10 Pulmonary Drug Delivery Systems for Biomacromolecules

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THE PULMONARY ROUTE OF DRUG DELIVERY

Demand for improved methods for the delivery of biopharmaceutical agents initiated by the Human Genome Project (Olson 2002) has resulted in the development of numerous technologies. Although considerable effort has been spent on finding ways to deliver the biomacromolecules by the convenient gastrointestinal, nasal, buccal, and transdermal routes, these body surfaces are fundamentally impermeable and have restricted surface area, limited residence time; or other factors make the efficiency of penetration very low to all but the most potent small peptides. Of the small peptides absorbed through these routes, many have specific uptake mechanisms (Russelljones 1996), thus, the potential for widespread biomacromolecular drug delivery via these routes is difficult.

Two distinct areas of application of the pulmonary route for systemic delivery are inhalation delivery for situations where (1) rapid absorption of drugs is required than can be achieved by other noninvasive routes, especially by oral administration; and (2) absorption by other noninvasive routes is quite low (Gonda 2000). Attempts to deliver macromolecules through the lung for systemic effect have achieved mixed success. To date, the most successful example of delivery of biologically derived macromolecules is insulin. This work has been so successful that an inhaled formulation has reached phase III clinical trials (Laube et al. 1998). Other large biologically active proteins have also been delivered via the airways either to experimental animals or to humans. Proteins include recombinant human granulocyte colony stimulating factor (rhG-CSF) (Niven et al. 1993, 1995; Machida et al. 1996), interferon-α (INF-λ) (Giosue et al. 1996), interleukin-2 (IL-2) (Huland et al. 1992), and human growth hormone (hGH) (Patton et al. 1989–1990; Colthorpe et al. 1995; Wall and Smith 1997).

We present the current status of delivery of biotechnology-derived products by the pulmonary route of administration. Examples are included to demonstrate the usefulness of this approach for systemic delivery of therapeutic peptides, proteins and poly(nucleic acids) (PNAs). When possible, factors with a significant effect on observed performance results are discussed. This review will not be exhaustive, and further reading is suggested. The ideas and examples presented are intended to give a point of reference for the reader to gain an understanding of the possibilities of pulmonary drug delivery and to initiate the search for information.

Pulmonary drug delivery is one of the more underutilized and potentially beneficial routes of drug delivery available. Pulmonary drug delivery has been in use since the early 1950s, with many drugs absorbed by inhalation, although technological problems have prevented more drugs being administered via this route. Technology problems associated with pulmonary drug delivery are being overcome with advances in design. Delivery of a biologically derived agent by the pulmonary route requires an understanding of the anatomy and physiology of the lungs to determine the methods that can be used to administer a drug as well as potential problems that may be encountered.

ANATOMY AND PHYSIOLOGY OF THE RESPIRATORY SYSTEM

To administer a drug using adsorption in the lungs, the drug molecules must first arrive in the lungs. For healthy humans, there are two routes that lead into the lungs. The first leads through the nose and the second through the mouth; the nose and mouth along with the pharynx and associated structures are referred to as the upper respiratory system (Figure 10.1). The upper respiratory system leads directly into the lower respiratory system: larynx, trachea, bronchi, and lungs (Tortora and Grabowski 1993). Traditionally, pulmonary administration of drugs avoids the nose and nasal cavity by using the oral cavity; however, this is not always the case. The main reason for using oral and not nasal administration is that the nasal cavity creates a very turbulent environment that may cause an aerosolized particle to contact the

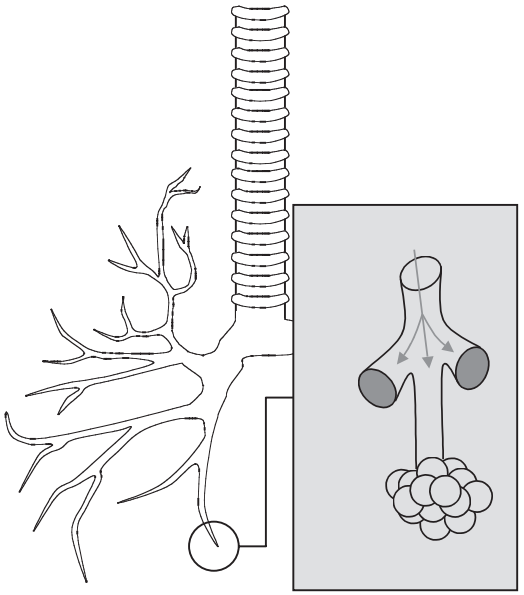

FIGURE 10.1 Schematic representation of the respiratory system. The trachea leads into the bronchioles that branch and finally reach the alveolar sacs only on one side of the bronchial tree. *Inset* represents the alveolar tubules and alveolar sacs present at the terminal portions of the bronchial tree.

nasal mucus and epithelium. This disadvantage for pulmonary administration is actually capitalized upon when using nasal delivery.

By avoiding the turbulent and restrictive environment of the nasal passage, upper respiratory deposition of aerosol particles for pulmonary delivery is very low (Cheng et al. 1999). Air that reaches the larynx from the nasal passage or from the oral cavity proceeds along the same route through the trachea to the lungs. The trachea splits into the two primary bronchi, left and right; the primary bronchi split into secondary bronchi, tertiary bronchi, and bronchioles. Secondary bronchi each lead to a lobe of the lung: superior, middle, and inferior lobes on the right and superior and inferior on the left. The bronchioles lead into terminal bronchioles that then lead into respiratory bronchioles. This network of branching bronchi and bronchioles is similar to the branching of a tree, thus it is referred to as the bronchial tree.

Analogous to the leaves of a tree are the alveolar sacs of the lungs; leaves convert sunlight to energy while the alveolar sacs allow gas exchange for the future production of energy for the organism (Patton 1996). It is at this point in the bronchial tree that most absorption of a drug takes place, although a molecule may not undergo transport exclusively at this point. This is not unexpected, as the alveoli are designed for optimal transport of molecules into the bloodstream. Only two cellular layers are present that allow molecular transport (Figure 10.2). Along with the alveolar epithelium and capillary endothelium, there is a bilayer of basement membrane,

