelial cells.

Clinical trials with children and adults have demonstrated that intranasal administration of FluMist reduces the incidence of influenza and is well-tolerated, with rhinorrhea or nasal congestion and sore throat occurring more frequently than with placebo.[50]

Other investigators are developing spray-dried formulations that contain whole inactivated virus or split-subunit vaccine for aerosolization and mucosal vaccination of the pulmonary tract. These investigators use biocompatible excipients already approved for human use to deliver microparticles of vaccine to bronchial-associated lymphoid tissue. Their studies show that this approach is more effective than parenteral or nasal administration in triggering specific immunity in animals.[51]

Experience With Measles Vaccine Aerosol. Measles accounts for 5% of the global mortality among children $<$ 5 years old.[52] Over a decade ago, in Mexico, Albert Sabin, Jorge Fernandez de Castro, and their associates proved the feasibility of vaccination with aerosolized measles vaccine.[3–4] Since then, other trials have demonstrated that measles vaccine administered via aerosol provides a superior boosting response, compared to vaccination by injection in school-age children.[53,54] Results from another trial in 12 month-old children showed that percent seroconversion (an indicator of vaccination) was high, but not as high with inhaled vaccine as with injected vaccine.[55]

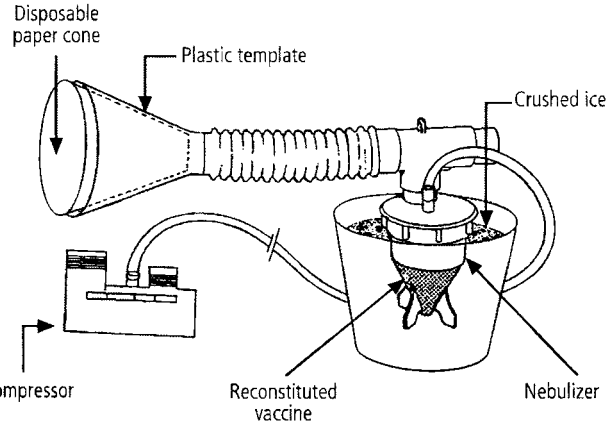

Fig. 9. Delivery system used to administer aerosolized measles vaccine in clinical trials in the 1980s and early 1990s in Mexico. This nebulizer system proved that immunization against measles was feasible via the aerosol route. (From Reference 53, with permission.)

Fig. 10. Delivery devices selected by the World Health Organization to administer aerosolized measles vaccine in upcoming clinical trials.

Additional studies are underway to determine if the dose administered to this age group needs to be increased to obtain higher percent protection.

Because of its needle-free delivery, the World Health Organization (WHO) has decided to explore the possibility of using an aerosolized measles vaccine in its mass immunization campaigns. The WHO decided to aerosolize the liquid formulation that is currently licensed for injection therapy and has proven effective via that route of administration in earlier studies. This is the Edmond Zagreb strain of measles vaccine. This choice meant that the WHO did not have to reformulate the vaccine, which could have resulted in years of additional testing. If feasible, they plan to test a dry powder formulation in later trials.

Choice of Delivery Device

Experience With Influenza Aerosol Vaccine. The third challenge to the development of inhaled vaccines is the choice of delivery device. In order to avoid the possibility of adverse effects related to exposure of the lung to influenza vaccine, MedImmune opted to deliver their liquid formulation directly into the nasal cavity via nasal sprayer.

