Expert Opinion

Disease guided optimization of the respiratory delivery of microparticulate formulations

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Background: Inhalation of microparticulate dosage forms can be effectively used in the treatment of respiratory and systemic diseases. *Objective*: Disease states investigated for treatment by inhalation of microparticles were reviewed along with the drugs' pharmacological, pharmacokinetic and physical chemical properties to identify the advantages of microparticulate inhalation formulations and to identify areas for further improvement. *Methods*: Microbial infections of the lung, asthma, diabetes, lung transplantation and lung cancer were examined, with a focus on those systems intended to provide a sustained release. *Conclusion*: In developing microparticulate formulations for inhalation in the lung, there is a need to understand the pharmacology of the drug as the key to revealing the optimal concentration time profile, the disease state, and the pharmacokinetic properties of the pure drug as determined by IV administration and inhalation. Finally, *in vitro* release studies will allow better identification of the best dosing strategy to be used in efficacy and safety studies.

Keywords: Aerosol, amphotericin B, asthma, cystic fibrosis, fungal infection, infection, insulin, lung cancer, microparticles, transplantation, tuberculosis

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1. Introduction

The process of optimizing respirable, microparticulate dosage forms or any other drug delivery system must first begin with a clear goal. As a review that is intended to stimulate improved formulations, the ultimate goal is the development of ideal respiratory delivery systems. The ideal formulation for respiratory drug delivery is an aerosol because of overwhelming patient preference. An aerosol is defined as a dispersion of solid or liquid particles in air. Aerosol formulations will generally provide an immediate release and rapid absorption of a drug, which should be designed to deliver a controlled and accurate dose. However, the focus of this review is the use of microparticulate formulations, which can extend the duration of drug delivery. At present, the ideal respiratory drug delivery system has not been produced even for those formulations intended for immediate release. Therefore, sustained delivery of drug within a formulation composed of relatively small, respirable particles poses a monumental challenge.

Nevertheless, therapeutic success can be achieved with non-ideal drug delivery systems and remarkable progress has been made in treating a number of disease states. These include drug therapy for microbial infections, chemotherapeutic agents for the treatment of lung cancer, diabetes, and bronchodilators, and steroids for asthma. In this review, the salient features of the biopharmaceutics of drug delivery to the lung are discussed first. Thereafter, disease states that have been studied for respiratory delivery are examined along with the drugs that have potential therapeutic activity. In this context, each drug delivery system is evaluated and the progress and

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shortcomings in achieving ideal drug delivery with the use of microparticulate formulations are critically assessed.

2. Biopharmaceutics of aerosol delivery of drug to the lower respiratory tract

The discussion is limited to drugs delivered to the lower respiratory tract or the lung. The upper respiratory tract is not included because it represents a different administration site with distinct requirements for aerosol delivery.

Because of patient preference, it is desirable to have the aerosol delivery take place in a short time period. Thus, there is a conscious effort to administer the entire dose in a single inhalation. The aerosol generating devices currently available, such as the metered dose inhaler (MDI), dry powder inhaler (DPI) and the soft spray, electrohydrodynamic atomizer, generally can deliver drug in a single breath. On the other hand, nebulizers, including jet and ultrasonic devices as well as the newer vibrating plate aerosol generator, take longer and are therefore less desirable. The requirement of a short administration time introduces another limitation in that continuous administration can not be used to extend the duration of drug delivery. Thus, there remain two options to maintain the desired concentration of the drug in the lung. Either the drug must be selected to have a low rate of lung clearance or the release rate of drug from the formulation must be slow.

In considering these two options, it should be noted that the lung is generally very permeable to most compounds. As shown in early studies by Shanker *et al.* (as reviewed by Sakagami) [1], the rate of absorption of free drug is rapid, which is not surprising and entirely consistent with the gross and microscopic anatomy of the lung. Specifically, the area of the lung is typically quoted to be about 100 m^2. The vast majority of the lung area consists of a single cell layer, and most cells are flat to reduce the distance between the epithelial fluid and the blood stream. Larger molecules, such as proteins, immunoglobulins and DNA, represent exceptions to the general rule of high lung permeability. Due to this, maintaining the concentration in the lung requires a slow release rate from the formulation for most compounds.