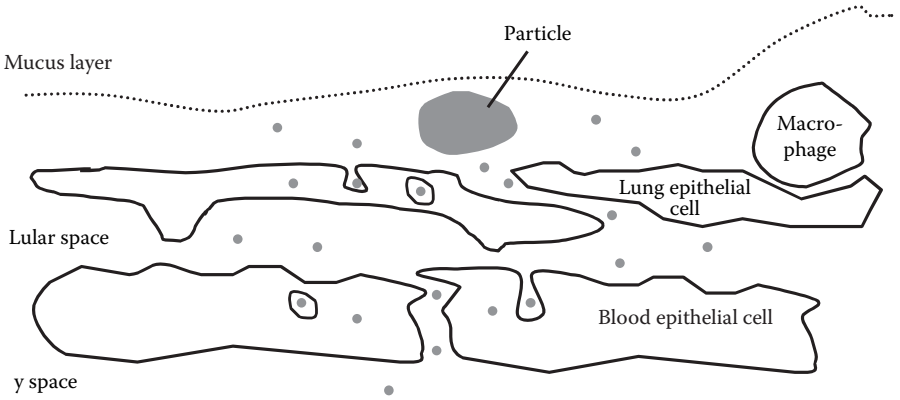

FIGURE 10.2 Schematic representation of alveolar cells and possible mechanism of transport of molecules from the alveolar space into the circulation. Particles will release molecules of interest (*gray circles*) into the mucus in which the particle is embedded. The molecule can either be lost in the mucus, taken up by alveolar macrophages by phagocytosis or diffusion, taken up by alveolar epithelial cells by passive or active transport, or bypass the alveolar cells via paracellular transport depending upon the properties of the drug. Once a molecule has reached the extracellular space, the same mechanisms are possible for transport from the extracellular space into the blood. Molecules in the extracellular space may also reach to circulation via the lymph.

which consists of alveolar epithelial basement membrane and capillary basement membrane. This distance, approximately 0.5μm, is one of the narrowest barriers for molecular transport into the body.

For this reason, the lungs represent an attractive route for drug delivery mainly due to the high surface area for absorption: 100–140 m^2 in humans (Altiere and Thompson 1996). Not only is the area for absorption high, but extensive vascularization of the alveolar epithelium allows efficient molecular transport. More important for small molecules than for biomacromolecules, the vasculature of the lung does not lead directly into the liver. Vessels leave the lungs and reenter the heart for distribution to the remainder of the body. Most biomacromolecules do not undergo the so-called first-pass effect, but many small biomolecule-derived molecules will have higher apparent absorption simply due to the lack of immediate hepatic clearance or metabolism.

Despite the fact that most of the alveolar surface is composited of alveolar epithelium, three primary types of cells are present in the alveoli: type I alveolar cells, type II alveolar cells, and alveolar macrophages. Type I alveolar cells are also referred to as squamous pulmonary epithelial cells and are the continuous lining of the alveolar sac. Type II alveolar cells are also referred to as septal cells. Type II alveolar cells secrete the alveolar fluid that is necessary to keep the surface moist and to maintain surface tension of the alveolar fluid; surface tension is necessary to keep the alveoli from collapsing. Alveolar fluid is a suitable environment for proteins when compared to the low pH and high protease levels associated with the intestine

and oral drug delivery, although some proteolytic activity does exist (Yang et al. 2000). Transport of molecules across the mucus of the intestine may even be slower than the transport in the surfactant layer of the lungs. Lung surfactant is particularly devoid of protein content. Many suggestions have been proposed for this lack of protein in the fluid of the lung, but cellular uptake/renewal and fluid flow/regeneration have been cited as potential reasons. If cellular uptake/renewal is the main mechanism, then molecular delivery following pulmonary administration is very promising. A hypothetical model for absorption of macromolecules across alveolar type I cells is shown in Figure 10.2 (Patton 1996).

LIPID-BASED PULMONARY DELIVERY

Liposomes and micelles are lipid vesicles composed of self-assembled amphiphilic molecules. Amphiphiles with nonpolar tails (i.e., hydrophobic chains) self-assemble into lipid bilayers, and when appropriate conditions are present, a spherical bilayer is formed. The nonpolar interior of the bilayer is shielded by the surface polar heads and an aqueous environment is contained in the interior of the sphere (Figure 10.3A). Micelles are small vesicles composed of a shell of lipid; the interior of the micelle is the hydrophobic tails of the lipid molecules (Figure 10.3B). Liposomes have been the primary form of lipid-based delivery system because they contain an aqueous interior phase that can be loaded with biomacromolecules. The ability to prepare liposomes and micelles from compounds analogous to pulmonary surfactant is frequently quoted as a major advantage of liposomes over other colloidal carrier systems.

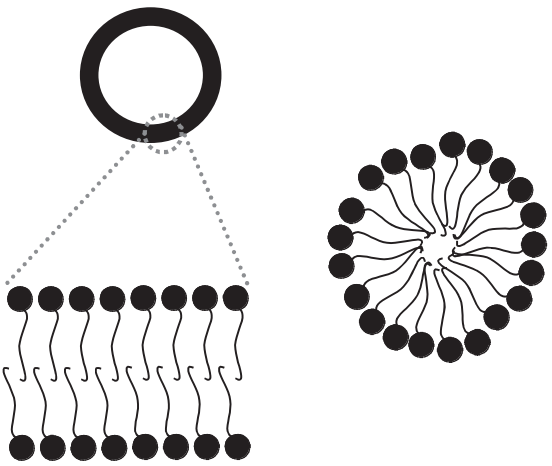

FIGURE 10.3 Schematic presentation of lipid based drug delivery systems. Micelles (A) are composed of a solid lipid core with the polar heads exposed to the aqueous environment. Liposomes (B) are particles with a lipid bilayer surrounding an aqueous core. Drug can be encapsulated in the hydrophobic regions of the lipid particle, in the aqueous environment of the liposome, or adsorbed to the surface of the lipid particle.

Most materials used to produce liposomes are derived from natural materials, thus are thought to be safe when administered. Generally, however, phospholipids administered in liposomal form are cleared from the lungs more slowly than comparable doses of lung surfactant (Oguchi et al. 1985). Many macromolecules have been incorporated into liposomes in order to improve their pulmonary delivery. Some lipid-entrapped macromolecules have been tested in animal models and human volunteers to determine efficacy (Kellaway and Farr 1990).

Insulin is the protein that has been most investigated for pulmonary administration. Insulin levels are not maintained in diabetic patients, and precise control over blood glucose levels is needed. Insulin is a small protein, 5.8 kDa, which is composed of two chains that are covalently linked by an interchain disulfide bond. Currently, insulin is administered by injection, several times a day for many diabetics. The ability to deliver insulin via a noninvasive route would free diabetics from inconvenient, invasive insulin delivery methods and possibly eliminate secondary problems associated with diabetes, such as diabetic retinopathy.