Experience With Measles Aerosol Vaccine. For measles vaccine, nebulizer delivery is efficacious. This was demonstrated during the 1980s and early 1990s, when extensive field trials were conducted with an aerosolized formula of the Edmond Zagreb live attenuated measles vaccine. Figure 9 shows the aerosol device that was used.[53] Although that nebulizer system, also known as the classic Mexican device, demonstrated the feasibility of immunizing children against measles via the aerosol route, it lacks portability and requires an outside energy source for operation. The WHO has therefore conducted an extensive search to replace the classic Mexican device and has identified at least 3 devices that meet their criteria for replacement. These devices will be manufactured by Omron, Trudell, and Aerogen and are shown in Figure 10. Each has demonstrated performance characteristics that are similar to that of the classic Mexican device (ie, similar particle size, output, and vaccine potency) and have met specific usability and logistical criteria, including the safe dosing of numerous individuals with the same device and the capacity for aerosolization over many hours without the need of any outside energy source. These 3 devices will be included in the upcoming phase I and II clinical trials conducted by the WHO in India and Mexico. The possibility of delivering a combination aerosol vaccine to protect against measles and rubella is also being explored.

Vaccines Against Inhaled Bioterrorism Agents

Other diseases are being targeted for prophylactic vaccination via inhalation, including diseases that could be precipitated by the intentional release of airborne pathogens in a bioterrorist attack. Two such pathogens under study are *Bacillus anthracis* and *Francisella tularensis*. Inhalation of these bacteria has been shown to cause severe infection and death in humans.[56–58] Several laboratories are currently testing aerosol formulations (both liquid and dry powder) of vaccines against these pathogens in animal models.[59–60]

Summary

The role of aerosol therapy is clearly expanding beyond its initial focus on the treatment of asthma and COPD. This expansion has been driven by the Montreal protocol and the need to eliminate CFCs from traditional pMDIs, by the need for delivery devices and formulations that can efficiently and reproducibly target the systemic circulation for the delivery of proteins and peptides, and by developments in medicine that have made it possible to consider curing lung diseases with aerosolized gene therapy and preventing epidemics of influenza and measles with aerosolized vaccines.

The best example of the expanding role of aerosol therapy is the development of insulin as an aerosol to treat diabetes. Its progress toward Food and Drug Administration approval has been associated with unprecedented research and innovation in terms of new devices and formulations for both liquid and dry powder aerosols. Because it will be used as a long-term treatment for diabetes, demonstration of long-term safety in terms of its effect on lung function has been paramount to development. Finding ways to increase bioavailability should lower the costs of manufacturing and increase the use of this exciting innovation as an alternative to injection therapy for treating diabetes.

Compared to systemic drug delivery via the aerosol route, successful aerosolized gene therapy to correct the ion-transport deficiency in CF and to treat lung cancer appears to be many more years away. However, most of the problems have now been identified and solutions can be examined and tested. Hopefully, improvements in delivery technology and formulations will become available in the not-too-distant future, making the dream of curing lung diseases with aerosolized gene therapy a reality.

Aerosol immunization is a promising new method for vaccination. It has already been used in large populations and appears to be a feasible method for mass vaccinations. The benefits of this mode of vaccination clearly outweigh the small amount of risk. Improvements in delivery to infants and in the development of vaccines that are stable at the ambient temperatures of the tropics could make this the preferred route of administration for a number of vaccines.

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Discussion

Anderson: Even if you increased delivery of the CF aerosols, I think the mucus layer in the biofilm would prevent penetration of the vector. Is there anything known about that? Are there strategies to deal with it?

Laube: That's so important. As I mentioned, there are many factors that constrain gene-transfer efficiency. There are laboratories concerned about the mucus barrier, which is very thick. Even if you get enough drug into the lungs, how do you get it past that thick barrier into the epithelial cell? Some researchers are studying formulations that increase particle uptake through the mucus layer, that hold it there longer, or that push it deeper into the mucus so it is not stuck on the surface and removed by mucociliary clearance.

And there are other problems. The junctions between the epithelial cells are tight, which keeps toxic substances from getting below the apical surface. Unfortunately, most of the vectors that have been developed for gene delivery have their receptors on the basal-lateral surfaces of epithelial cells, which requires getting through those tight junctions. We need vectors that carry the material to the receptors on the apical surface, so that if they get through the mucus, they have a higher probability of entering the cell. Some researchers are looking at RSV, which has its receptors on apical surfaces. If we could inactivate the RSV and load on the genetic material, that might be one way to improve gene transfer efficiency.