Another underlying complexity that adds to the challenge of formulating drugs for continuous delivery is the well-developed clearance mechanisms for removing particulate matter from the lung. The problem is further compounded by the interplay between the site of deposition and clearance rate, although this perhaps opens a door of opportunity. The lower respiratory tract is divided into the conducting airways and pulmonary airways, which, as their names imply, provide a pathway to move air from the trachea of low surface area to the peripheral alveolar region of high area. With these physiologically distinct functions come physiologically unique properties and clearance mechanisms. For particles deposited in the conducting airways, the mucociliary system rapidly removes particles such that a negligible mass will remain in the lung 24 h after dosing. As such, the free drug concentration in the lung is determined by the residence time of the formulation in the lung, the release rate, and the permeability of the lung to the drug.

For drugs within particles deposited in the pulmonary region, the free drug concentration also depends on the residence time, release rate and lung permeability, but the environment is distinct. This leads to quantitative differences. The lining of the pulmonary region has lipid-rich lung surfactant and macrophages. Solubilization of the drug in lung surfactant can lead to incorporation in the type II cells and thereby a longer residence time. Particles that do not dissolve may interact with lung surfactant and/or be engulfed by macrophages. This leads to a relatively slow clearance rate. However, the lung surfactant can enhance the dissolution rate, and with the thin alveolar membrane the rate of drug clearance may be relatively rapid.

The slow clearance rate of particles from the pulmonary region of the lung provides an opportunity for long term delivery, as numerous toxicological studies have demonstrated that insoluble particles may remain within the peripheral lung for weeks or even months after deposition [2]. As such, it remains an attractive area to target sustained release formulations. However, nanoparticles appear to be able to translocate across lung tissue and then gain access to the lymph or systemic blood circulation [3].

3. Identifying an ideal drug delivery system

The ultimate goal of formulation development is the optimization of the delivery of drugs. Optimized drug administration entails delivering the drug to the correct location and providing an effective concentration for the required length of time thereby achieving the therapeutic objective with no side effects. Location is dictated by the disease state, and the required concentration and duration are dictated by the inherent pharmacological properties of the drug. The challenge lies in manipulating the formulation to produce a release rate appropriate for the pharmacokinetic properties of the drug that will result in a concentration-time profile at the site of action that will meet the pharmacological requirements. This must of course be in a product that will be readily accepted by the patient, otherwise non-compliance will result in failed drug delivery.

The work in the area of respiratory drug delivery has been contributed by scientists with strong backgrounds and in-depth knowledge of technology. In addition, the ever increasing importance of intellectual property causes research to focus on the development of novel, non-obvious delivery systems that can be readily reduced to practice. Thus, reading the literature can generate the impression that the drug delivery systems are invented as solutions looking for therapeutic problems.

An alternate perspective is to state the therapeutic problem and then identify the ideal drug delivery solution. However,

even with diseases that appear to have a straightforward therapeutic objective, it is difficult to identify an optimal delivery system because the optimal concentration-time profile is unknown. For example, the minimum effective concentration of antibiotics can be determined *in vitro* and the pharmacokinetics following aerosol inhalation can also be readily assessed, so it would seem that an ideal delivery system can be easily identified. However, even with the use of tobramycin, where such studies have been reported, neither the required concentration nor duration of therapy is known with certainty [4]. Thus, selecting the method of drug delivery becomes a relatively complex process despite the limited number of active agents for treating lung diseases. The fundamental problem is that there is an infinite set of possible concentration-time profiles that will result in failure and perhaps either no possibilities or an infinite set of possibilities that will lead to success. Moreover, it may take years, as in clinical studies addressing tuberculosis (TB) or lung cancer, to determine whether success has been achieved. It is within this realm that the respiratory delivery of particles falls.

Finally, a recent and extensive review has been written that describes the various means for generating microparticles [5]. The article places a special emphasis on the advantages and disadvantages of each technology with respect to the engineering and manufacturing methods of generating aerosols. This review will complement rather than repeat that effort by taking the perspective of the final therapeutic objective. That is, the disease state is described and then the advantages and disadvantages of the formulations that have been used are provided.

4. Disease states

Respiratory drug delivery provides an inherent advantage over other routes of administration in several disease states. Most of these involve diseases of the lung, but inhalation may also be an attractive alternative for drugs that can not be readily given by the oral route. Significant advances in developing respiratory drug delivery systems are evident in the improved treatment of a number disease states. However, none is ideal. Thus, ample opportunities remain in this field for improving health through the development of better respiratory delivery systems.