Liposomes were formed from 1,2-dipalmitoylphosphatidylcholine (DPPC) and cholesterol (Chol) and the effect of liposomal entrapment on pulmonary absorption of insulin was related to oligomerization of insulin (Liu et al. 1993). Instillation of both dimeric and hexameric insulin produced equivalent duration of hypoglycemic response. However, the initial response from the hexameric form was slightly slower than that from dimeric insulin, probably due to lower permeability across alveolar epithelium of the hexameric form caused by larger molecular size. The intratracheal administration of liposomal insulin enhanced pulmonary absorption and resulted in an absolute bioavailability of 30.3%. Nevertheless, a similar extent of absorption and hypoglycemic effects was obtained from a physical mixture of insulin and blank liposomes and from liposomal insulin. This suggests a specific interaction of the phospholipid with the surfactant layer or even with the alveolar membrane.

Charge on the surface of a liposome can be controlled by the addition of specific molecules to the liposome composition; charge can influence both the interaction of the lipid with the lung and with the molecule being delivered. Physical mixture of insulin with positively charged liposomes was observed to show a strong hypoglycemic effect. Negatively charged and neutral liposome physical mixtures with insulin showed a lower hypoglycemic effect, but still had a significant response when compared to insulin alone (Figure 10.4.) (Li and Mitra 1996). Greater bioavailability of insulin observed using positively charged liposomes is suggested to be due to the membrane destabilizing effect of stearylamine. Stearylamine is one of the more toxic components that have been traditionally added to liposomes as a positive charge-inducing agent due to its basic nature. Unfortunately, the more toxic charge-inducing agents are the most effective at transferring proteins. This fact is not unexpected since the mechanism that is expected to allow molecular transport is the destabilization of cell membranes; toxicity from lipids is also typically by a destabilization mechanism.

Phospholipids, such as DPPC, act as absorption enhancers in the lung. A significantly higher reduction in blood glucose levels was observed with a DPPC-insulin physical mixture compared to liposome-insulin following intratracheal instillation into rats (Figure 10.5) (Mitra et al. 2001). In this study, insulin alone, 1 U/kg, resulted

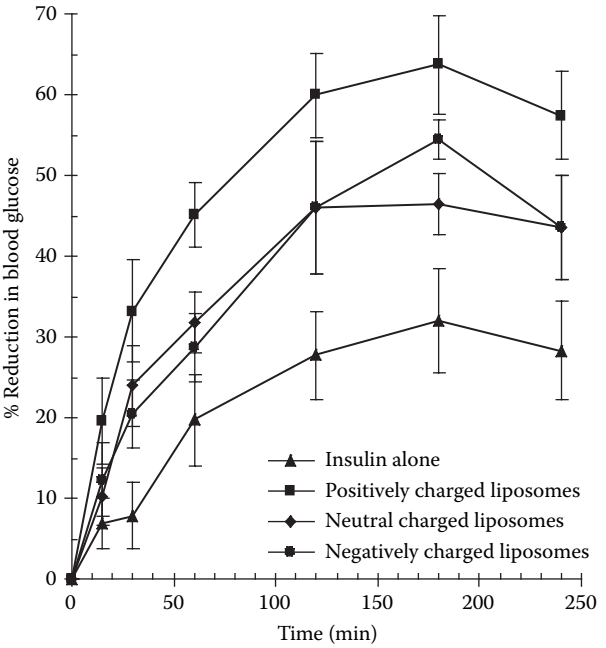

FIGURE 10.4 Reduction in blood glucose following pulmonary administration of 1 U/kg insulin (Å), insulin encapsulated in positively charged liposomes (■), insulin encapsulated in negatively charged liposomes (●), insulin encapsulated in neutral liposomes (♦). (Adapted from Li and Mitra 1996).

in a 32% reduction in blood glucose after 3 hours, while an insulin-liposome mixture resulted in a 50% reduction in blood glucose. The highest hypoglycemic effect was obtained with a DPPC-insulin physical mixture, generating a 73% reduction. This suggests that the physical mixture of lipid and protein may actually be a more efficient transport mechanism, potentially due to entrapment of insulin in the liposome that cannot escape. This was just one case of increased delivery by physical mixture with lipid, but the idea that entrapment may not be necessary cannot be overlooked when delivering biomacromolecules.

Not only will the charge of a lipid and the composition of lipids affect the delivery of biomacromolecules, but the size of the liposome may alter the transport. Mixtures of insulin with three different diameter (1.98 μm, 0.4 μm, and 0.1 μm) neutral liposomes (DPPC: Chol) resulted in similar overall hypoglycemic effects to insulin alone. Contrary to this finding is the fact that pulmonary absorption of liposomal [^{3}H] terbutaline, a small molecule, has been reported to be dependent on both composition and size of the liposomes used (Abra et al. 1990). Differences in the absorption mechanism may be the explanation for this contradictory evidence; further studies are needed to clarify this and other uncertainties about the uptake mechanism of macromolecules (Patton 1996).

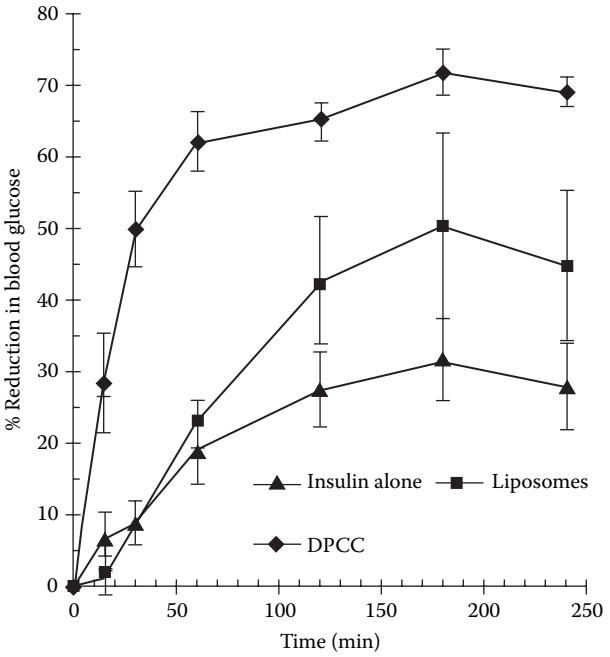

FIGURE 10.5 Reduction in blood glucose following pulmonary administration of 1 U/kg insulin (Å), insulin encapsulated in liposomes (■), insulin physical mixture with DPCC particles (♦). (Adapted from Mitra et al. 2001).