Geller: I've been thinking about this problem in CF and similar diseases in which aerosol distribution is erratic and patchy. Some of the new therapies, such as gene therapy, anti-elastases, and antibiotics, are expensive, and some require milligrams of drug to reach the lung, not just micrograms, as with steroids and bronchodilators. Dr Smaldone mentioned the approach of controlling the breathing pattern to increase the spread or regional distribution of aerosol. Another way would be to add a compound that improves the spreadability and peripheral distribution of the drug; that would be especially useful for infants, in whom aerosol tends to deposit centrally. Have you seen anything about compounds that could improve spreadability within the lungs?

Laube: If drugs could get it into the surfactant layer, that might help. Or if we could deliver the drug mixed with a surfactant-like material, that might make it more spreadable.

Rubin: There have been several studies on co-administering surfactant with some of these medications.[1–3] There is interest in incorporating surfactants with spreadable materials as a way to enhance distribution of these particles. It might also enhance the distribution through the mucus layer.

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Ahrens: Regarding peptides, which are the leading edge of the field of systemic drug delivery, you talked about their relatively poor systemic bioavailability. Pfizer/Nektar insulin DPI actually delivers about 60% of the nominal dose to the lung, but far less than that actually shows up in the systemic circulation. You also talked about the small effects on pulmonary function that have been seen with this formulation. I'm wondering what happens to the rest of the insulin and whether this unaccounted-for insulin, or excipients such as mannitol, cause the small effects on pulmonary function.

Laube: I don't think there's any published data on what happens to drug that is not absorbed into the blood. Insoluble particles may be phagocy-

tosed and removed. Drugs that dissolve when they deposit are absorbed to some extent. Unabsorbed drug may be degraded by proteases or other enzymes, but this isn't clear.

Atkins: You mentioned that there's a difference in the insulin permeation between smokers and asthmatics, but which has the greater permeation?

Laube: Smokers absorb more insulin, and asthmatics absorb less insulin than nonsmokers and nonasthmatics.

Atkins: How big is the difference? How will this be handled? With a short-acting β agonist the patient gets quick feedback, because the symptoms are relieved, so the patient can titrate dose to response, but with insulin how is the patient going to know? Is a smoker at one end of the spectrum or the other end of the spectrum?

Laube: That's a big issue. Smokers' lungs are very leaky to inhaled substances. Smokers absorb more ^{99m}technetium chelated with DTPA than do nonsmokers. I don't know how much more insulin smokers absorb than nonsmokers, but I think it is an important difference, so diabetics who are smokers, and/or asthmatics, will probably need different doses of inhaled insulin than nonsmokers and nonasthmatics.

Nikander: A study with terbutaline with healthy nonsmokers and smokers showed that the peak plasma concentration was about twice as high in smokers as in nonsmokers.[1]

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Fink: My recollection was that it was a 2–3-fold difference.

Hickey: My concern is not that there is a difference between smokers and asthmatics in general, but that a change in the individual's disease state or smoking habits could be life-threatening if they are taking insulin. Is there anything known about individual variability?

Leach: There are a lot of questions about inhaled insulin. I would like to add some comments based on my previous experience. First, I would like to correct a misconception about D_{LCO}. The follow-up long-term studies seem to show no major safety concern regarding D_{LCO}. Some people don't really believe there ever was an important issue. The clinical technique for measuring D_{LCO} is not standardized, and the techniques differ widely. It is difficult enough to measure in laboratory animals, under highly controlled conditions. The D_{LCO} may or may not have decreased short-term, but safety studies indicate that it is not an issue long-term.