4.1 Infectious diseases

Infectious diseases of the lung offer a clear example where local delivery should have an inherent advantage over systemic (oral or intravenous) delivery. When the microorganism, whether it is viral, bacterial or fungal, is limited to the respiratory tract, delivery of drug to the lung will yield a high local concentration at the site of action and reduced concentration in the systemic circulation. Together, the result will be high efficacy and low toxicity. This is particularly important for antibiotics that commonly perturb the normal flora of the gastrointestinal tract or have a narrow therapeutic index.

For spatial localization, antimicrobial agents have selectivity between the microbe and human cells by virtue of their mechanism of action. Thus, delivery to the lung is generally sufficient making more refined targeting unnecessary, although there is an exception for macrophages in TB as discussed below. However, the effective concentration and duration varies from agent to agent. That is, antibiotics may either be 'concentration-dependent', 'time-dependent', or 'concentration-time-dependent' agents [4]. For concentration dependent agents, the parameter that correlates best with the logarithm reduction in microbial number is the peak concentration. For these agents, aerosol delivery of drug that is immediately available is ideal, and no modification of the rate of release would be needed. That is, aerosol delivery provides for a very high location concentration that quickly dissipates, which effectively reduces the microbial count.

On the other hand, for agents that are time-dependent, the reduction in microbial count is best correlated with the time during which the concentration of agent exceeds a minimum effective concentration. Here, a sustained delivery should provide a better kill rate in extending the exposure and reducing the toxicity by reducing the peak concentration. The third category contains agents that have a kill rate that correlates with the AUC. Here too, controlled delivery can provide an advantage.

For determining the required drug concentration, it should be ascertained whether the drug is being used to treat an active infection, to act as a preventive agent for patients at risk of getting an infection, or to eradicate latent organisms or spores. Finally, reports of the pharmacokinetic description of drugs delivered to the respiratory tract encapsulated within microparticulate dosage forms must be interpreted cautiously. Measurement of drug levels rarely involves distinguishing between encapsulated drug and drug that has been released, that is, free drug. Thus, achieving high levels or even sustained levels of total drug is of no value, if the free drug concentration never exceeds the minimum effective concentration.

4.1.1 *Bacterial infections and aminoglycosides, tobramycin, gentamycin, and amikacin*

The first therapeutic problem to be considered is the repeated respiratory infections that occur with individuals suffering from cystic fibrosis (CF). As provided in more detail by Hagerman *et al.* [4], *Pseudomonas aeruginosa* is found in 50% of all CF patients and in about 80% of all patients 18 years and older. In younger CF patients, *Staphylococcus aureus* is predominant, with *Haemophilus influenzae* and *Burkholderia cepacia* also being significant.

The currently approved inhalation product for treating respiratory infections is a tobramycin solution (TOBI®, Chiron Corp), although studies have also been carried out with other aminoglycosides (amikacin and gentamycin).

Table 1. Microparticulate formulations of antibiotics for bacterial infections of the lung.

Drug	Formulation	Ref.
Tobramycin	Liposomes, microspheres	[11]
Tobramycin	Fluidosomes	[15]
^{99m}Tc-Tobramycin	Pulmonary surfactant	[109]
Tobramycin	Liposomes	[16]
Tobramycin	Lipid-coated particles	[18]
Tobramycin	Pulmonsphere	[17]
Amikacin	Liposomes	[110]
Amikacin	Liposomes	[111]
Ciprofloxacin	Spray-dried Liposomes	[112]

The absolute systemic availability of tobramycin following aerosol administration to humans ranged between 9.13 and 17.5% [6-8]. This was based on urinary recovery, which represents a reasonably good estimate of that absorbed from the lung due to the low oral availability and minimal metabolic clearance. In two different studies, the sputum concentrations normalized by dose in the nebulizer were 3.33 and 4.12 ug/ml/mg [6,9]. Similar results were obtained with gentamycin at 2.35 ug/ml/mg [10]. Reproducibility in the sputum concentrations was poor, which was suggested to arise from the low and variable oral availability. However, variability in patient inhalation is also a likely culprit.

The MIC of tobramycin against *P. aeruginosa* is 4 ug/ml but may rise to 100 ug/ml in purulent sputum of CF patients [4]. As the nebulizer dose of tobramycin can be over 600 mg, the sputum concentrations that can be achieved will easily exceed the required MIC (600 mg × 3 – 4 ug/ml/mg = 1800 – 2400 ug/ml). This is noteworthy and indicates the tremendous advantage direct inhalation delivery to the lung has over intravenous (IV) administration. This is particularly pronounced with tobramycin, as sputum levels are typically only 12 – 20% of the serum levels [6]. Thus, IV administration will only achieve the needed sputum concentrations at doses that also cause severe systemic side effects.