Proteins are transported across the pulmonary epithelium effectively, and poly(nucleic acids), such as DNA, are also transported. Nucleic acids can be efficiently expressed in the lungs by intravenous injection of cationic lipid and plasmid DNA complexes (Liu et al. 1995, 1997). The safety of intra-pulmonary cationic lipid delivery in healthy volunteers has been investigated (Chadwick et al. 1997). A single application of aerosol formulation of a cationic lipid [N^4-spermine cholesterylcarbamate (GL-67), dioleoyl phosphatidyl-ethanolamine (DOPE), dimystoyl phosphatidyl-ethanonamine conjugated with poly(ethylene glycol) (DMPE-PEG_{5000})] did not result in clinically detectable changes when given by nebulization into the lungs of healthy volunteers and provides an indication of a lipid dose tolerated in man. Cationic lipids are necessary to transfer anionic PNAs into cells as they bind to the anionic DNA macromolecules; anionic lipids repel anionic PNAs.

The therapeutic efficacy of either systemic or local pulmonary delivery of the IFN-γ gene was evaluated in a murine allergen–induced airway hyper-responsiveness (AHR) model (Dow et al. 1999) and it was found that a high efficiency of gene transfer could be achieved. For intratracheal administration cationic liposomes were prepared from a mixture of 1,2-diacylglycero-3-ethylphosphocholine (EDMPC) and cholesterol. Intravenous injections were prepared from 1,2-dioleyl-3-trimethylammonium propane (DOTAP) and cholesterol and compared with pulmonary administered

IFN-γ. Although the reason for using different cationic lipids in different routes was not mentioned, probably the size of liposomes would have had an impact on choosing alternate cationic lipids for administration via different routes. Intravenous IFN-γ gene delivery significantly inhibited development of AHR and decreased serum IgE levels, compared to control mice. Intratracheal IFN-γ gene delivery also significantly inhibited AHR and airway eosinophilia, but did not affect serum IgE levels. Treatment with IFN-γ was much less effective than IFN-γ gene delivery by either route. This is one example of an effective gene therapy that illustrates the potential of pulmonary lipid-based PNA delivery. Of major importance is the fact that careful selection of lipid composition when considering both protein and PNA delivery.

Pulmonary administration of PNAs has great potential for the same reasons that pulmonary protein and peptide delivery have been successful. Predominantly, the distance for transport and ease of administration of agents are the advantages of pulmonary delivery, but the formulation of labile molecules for eventual pulmonary administration as lipid-based aerosols may be problematic.

SOLID COLLOIDAL PARTICLES

Microparticles (MP) are small colloidal particles, with a diameter less than 1000 μm, and generally composed of a solid matrix in which a drug is dispersed and/or dissolved. Microparticles have been produced from a wide variety of biodegradable materials of both natural (e.g., chitosan, gelatin, albumin) and synthetic origin (e.g., polyesters of lactic acid, glycolic acid, and -caprolactone). Microparticles can be prepared in different sizes and many hydrophilic and hydrophobic drugs have been entrapped or incorporated with relatively high efficiency and different drug release rates can be achieved. In comparison to liposomes, MP may be more stable both *in vitro* and *in vivo*, therefore producing a slower release rate and a longer duration of action of the incorporated agent. Higher stability of MP may provide an opportunity for optimization of duration of pulmonary release and action. The following sections describe several of the most promising materials used to produce small colloidal particles and how they have been used for pulmonary drug delivery of biotechnology-derived products.

POLY(DL-LACTIDE-*CO*-GLYCOLIDE)

Poly(DL-lactide-*co*-glycolide) (PLGA) is a synthetic polymer that has been successfully applied in the medical field. PLGA is composed of lactic acid and glycolic acid monomers, which are natural molecules in the body. The only problem that has been associated with PLGA polymers is the fact that as the polymer degrades by water hydrolysis in physiologic conditions, acid is formed as the lactic and glycolic acid is liberated. Consequently, degradation of the incorporated molecules is possible, so care must be taken during formulation. To counteract this effect, buffering molecules are at times added to the formulation to keep the environment neutral and prevent acid catalyzed degradation of the incorporated molecules.

Rifampicin-loaded PLGA microparticles (RifMP) were studied for treatment of pulmonary tuberculosis (Suarez et al. 2001a,b). A guinea pig infection model has been adopted as a post-treatment screening method for antimicrobial effect. Rifampicin, alone or encapsulated in microparticles was delivered by insufflation or nebulization. There was a dose-dependent relationship between insufflated RifMP and burden of bacteria in the lungs. In addition, guinea pigs treated with RifMP had a smaller number of viable bacteria, reduced inflammation, and lung damage than control (lactose and saline), PLGA, or RIF treated animals. Nebulization was more efficient in reducing the number of viable microorganisms in the lungs at equivalent doses of RifMP than was insufflated. This is due to the deeper penetration of the nebulized MP when compared to insufflated MP. Results with a small molecule in a locally administered model suggested that PLGA microparticles should in fact be nebulized or aerosolized in order to be active in the lungs. It was still necessary to show that a biotechnology-derived agent could be administered by this method.

Poly(DL-lactide-*co*-glycolide) MP have successfully been used to deliver insulin via the pulmonary route (Kawashima et al. 1999). Insulin loading was much improved compared to the methods previously described (Masinde and Hickey 1993); incorporation of hydrophilic molecules, and specifically proteins, is typically quite low for PLGA MP. Eighty-five percent of the drug was released from the MP at the initial burst *in vitro*, followed by prolonged release of the remaining drug for a few hours. After nebulization, a prolonged hypoglycemic effect was achieved (48 h) compared to the nebulized aqueous solution of insulin (6 h); however, only 2.6% of the nebulized mist was delivered into guinea pig lungs during a 20 minute nebulization.

One important factor that has to be considered for all types of pulmonary delivery: particles deposit in the lungs based on their aerodynamic diameter (Equation 1). For a spherical particle, the aerodynamic diameter (d_{aer}) is equal to the product of actual diameter (d) times the square root of particle density (ρ) (Gonda 1992).

$$d_{aer} = d \cdot \sqrt{\rho} \tag{1}$$

Particles of aerodynamic diameter greater than 5 μm deposit primarily in the upper airways or mouth and throat region, while a significant percentage of those less than 1 μm do not deposit but are exhaled (Darquenne et al. 1997). Therefore, for optimum deposition in the alveolar region and systemic delivery, particles have to be between 1 and 5 μm.