On another point, it is thought that the insulin in the lungs that is not bioavailable is metabolized by endogenous enzymes such as insulin degrading enzyme. Also, I don't think that the majority of inhaled insulin reaches the alveoli. The particles are too large to make it down to the alveoli, which in this case may be a good thing because of the relatively tight junctions between the alveolar epithelial cells. By contrast, the epithelial cell junctions in the conducting airways are much less tight and therefore probably allow penetration of larger molecules. Given the average particle size of inhaled insulin, it is more likely that the majority of drug is deposited in the conducting airways.

Laube: What about absorption across the alveoli?

Leach: I don't think there was any data to support that it ever got to the alveoli.

MacIntyre: It's well known that smokers have looser tight junctions, which goes along with the idea that most of the action is happening in the conducting airways.

Smaldone: It sounds like the asthma data and the COPD penetration data suggest that the bioavailability depends on the penetration. The data from the MannKind company suggests that aggregates of insulin might affect its permeability, because their excipient appears to prevent aggregation, and they found increased bioavailability.

Laube: Are you referring to the Technosphere technology?

Smaldone: Yes. The excipient surrounds the insulin, and they claim it increases bioavailability.

Laube: They claim 26% bioavailability.

Smaldone: The site of deposition might also be important. It may not get to the alveoli. Maybe it aggregates in the airways and that's why only 10% of drug will get across instead of 20%. Manipulating the molecule might increase bioavailability. Some companies are studying nanoparticles for drug delivery.

Laube: That's the Dura group, now part of Elan Pharmaceuticals.

Smaldone: What about nanoparticle toxicity? There's literature on it in the toxicology journals.[1,2] With some substances the toxicity of the particle is size-dependent; that is, larger particles are not that toxic, but nanoparticles of the same substance are more toxic, perhaps because of the concentration of the material or to how it bioreacts. Nanoparticles are very reactive. Should we worry about nanotoxicity?

REFERENCES

1. Oberdorster G. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health 2001;74(1):1–8.
2. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113(7):823–839.

Martonen: I think that question is well founded. Aerosol science and technology terminology has evolved, and what used to be called "ultra-fine" aerosols are now called "nanoparticles." There's a lot of literature in inhalation toxicology that establishes that the toxicity of ultra-fine aerosols or nanoparticles is independent of chemical composition, though we don't know why that's so. That idea is not new. The health effects of asbestos, for instance, are independent of its chemical composition; the health effects are a function of the shape and size of the asbestos fibers. That principle applies to ultra-fine particles. The mechanism of that is the subject of very intense arguments at inhalation toxicology meetings, but the 2 basic lines of reasoning are (1) there are so many nanoparticles and they have such a large surface area, they can function as carriers of toxic components of the atmosphere, and (2) they can act as irritants in the lungs, like sandpaper. If you ask 10 inhalation toxicologists whether toxicity is independent of chemical composition, you'll get five who say "yes" and five who say "no," which is startling. The pharmaceutical industry is going to have to take that into consideration.

Hickey: In the absence of any evidence about this point, the drug nanoparticles are the same as the environmental exposures. The key issue is the residence time of the particles. Many of the particles you talked about don't dissolve very readily, and I think some of these nanoparticles are intended to dissolve almost instantly, so there will be no nanoparticle to cause a problem. Residence time might be an issue with slow-release particles that do not immediately dissolve.

Leach: Most toxicologists would agree that it is a question of solubility. We have researched diesel exhaust and other ultra-fine particles, and most researchers suspect that it's the insoluble ultrafine particles that cause the problems. Most people in the pharmaceutical world don't want to make inhaled drugs that are in the nanoparticle size range, because they can be easily exhaled. Regarding the various inhaled insulin technologies, there is probably some equilibrium between monomer, dimer, and hexamer forms. It seems likely that monomer will cross the lungs more readily than hexamer and probably more readily than dimer. Some of the inhaled insulin technologies in development use insulin that is protected by a shell, and when the drug particle reaches physiologic pH, it releases the insulin immediately. It may be that most of the insulin is monomeric and therefore could go across the tight junctions quickly, reducing the opportunity for degrading enzymes to metabolize the insulin.