In CF patients, the pharmacokinetics has been determined following nebulized TOBI [6]. A biexponential model was used to fit the serum data yielding initial and terminal half-lives that ranged from 0.077 to 2.85 h and 5.95 to 19.3 h, respectively. Cooney *et al.* [7] also reported a mean absorption time of 0.25 – 2.5 h following aerosol administration to normal patients. The variability in the initial half-lives may be related to differences in the site of lung deposition, whereas the variability in the terminal half-lives may be a result of differences in renal function. For the initial clearance, the rate of drug absorption from the lung to the systemic circulation would be expected to be a function of the area of deposition. In addition, when only 5 – 10% of the dose is deposited, small changes in the percent deposition will lead to large changes in sputum and serum levels.

If it is desired to have a constant lung level of tobramycin, the above studies form a good starting point to assess the required delivery rate. Scant information is available on the clearance rate of tobramycin from the lung, but the information from Mukhopadhyay *et al.* [9] and Geller *et al.* [6] can be used. Following aerosol delivery, the peak serum concentration was 1.27 ± 1.07 mg/l, and the systemic clearance was 6.98 ± 2.89 l/h. Thus, to maintain this peak concentration, the required zero order delivery rate is found from the product of the concentration and clearance rate, (1.27 × 6.98) mg/h = 8.86 mg/h. There is no data on lung clearance, but the terminal half-life appears significantly longer than the initial half-life. Thus, the sputum concentration can be assumed to be at equilibrium with the serum concentration, albeit at perhaps 5 – 10 times the concentration. As such, with a serum concentration of 1.27 ug/ml, the lung concentration may optimistically be maintained at 12.7 ug/ml, which is well below the required MIC of 100 ug/ml.

Despite the expected failure to achieve a sputum concentration of 100 ug/ml, therapy may be beneficial for select patients even at the lower tobramycin concentrations. However, delivering nearly 9 mg of drug every hour by aerosol would require 216 mg (= 24 h × 9 mg/h) of drug in a once a day delivery system and enough excipients to prolong the release of this water soluble compound for 24 h. Again, even with a 50% drug load in the formulation, the total weight of the dosage form is now at 432 mg. Finally, the deposition fraction must be taken into account, which was only 17% [8] and leads to a total mass required at 2,541 mg (= 432 mg/0.17). This mass of drug can not be readily administered to the lung and, if attempted, may result in increased side effects, such as coughing and thereby poor patient acceptance. Thus, within the framework of optimization, it may not be realistic with current technology to achieve a constant delivery of tobramycin in a once a day dosage form.

The analysis does reveal the areas that need to be addressed to improve the chance of therapeutic success. First, the administration can be limited to those patients with lower MICs. The deposition fraction, dosing frequency and possibly the drug load can also be increased. An alternative strategy is to develop a formulation for preventive therapy rather than treating active infections. The thinking is that a lower concentration of tobramycin is needed to prevent bacterial growth than is needed to kill.

In **Table 1**, the studies involving the preparation of tobramycin in respirable microparticles are given. One of the earliest studies was carried out by Poyner *et al.* [11], who prepared both liposomes and polymeric microparticles. The main finding of this study was that both formulations can sustain the release of tobramycin, although there was an initial burst effect in the first 12 h and release was not complete even at 240 h. A second finding was that the

percent distribution to the lung was higher and to the kidney lower with intratracheal (IT) administration in comparison to IV; significant but fully expected.

Liposomes are often used to prolong the delivery of drug. A very thorough review was written that covers the early efforts in the delivery of liposomes to the respiratory tract [12]. For liposomes, it is important to consider the inherent deposition and clearance of the lipid components as originally studied by Farr *et al.* [13]. Pulmonary deposition of multi-lamellar and small unilamellar vesicles, when delivered by a jet nebulizer, was dependent on droplet size. Short term clearance of both liposome populations was typical of mucociliary transport, resulting in statistically equivalent retention at 6 h. Subsequent retention data suggested that faster processes than those described for insoluble particulate were contributing to the clearance of alveolar deposited liposomes.