In an attempt to increase the amount of particles retained in the lungs, large porous particles with low density ($\rho < 0.1$ g/cm^2) have been designed (Edwards et al. 1997). The particles were composed of 50% lactide and 50% glycolide. Porous and nonporous particles loaded with testosterone were aerosolized into a cascade impactor system from a dry powder inhaler (DPI) and the respirable fraction was measured. Nonporous particles (d = 3.5 μm, ρ = 0.8 g/cm^3) exhibited a respirable fraction of 20.5 ± 3.5%, whereas 50 ± 10% of porous particles (d = 8.5 μm, ρ = 0.1 g/cm^3) were respirable, even though the aerodynamic diameter of the two particle types were nearly identical. Porous particles as a consequence of their large size and low mass density can

aerosolize from a DPI more efficiently than smaller, nonporous particles, resulting in higher respirable fraction. Insulin encapsulated in porous ($d = 6.8$ μm, $d_{aer} = 2.15$ μm) and nonporous ($d = 4.4$ μm, $d_{aer} = 2.15$ μm) PLGA particles and administered in rats by force-ventilation resulted in activity of insulin in both cases. For large porous particles, insulin bioavailability relative to SC injection was 87.5%, whereas the small nonporous particles yielded a relative bioavailability of 12% after inhalation. This dramatic increase in insulin activity should be achievable for other biologically derived molecules. One particular aspect of increased insulin bioavailability that must be examined is the fact that large particles can avoid phagocytic clearance from the lungs until they have delivered their therapeutic dose. For nonporous particles, 30 ± 3% of phagocytic cells contained particles immediately after inhalation, compared to 8 ± 2% for large porous particles. This again is a dramatic difference in phagocytosis. The combined effect of greater respirable fraction and lower phagocytosis should greatly improve the efficiency of pulmonary biomolecular delivery.

Although PLGA microparticles, in general, offer several advantages including providing sustained delivery of drug from a biocompatible and nontoxic polymer, some limitations as a carrier for drugs in the lungs do exist. First, small PLGA microparticles degrade over a period of weeks to months, but typically deliver drugs for a shorter period of time (Batycky et al. 1997; Edwards et al. 1997). Therefore, unwanted build-up of polymer in the lungs upon repeat administration may occur. Also, the hydrophobic surface of PLGA MP can cause rapid opsonization (protein adsorption), resulting in a rapid clearance by alveolar phagocytic cells (Tabata and Ikada 1988). However, this problem was solved by large porous PLGA microparticles with low mass density (Edwards et al. 1997). Finally, bulk degradation of PLGA microparticles creates an acid core, which can damage pH-sensitive drugs such as peptides and proteins (Mader et al. 1996). Depending on the compositions of the MP and the desired dosing regimen, PLGA MP may be an optimal delivery vehicle, but for frequent administrations, high molecular weight PLGA may not be an optimal choice.

POLYCYANOACRYLATES

An alternative to PLGA for the production of MP is poly(alkyl cyanoacrylates) (PCNA). PCNAs are a type of polymer that is produced under mild conditions by anionic polymerization. PCNAs have low toxicity and are used in medical applications, including surgical adhesives. These polymers also degrade rapidly in the body when compared to PLGAs, decreasing the residence time for the particles after drug delivery has been completed. These advantages over PLGAs have been exploited for delivery of insulin.

Prolonged hypoglycemic effect of insulin was reported after using poly(butyl cyanoacrylate) microparticles with a mean diameter of 254.7 nm (Zhang et al. 2001). Insulin-loaded poly(butyl cyanoacrylate) microparticles were prepared by emulsion polymerization in the presence of insulin. Insulin-loaded microparticles were administered intratracheally to normal rats. The duration of glucose levels below 80% of baseline was maintained for a longer period when insulin was administered in

microparticles than when insulin was administered as a solution at all doses. Relative pharmacological bioavailability of the insulin loaded in microparticles was 57.2% compared to the same formulation by subcutaneous administration. Nevertheless, insulin solution resulted in decreased glucose levels compared to insulin-loaded microparticles after single intratracheal administration. The equivalent initial decrease in glucose levels is explained by some permeability of the lung epithelium to insulin, but the prolonged glucose level decrease suggests that PCNA microparticles can be used for extended delivery of biologically derived molecules.

GELATIN

Gelatin is a naturally derived polymer that has been used to produce microparticles for pulmonary delivery. The natural derivation and biodegradability of gelatin have pushed it to the forefront as a material for producing pulmonary drug delivery systems. Gelatin must, however, be crosslinked by synthetic means to produce MP. Typically, gluteraldehyde is used to crosslink gelatin into a solid matrix. Gluteraldehyde is a toxic molecule and can retain activity in the MP. For this reason, care must be taken to fully remove unreacted gluteraldehyde prior to administration. Gluteraldehyde, after reacting with gelatin, may revert to form reactive gluteraldehyde over time; even when care was taken to remove or react all gluteraldehyde, free, reactive gluteraldehyde may be present. Gluteraldehyde may also react with the molecule being incorporated, thus decreasing the activity of the molecule or changing the activity altogether. For this reason, biomolecules must be added to the MP after the particle has been formed. Typically, loading efficiency is quite low and the release period is not typically more than a few hours. For this reason, residual gelatin MP may be problematic as described with PLGA MP. Control of gluteraldehyde crosslinking can be used to decrease the time for complete degradation, but not completely.

Gelatin MP loaded with salmon calcitonin have been investigated (Morimoto et al. 2000). Positively and negatively charged gelatin microparticles with different sizes were prepared. *In vitro* release studies showed that the release of salmon calcitonin from negatively charged gelatin microparticles was slower than from positively charged gelatin microparticles indicating a potential ionic interaction with the MP. Cumulative calcitonin release from negatively charged gelatin microparticles reached approximately 40% after 2 hours and then leveled off compared to 85% in positively charged microparticles. In addition, the release profiles were not influenced by particle size. Pulmonary absorption of salmon calcitonin from positively and negatively charged gelatin microparticles were estimated by administering the formulation intratracheally (via tracheotomy) in rats. Results indicated that salmon calcitonin in positively and negatively charged gelatin microparticles reduced plasma calcium levels greater than salmon calcitonin in PBS (pH 7) (Figure 10.6). Administration of salmon calcitonin in positively charged gelatin microparticles with smaller size particles led to higher pharmacological availability.

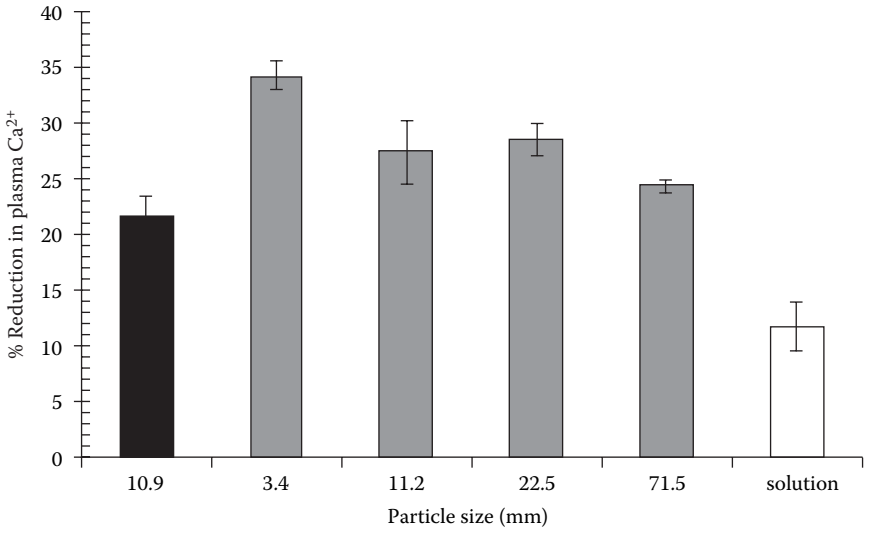

FIGURE 10.6 Hypercalcemic effect in rats following administration of 3 U/kg of salmon calcitonin in solution (*white fill*) and negatively charged (*black fill*) or positively charged (*gray fill*) gelatin microspheres of indicated size. The time to reach maximum effectiveness ranged from 0.5 to 1.5 hours. (Adapted from Morimoto et al. 2000).

POLY(ETHER-ANHYDRIDES)

A relatively new class of polymers, polyether-anhydrides (PEAs), for pulmonary delivery has been reported (Fu et al. 2002). PEAs are hydrolyzed in a fashion similar to PLGAs, but due to the lability of anhydride bonds, PEAs have significantly faster degradation rates than PLGAs. By controlling the ratio of anhydride to ether bond, the degradation rate can be tuned. These polymers are composed of the monomers sebacic acid (SA) and PEG. Microparticles prepared from poly(SA-co-PEG) using a double-emulsion solvent-evaporation method. Large, low-density particles with aerodynamic diameters between 1.9 μm (30% PEG) and 3.7 μm (0% PEG) have been produced. Various amounts of PEG (5–50% by mass) were incorporated into the backbone of new polymers. The variations in PEG allowed tuning of particle surface properties for potentially enhanced aerosolization efficiency. The PEG also decreased particle clearance rate by phagocytosis in the deep lung; steric stabilization was proposed as the mechanism of phagocytosis control. Drug delivery from these particles have not been examined *in vivo*, however, cell culture models have shown that the particles do affect the transepithelial resistance in some models, but not others (Fiegel et al. 2003). Because of the biocompatibility and degradation rates of these polymers, these materials may, in fact, be excellent for use as pulmonary drug carriers.

DIKETOPIPERAZINE DERIVATIVES

A composition based on diketopiperazine derivatives (3,6-bis (N-fumaryl-N-(n-butyl) amino-2, 5-diketopiperazine) has been investigated as a pulmonary drug delivery system, termed Technospheres™ (Pharmaceutical Discovery Corp., Elmsford, NY) (Pohl et al. 2000; Steiner et al. 2002). The diketopiperazine derivatives self-assemble into microparticles at low pH with a mean diameter of approximately 2 μm. During self-assembly, diketopiperazine derivatives microencapsulate peptides present in the solution. Insulin incorporated in diketopiperazine derivatives (TI) was administered to five healthy humans by the use of a capsule-based inhaler with a passive powder deagglomeration mechanism. Relative and absolute bioavailability of TI in the first 3 hours (0–180 min) were 26 ± 12% and 15 ± 5%, and for 6 hours (0–360 min) 16 ± 8% and 16 ± 6%, respectively (Steiner et al. 2002). The time to peak action for glucose infusion rates was shorter with both IV (14 ± 6 min) injection and inhalation (39 ± 36 min), as compared to SC administration (163 ± 25 min). This rapid absorption of insulin would be beneficial for diabetic patients who need to rapidly affect their glucose levels.

By the use of a breath-powered unit dose dry powder inhaler, which was adapted to the physical properties of TI, relative bioavailability was 50% for the first 3 hours and 30% over the entire 6-hour period in 12 healthy volunteers (Pfutzner et al. 2002). However, although the studies demonstrated pulmonary administration of TI has the advantages of fast onset of action, short duration of action, and lower variability over the SC injections of insulin; no attempt has been made to compare pulmonary administration of insulin alone with the same inhaler device. This method of encapsulating biomacromolecules has some advantages and must be considered when electing to deliver a molecule.

POLYETHYLENEIMINE (PEI)-DNA COMPLEXES

Aerosol delivery of genes has been drawing more attention during recent years due to a targeted and noninvasive approach to the treatment of a wide range of pulmonary disorders. Polycations have been reported to be effective vectors for transfecting cells *in vitro* and *in vivo* (Boussif et al. 1995, 1996). PEI-DNA complexes have been administered through the pulmonary route via aerosol as a means of gene therapy and genetic immunization (Densmore et al. 2000). *In vivo* transfection efficiencies of liposome-DNA and PEI-DNA complexes after aerosol and nasal delivery were compared, and the results indicated PEI was a 10-fold better vector for expression in the lungs than bis-guanidiunium-tren-cholesterol (BGTC): DOPE and compared even more favorably to other cationic lipid formulations. Aerosol delivery resulted in about 20-fold greater transfection efficiency in the lungs than in the nose for PEI-based formulations. Genetic immunizations by aerosol and instillation administration of PEI-cytomegalovirus promoter-human growth hormone (pCMV-hGH) plasmid vaccines has been studied in mice with IM injection of naked DNA as a positive control (Figure 10.7). Antibody induction by genetic immunization with PEI-pCMV-hGH in animals exposed to the PEI-pCMV-hGH IM injection showed the levels of the antibody response persisted through 8 weeks from a single administration of th

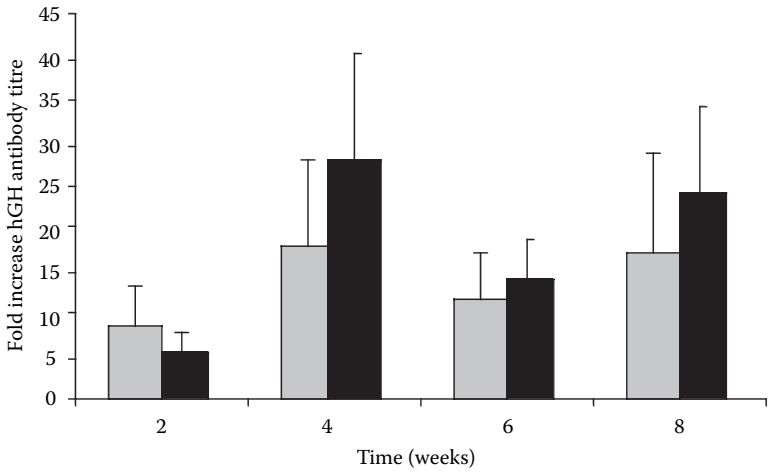

FIGURE 10.7 Antibody to hGH produced in mice following PEI-pCMV-hGH plasmid immunization via IM (*black fill*) and aerosol (*gray fill*) administration detected 2, 4, 5, and 8 weeks following induction. Fold increase is the number of times greater than untreated control animals. (Adapted from Densmore et al. 2000).

plasmid-PEI preparation and were essentially equivalent to those achieved with IM injection. This suggests that PEI-DNA complexes could be used for pulmonary administration of genes. Poly(ethyleneimine) is more efficient than liposomes at transferring the DNA to the cells, but may be more toxic. Alternative polymers are being investigated by many groups to create a less toxic polymeric carrier for gene transfer.