Lagace and co-workers [14,15] developed a low phase transition temperature liposomal-tobramycin formulation for delivery to the lung. They carried out IT administration and demonstrated that a chronic pulmonary infection of mucoid *Pseudomonas aeruginosa* could be eradicated in an animal model [14]. In a subsequent study, the same liposomal formulation was administered as a dry powder aerosol using free antibiotic as a control, with a chronic infection established by IT administration of 10^5 colony forming units (cfu) of a mucoid variant of *P. aeruginosa*. Sixteen hours after a single treatment, the cfu counts were 4.31×10^5 cfu/lungs with liposomes, 1.32×10^8 with free antibiotic and 3.02×10^8 cfu/lungs in the untreated [15]. The authors suggested that sufficient deposition occurred to treat chronic pulmonary infection caused by *Pseudomonas*.

Marier *et al.* [16] compared the pharmacokinetics and pharmacodynamics of tobramycin in rats. Following IT administration of tobramycin, liposomes caused a reduction in the rate constant of absorption from the lungs to the systemic circulation from 1.64 to 0.57/h corresponding to an increase in $t_{1/2}\alpha$ from 0.13 to 0.68 h. The $t_{1/2}\beta$ also increased from 14 to 34 h. The IT administration was associated with more reproducible lung levels. There appeared to be greater activity against *P. aeruginosa*, but the results were not impressive, as the number of animals with a bacterial count of less than 10^3 cfu only increased from 9.4 to 28%. The lack of impressive results may be related to a lower free concentration, which was sacrificed to achieve the increased duration.

Newhouse *et al.* [17] examined the effect of tobramycin in the patented formulation of PulmoSphere, which consists of porous particles. In a dose escalation study in humans, the percent deposition was increased to 35% in comparison to the 5% observed with nebulized tobramycin solution. The peak serum concentration was higher at 0.6 ug/ml in comparison to the 0.28 ug/ml observed in the controls. The AUC was also higher. Although the formulation was well tolerated and 150 mg could be given in 6 inhalations (equivalent to 81 mg tobramycin base), the low serum levels may correspond to relatively low lung concentrations that would be insufficient for significant antibacterial activity.

Pilcer *et al.* [18] reported the development of a dry powder inhalation system consisting of lipid-coated particles of tobramycin. In principle, the kinetics of release will be zero order provided that the diffusion through the coat remains the rate limiting step. This is in contrast to liposomes or drug dispersed in microparticles, where the release kinetics are expected to be a first order or dependent on the square root of time. The lipids were a mixture of cholesterol and phospholipon (hydrogenated soy phosphatidylcholine with about 85% distearoyl phosphatidylcholine and 15% dipalmitoyl phosphatidylcholine). Although progress was made toward the development of a DPI formulation, no release data was provided, and the percent load was only 10% at the maximum level, which severely limits the mass of tobramycin that could be delivered to the lung.

4.1.2 *Tuberculosis and rifampicin, isoniazid and pyrazinamide*

TB is the second therapeutic problem considered. An astronomical 1.86 billion people in the world (32% of the world population) are infected with TB, and there are over 3 million associated deaths each year [19]. This chronic, communicable disease is caused by *Mycobacterium tuberculosi* and is most often spread by inhalation. About 75% of the infections are localized within the lung. In the 1980s, there was an optimistic hope that TB would be eradicated in the twenty-first century. Unfortunately, the disease has rebounded with the emergence of resistant strains, which pose a threat to health care workers not only in third world countries, but also in the US.

These statistics become even more incredible, when realizing that there exists effective oral drug therapy. The primary, first line drugs, isoniazid (INH), rifampicin (rifampin) and pyrazinamide, can be used in combination and all are available in relatively inexpensive generic forms. However, the doses are very high and range from 2.5 to 25 mg/kg [20]. Furthermore, a long duration of therapy (2 – 6 months) is required to irradiate the slow growing micobacteria that reside within macrophages or encapsulated lipid nodules. The high doses and long duration lead to severe side effects (e.g., liver and renal toxicity and peripheral neurotoxicity). Finally, patients become asymptomatic with just a few weeks of therapy. Feeling better and encountering unpleasant side effects can lead patients to become non-compliant, which has been a significant factor in the development of resistant strains.

INH, rifampicin and pyrazinamide are all very well absorbed with oral administration, although rifampicin has a significant first pass effect with enterohepatic recycling (**Table 2**) [21]. The time of maximum concentration occurs in 1 – 3 h. The drugs are all widely distributed, but only rifampicin is significantly protein bound (85%). Each is metabolized in a

Table 2. Summary of the pharmacokinetic measures, median (minimum-maximum) for antituberculin drugs [113].