POLY(ETHYLENE GLYCOL) CONJUGATES

Poly(ethylene glycol) (PEG)-protein conjugates have become of interest in medical applications due to the ability of PEG to protect a protein from degradation and recognition. PEG is covalently linked to the protein through amino groups, carboxylic groups, or thiol groups depending on the biomacromolecule. Care must be taken as modification of a protein at or near the active site will decrease the biological activity of the protein; although in some cases the slight decrease in activity is insignificant when compared with the protection from degradation and clearance. Even modification at a site far from the active site has devastating consequences on the activity of many proteins, so care must be taken and each individual protein must be examined.

The pulmonary delivery of rhG-CSF (18.8 kDa) and two PEG conjugates of rhG-CSF (P1, 81.5 kDa and P2, 146.8 kDa) was investigated in rats (Niven et al. 1994). Comparison of white blood cell responses after IT instillation of 500 μg/kg P1 and P2 and rhG-CSF alone, demonstrated a greater response for more substituted PEG-rhG-CSF than rhG-CSF alone. The plasma concentration vs. time curve showed

a T_{max} at 160 ± 65 minute for rhG-CSF versus 347 ± 183 minute and 210 ± 83 minute for P1 and P2, respectively. However, lower bioavailability was obtained for PEG-rhG-CSF. All bioavailability values (500 μg/kg and 50 μg/kg) were less than 30%, and those of both PEG proteins were less than 5%. There was an apparent inverse relationship between the extent of absorption and the size of the protein. However, the absorption of rhG-CSF at the two doses was markedly different (3%, 50 μg/kg vs. 27.4%, 500 μg/kg) suggesting absorption may be influenced by other clearance mechanisms. Even though the absorption of PEG-rhGH-CSF is low, biologic activity is present. Again, this is a positive sign for pulmonary biomolecular delivery. This field is in its infancy of development and more research is needed to determine the exact mechanisms of biomolecular transport in the alveolar epithelium. However, it is clear that by use of appropriate modification or encapsulation, the uptake of biomolecules can be increased in the lung.

FACTORS AFFECTING PULMONARY DOSING

There are some factors that must be considered when comparing different formulations with respect to efficiency and bioavailability, and the results have to be interpreted very carefully. There are differences between inhaled aerosols and instilled liquids. Comparison of the pharmacokinetics following IV insulin administration with the pharmacokinetics following IT and aerosolized insulin administration in rabbits showed improved peripheral lung deposition after aerosol administration (Colthorpe et al. 1992). The penetration index (peripheral vs. central deposition) was significantly higher for the aerosol treatment (1.52 ± 0.36) compared to IT treatment (0.32 ± 0.08). Aerosol formulation administration produced a bioavailability of nearly 10-fold greater than produced by IT instillation (Table 10.1). It follows that more test macromolecules will be trapped in the mucus layer of the conducting airways of the central rather than peripheral deposition pattern and therefore will be cleared faster by mucociliary clearance.

TABLE 10.1
Insulin Pharmacokinetic Parameters by Different Routes of Administration (mean™ SD, n = 4)

Dose	IV (0.1 IU/kg)	IT Instillate (5.0 IU/kg)	Aerosol (300 IU/ml nebulized for 4 min)
t_{max} (min)	—	11.3 ± 4.8	12.5 ± 2.9
$t_{1/2}$ (min)	3.0	49	69
F (%)[a]	100	5.6 ± 3.3[b]	57.2 ± 28.5[b]

[a]F-values are calculated relative to 100% after IV dosing.
[b]Significantly different ($P < 0.05$)
Adapted from Colthorpe et al. 1992.

In general, IT instillation is an easy, rapid, and relatively inexpensive method for testing pulmonary drug delivery. It requires a small amount of drug for efficient administration and provides precise and noninvasive dosing. Intratracheal administration is best used to determine whether a test macromolecule is absorbed from the lung (El Jamal et al. 1996). In contrast, aerosol delivery is expensive, inefficient, technically challenging, and difficult to precisely quantify the delivered dose in animals (Brown and Schanker 1983). However, this technique has the advantages of being physiologically relevant and providing a more even distribution of test molecules in the lung than intratracheal administration (El Jamal et al. 1996). Even when the appropriate method of drug installation is chosen, care must be taken to appropriately choose an animal model.

Species differences in lung structure and function illustrate that caution must be exercised in extrapolating to the human lung (Hickey and Garcia-Contreras 2001). Compared to other species (rats, dogs, and baboons), human lungs were found to contain a greater number of macrophages, alveolar type II, endothelial, and interstitial cells (Table 10.2) (Crapo et al. 1983). The thickness of interstitium and

TABLE 10.2
Morphometric Parameters in the Alveolar Region of Normal Mammalian Lungs

	Sprague Dawley Rat	Dog	Baboon	Human
Body weight, kg	0.36 ± 0.01	16 ± 3	29 ± 3	79 ± 4
Lung volume, ml	10.55 ± 0.37	1322 ± 64	2393 ± 100	4341 ± 284
Surface area,[a] m^2/both lungs				
Alveolar epithelium				
Type I	0.387 ± 0.025	51.0 ± 1.0	47.7 ± 7.7	89.0 ± 8.0
Type II	0.015 ± 0.002	1.0 ± 0.2	1.9 ± 0.3	7.0 ± 1.0
Capillary endothelium	0.452 ± 0.035	57.0 ± 2.0	38.6 ± 9.5	91.0 ± 9.0
Tissue thickness, μm				
Harmonic mean, air-to-plasma	0.405 ± 0.017	0.450 ± 0.007	0.674 ± 0.055	0.745 ± 0.059
Total Lung Cells, %				
Alveolar type I	8.9 ± 0.9	12.5 ± 1.7	11.8 ± 0.6	8.3 ± 0.6
Alveolar type II	14.2 ± 0.7	11.8 ± 0.6	7.7 ± 1.0	15.9 ± 0.8
Endothelial	42.2 ± 1.1	45.7 ± 0.8	36.3 ± 2.4	30.2 ± 2.4
Interstitial	27.7 ± 1.8	26.6 ± 0.7	41.8 ± 2.7	36.1 ± 1.0
Macrophage	3.0 ± 0.3	3.4 ± 0.6	2.3 ± 0.7	9.4 ± 2.2
Alveolar Surface Coverage, %				
Alveolar type I	96.2 ± 0.5	97.3 ± 0.4	96.0 ± 0.6	92.9 ± 1.0
Alveolar type II	3.8 ± 0.5	7.1 ± 1.0	4.0 ± 0.6	7.1 ± 1.0

[a] Type I surface area (SA) is the surface area of the basement membrane under type I cells; type II SA is the air surface of type II cells excluding the extra SA contributed by microvilli; endothelial SA is the luminal surface of the endothelial cells.