Drug	2-h level (mg/l)	T_{max} (h)	C_{max} (mg/l)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ ((mg h)/l)
Rifampicin	4.4 (0 – 14.8)	2.5 (1.0 – 8.0)	5.9 (1.3 – 14.9)	1.9 (0.9 – 4.7)	25.6 (5.6 – 80.1)
Isoniazid	5.0 (0.4 – 13.6)	1.5 (0.5 – 4.0)	6.5 (0.5 – 15.0)	2.89 (1.0 – 8.4)	32.5 (3.5 – 87.2)
Pyrazinamide	49.6 (1.3 – 86.6)	2.0 (0.5 – 4.1)	52.7 (1.5 – 91.8)	5.9 (2.7 – 12.9)	499.7 (18.7 – 1,246)
Ethambutol	3.1 (0.8 – 9.8)	3.0 (1.0 – 6.1)	5.0 (0.2 – 10.4)	2.6 (1.1 – 8.1)	24.9 (6.9 – 54.0)

somewhat complicated manor. The terminal half-life for INH can range from 0.75 to 1.8 h in rapid acetylators but ranges from 2 to 4.5 h in slow acetylators. Pyrazinamide has a terminal half-life of 9.5 h with only 3% excreted unchanged. Rifampicin has a terminal half-life of 2 – 5 h with initial dosing, which can be reduced significantly with repeated dosing due to enzyme induction.

In contrast to the concentration-dependent killing of the aminoglycosides, the predictor of kill rate with TB is less clear [2,22]. In a recent study, rifampin exhibited concentration-time AUC-dependent killing *in vitro*, with the potency increasing steadily over a 9-day exposure period [22]. In a murine aerosol infection model with dose-ranging and dose-fractionation over six days, the pharmacodynamic parameter that best correlated with a reduction in bacterial counts was found to be the AUC/MIC ($r^2 = 0.95$), whereas the maximum concentration in serum/MIC ($r^2 = 0.86$) and the time that the concentration remained above the MIC ($r^2 = 0.44$) showed lesser degrees of correlation.

In light of the correlation with minimum drug levels, controlled delivery appears to be a reasonable goal and has prompted investigations into the inhalation delivery of drug. It would seem that a localized delivery method would be intuitively attractive for a disease that is very often restricted to the lung. More recently, efforts have been directed toward targeting the dormant or slow growing bacteria that reside within alveolar macrophages. Microorganisms within macrophages appear to require much higher concentrations for eradication, which makes targeting particularly appealing.

Although treatment of TB by drug encapsulated microparticles was suggested in the 1990s, not much effort was made until the work of Bain *et al.* [23] who examined the release of rifampicin from spray-dried microspheres composed of poly [D,L] lactide. A drug load of 20 w/w% was achieved in particles that had a mean diameter between 2 and 3 um. The effect of a number of processing variables on the release rate was examined. **Table 3** contains a listing of the various microparticulate formulations that have been used. Zhang *et al.* [24] also encapsulated rifampicin in polylactic acid and found a favourable distribution to the lung following IV administration.

O'Hara and Hickey prepared poly(lactide-co-glycolide) (PLGA) microspheres with rifampicin and achieved a load of 50% [25]. They subsequently tested the efficacy in a guinea pig model [26,27]. They found that the PLGA encapsulated rifampicin, either given as single or double doses, reduced the number of viable bacteria, inflammation and lung damage as compared with lactose-, PLGA-alone or rifampicin-treated animals 28 days post-infection. In a related report, they also showed that rifampicin-PLGA delivered by insufflation or nebulization affected the early development of pulmonary TB, indicating that this may be an effective preventive approach. More recently, Garcia-Contreras *et al.* [28] compared the efficacy of inhalation delivery rifampicin-loaded PLGA microspheres with rifampicin suspension. They also found that the microparticles resulted in lower bacterial counts.

Sharma *et al.* [29] carried out encapsulation studies of isoniazid and rifampicin [1:3 ratio] using PLGA. The particles were administered by IV, gavage, tracheal instillation and inhalation, and the serum and BAL levels were determined at 5 min or 30 min. Higher levels of durg in the microphages were observed following inhalation despite using one-tenth the dose. Macrophage uptake of microparticles containing INH and rifampin has since been examined in more detail [30].