Adapted from Crapo et al. 1983.

pulmonary capillary endothelia were also significantly greater in human lungs than in lower primates and mammals. However, despite these differences, an overall similarity in the characteristics of individual lung cells was observed (Crapo et al. 1983).

Intracellular pH in alveolar epithelial cells may also vary among species. Several different values for baseline steady state intercellular pH have been published for alveolar pneumocytes (Lubman and Crandall 1992). The baseline intracellular pH of isolated alveolar type II cells has been reported to be between 7.07 (Nord et al. 1987) and 7.36 (Sano et al. 1988) for rats and 7.22 for rabbits (Finkelstein and Brandes 1987). Secretions stored in mucous tubules of glands in the respiratory tract vary among species. Mucous tubules in the mouse form mainly sialomucin and to a smaller extent sulfomucin. In rat tracheolaryngeal glands, the mucus tubules, which predominate, produce and store sulfomucin in abundance. Human respiratory tract mucous tubules, on the other hand, show roughly equal proportions of sulfated and of nonsulfated, sialylated mucosubstances through these structures (Spicer et al. 1983; Hickey and Garcia-Contreras 2001). Cytochrome P-450 monoxygenase activity has been demonstrated in the mouse, rat, hamster, rabbit, and pig. No data are available regarding the activity in other species such as humans, guinea pigs, dogs, cats, and sheep (Plopper 1983; Hickey and Garcia-Contreras 2001). These differences between animal species and humans makes the interpretation of results more complicated, so choice of species for animal studies must be carefully chosen with no specific animal suggested to date for a generic test subject. But species-to-species differences in lung properties are not the only factor that must be examined; there are various types of inhalers, the selection of which greatly influences absorption.

The pulmonary deposition of an aerosol depends on (Matthys and Kohler 1985)

- Type of aerosol generator (physical principle, outlet geometry, and outlet speed)
- Properties of the particles (size, density, shape, solid, fluid, hygroscopicity, and electric charge)
- Route and mode of application (by mouth, nose, tracheostoma, bolus, continuous)
- Breathing maneuver (tidal volume, frequency) and duration
- Morphometry of the upper and lower airways (degree of obstruction)

Aerosols can be generated by three main drug delivery systems: nebulizers, pressurized metered dose inhaler (pMDI), and dry powder inhaler (DPI).

Nebulizers are usually prescribed to patients who are unable to operate other inhalation devices, for example because of poor hand-lung coordination (Nikander 1997). Use of nebulizers have some limitations including relatively long treatment times and lack of portability. Nebulizers use aqueous solutions of drugs, so drug instability in aqueous solutions via hydrolysis would preclude the use of nebulizers. Many colloidal particles can be used to circumvent aqueous degradation, and thus could be used in nebulizers. In addition, the process of nebulization exerts high shear stress on the compounds, which can lead to protein denaturation. Furthermore, the droplets produced by nebulizers are rather heterogeneous, which results in poor drug delivery to the lower respiratory tract (Agu et al. 2001).

MDIs utilize propellants (chloroflurocarbons and increasingly, hydrofluroalkanes) to atomize drug solutions resulting in a more uniform spray and improved effectiveness compared to nebulizers (Agu et al. 2001). Easy handling and convenience to the patient are other advantages of these devices; however, some proteins and peptides are susceptible to denaturation when they come into contact with propellants. The large air–liquid interface that is constantly being generated during aerosolization may also cause denaturation of macromolecules (Banga 1995). The high exit velocity of drug aerosol can lead to high levels of oropharangeal impaction, and the need for users to coordinate the pMDI valve actuation with their breathing maneuver make MDIs a difficult apparatus for some users (Smart 2002). Improvements on the design of MDIs may make this type of device more highly applicable.

DPIs represent a significant advance in pulmonary delivery technology. They are potentially suitable for delivering a wider range of drugs than MDIs, including biopharmaceutically derived therapies for systemic applications such as peptides and proteins. DPIs have a key advantage over pMDIs because they are breath-actuated and therefore do not require patient coordination. Since the production of an aerosol with a DPI is independent of the breath of the user, less shear stress and no propellants are needed for production of the aerosol. Many modifications of DPIs are currently available with more being designed.

Even when the appropriate inhaler is chosen, the influence of the disease state cannot be ignored. Disease states can influence the dimension and properties of the airways and hence the disposition of any inhaled drug. Thus, great care must be taken when extrapolating the findings based on intratracheal administration to different animal species in order to predict deposition profiles after inhalation of aerosol formulations by patients suffering from airway disease. DPIs are not appropriate in many diseases when the ability to have sufficient airflow is hindered. Since many diseases that we would like to treat via pulmonary administration of biomolecules cause a decrease in airflow, we must be careful in the decision of which type of inhalation mechanism to choose.

CONCLUSIONS

In this chapter, different delivery systems such as liposomes and microparticles for pulmonary administration of macromolecules were reviewed. However, comparison of various delivery systems is problematic due to different aerosol devices, methods of administration (inhalation vs. IT) and animal models. The past 10 years have seen dramatic changes in delivery of drugs by inhalation and there is good evidence to show that pulmonary administration of drugs with poor oral bioavailability can be very effective in achieving significant plasma concentrations. Few adverse effects have been noted in the clinical trials conducted to date, and the toxicology studies have, in general, produced only modest lung effects. The limited data available also suggest if SC delivery has been well tolerated immunologically, there is a reasonable expectation that inhalation delivery may also be well tolerated (Wolff 1998). This may be of particular benefit for many biologically derived substances, which are currently given parenterally, without the associated complications and discomfort.

The field of pulmonary administration of biomacromolecules is just now in its infancy, and much promise is held in this field. By carefully examining the methods and types of delivery that are possible we may be able to better design easy to use and inexpensive methods for delivering biotechnology-derived products. This chapter was neither all-inclusive nor comprehensive, but was intended to be a starting point for the examination of pulmonary administration of biotechnology-derived drugs.

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