In a study by Pandey *et al.* [31], INH, rifampicin and pyrazinamide were encapsulated into PLGA nanoparticles and administered to guinea pigs by IV, orally and nebulization. The authors stated that in each case, 'drugs were administered once in therapeutic dose combinations, for example rifampicin 12 mg/kg, isoniazid 10 mg/kg and pyrazinamide 25 mg/kg body weight'. In reporting the plasma levels, which were claimed to be 'in the free form', and the pharmacokinetic parameters, the absolute bioavailability following nebulization ranged from 6.5 to over 19. However, it is likely that these results represent encapsulated drug as shown in the subsequent study with alginate coated nanoparticles [32]. Similar results were obtained for these latter nanoparticles with little evidence that alginate coating prolonged the circulation time over the PLGA nanoparticles.

Investigators have also covalently linked wheat germ agglutinin to the PLG-nanoparticles to enhance the macrophage uptake [33]. Following aerosol delivery, rifampicin was detected in the plasma for 7 d, and INH and pyrazinamide were detected for 14 d. This formulation was found to be efficacious. In a recent publication by the same group [34], ethambutol was added, and significant efficacy was observed with no detectable toxicity.

Kurunov *et al.* [35] was an early group investigating the use of liposomes. They found twice weekly nebulized

Table 3. Particulate formulations of antituberculosis agents (Rifampicin, RIF; isoniazid, INH; pyrazinamide, PZA).

Agents	Formulation	Ref.
RIF, INH	Liposomes	[114,115]
RIF, INH	Liposomes	[116]
RIF	Liposomes	[37]
PZA	Liposomes	[117]
Rifampicin	Liposomes	[35,118,119]
INH, PZA, RIF	Liposomes	[38]
RIF, INH	Liposomes and microparticles	[120]
RIF	PLGA microspheres	[23]
RIF	PLGA microspheres	[25-28]
RIF	PLGA microspheres	[24]
RIF, INH	PLGA microspheres	[29,30]
RIF, INH, PZA	PLGA nanoparticles	[33]
RIF, INH, PZA	PLGA nanoparticle	[31]
RIF, INH,PZA	PLGA nanoparticle	[34]
PZA	Alginate nanoparticles	[32]
RIF	PLA	[24]
RIF	PLGA microspheres	[121]
INH	PLGA microparticle	[122]
RIF	Nanoparticle of conjugated rifampcin	[123]
RIF, INH, PZA	SLN	[39]
Para-aminosalicylic acid	PLGA nanoparticles	[40]

PLGA: Poly(lactide-co-glycolide).

liposomal rifampicin was equally effective compared to daily oral administration in a mouse TB model. Since then, Pandey *et al.* [36] encapsulated rifampicin and isoniazid, Vyas *et al.* [37] encapsulated rifampicin alone, and Justo and Moraes [38] compared the encapsulation efficiency of most of the first line antibiotics in liposomes. Pandey *et al.* [36] showed that nebulized liposomal rifampicin could maintain therapeutic drug concentrations in the plasma for 48 h following administration, whereas free drug was cleared within 24 h. Further selectively to macrophages was demonstrated in inhalation studies in rats at 30 min and 24 h by attaching O-SAP and MBSA to liposomes with entrapped rifampicin [37]. This group also explored the use of solid lipid nanoparticles for the delivery of rifampicin, INH and pyrazinamide [39]. The formulation yielded efficacy in the guinea pig TB model. Finally, Tsapis *et al.* [40] incorporated para-aminosalicylic acid into DPPC at an impressive 95% loading and exposed rats by insufflation. However, a relatively short duration was observed (about 3 h for lungs and < 3 h in plasma).

4.1.3 Fungal infections and amphotericin B

Fungal infections are rare in normal individuals, but become a significant risk for those patients who are immunocompromized [4,41]. These include solid organ transplant (SOT), haematogenous stem cell transplant (HSCT) recipients, and those with prolonged neutropenia secondary to chemotherapy for malignancy. The most common invading organism is *Candida* spp., but *Aspergillus* infections can also occur. As with TB, inhalation is a common means through which infection is spread. The mortality rates range from 50 to over 80% and, thus, there has been an ongoing effort to improve therapy as well as develop targeted prophylaxis.

The ideal prophylactic agent should i) be effective; ii) have an acceptable safety profile; iii) have broad spectrum antifungal activity; iv) be easy to administer; and v) have no significant drug interactions [41]. Amphotericin B is probably the most commonly used agent, but other agents are available, such as fluconazole, itraconazole, voriconazole, echinocandins and a number of azole antifungals. Among these agents, AmB is associated with electrolyte wasting and nephrotoxicity. Echinocandin is well tolerated, but like AmB, does not have significant oral bioavailability. Fluconazole and itraconazole are orally available, although absorption is unreliable, and GI side effects of nausea and vomiting are common. Voriconazole causes visual disturbances, dermatological reactions and abnormal liver tests. Given the nature of the disease and the undesirable characteristics of the active agents, respiratory delivery offers the possibility of improved therapy with reduced side effects.

Amphotericin B is poorly water soluble, and so IV formulations include surface active agents (see **Table 4**). The early formulation of amphotericin B was a complex with deoxycholate (AmB-DC, Fungizone). DC is a naturally occurring bile salt, which has a poor taste and potential to cause mucosal irritation. Thus, an ideal respiratory delivery system would not contain bile salts.

With IV administration of 1, 2.5, or 5 mg/kg/d of AmBisome, the maximal concentrations in serum (C_{max}) were 7.3, 17.2, and 57.6 µg/ml, respectively [42,43]. The lack of dose proportionality is associated with the extensive tissue distribution reflected in the steady state volume of distribution that ranged from 0.16 to 0.44 l/kg. The half-life of elimination fell from 10.7 to 8.1 to 6.4 h with the increasing doses cited above. This is deceptively short, however, as there is a very long terminal elimination phase associated with removal of AmB from tissues.

Animal studies have shown aerosol administration to be an effective means of both treating and preventing fungal infections [42,44-48]. In addition, distribution to the lung is more favourable with inhalation delivery when compared to IV [49,50]. The potency of AmB and its relatively long retention time in the lung reduces the challenge of developing an inhalation formulation for sustained delivery.

Table 4. Microparticulate formulations of antifungal agents.

Agent	Formulation	Ref.
Amphotericin B	Liposome	[124]
	Liposome	[125]
	Liposome	[49]
	Liposome	[126]
	Liposome	[48]
	AmB-DC	[52]
	AmBisome	[44]
	AmBisome	[127]
	AmBisome	[46,47]
	AmB-Egg PC complex	[128]
	Abelcet	[129]
	Abelcet	[130]
Itraconazole	Microparticles	[55,56]

Similar to the IV studies, the AmB level was 15.75 ug/ml in bronchial alveolar lavage 4 h after exposure [51]. In a related study, Marra *et al.* [52] determined the lung levels of AmB-DC 10 min following inhalation by BAL. The mean AmB concentrations following a 30 mg inhalation dose were 0.68 and 0.50 ug/ml in the upper and lower lobes. These are relatively low in comparison to the peak serum concentrations observed with IV dosing even after correcting the dose for body weight. These low levels may reflect poor deposition or inadequate recovery with BAL.

Not surprisingly, the IV doses were effective as shown in a corresponding clinical study, where the rate of invasive fungal infection ranged from 0 to about 23%, which was significantly lower than the control [41]. As the MIC values for non-resistant organisms appear to be near 1 ug/ml, the high concentrations of AmB are unwarranted for prophylaxis. There were side effects that included nausea, dysgusia, dysphagia, coughing, dyspnoea and bronchospasm, and the study had a 12 – 23% treatment discontinuation rate. This suggests that the dose may be reduced to minimize patient discomfort without sacrificing efficacy.

In a head to head clinical comparison, the lung concentration of drug given as the phospholipid complex (AmBisome) was 3.7 times greater than the deoxycholate complexed AmB [42]. In part, a higher dose was administered (50 vs 100 mg), but there was also enhanced lung deposition and prolonged retention, which contributed to the greater exposure. Of the 51 patients, two developed pulmonary infections and four developed extrapulmonary infections. In a following up study [53], the rate of infection was 14 and 12% for the DC-AmB and AmBisome formulations, respectively. The lipid based formulations were well tolerated with nausea, vomiting and taste dysfunction reported in only 3 of 381 patients [54]. The corresponding respiratory complications were also reduced or eliminated. A similar low incidence of side effects was observed with aerosol administration of AmB-DC and Abelcet [53]. Because of the high levels of AmB that were achieved for the purpose of prophylaxis, once a week dosing was sufficient to obtain these good results. An alternative strategy would be to develop a sustained release dosage form that would reduce the peak concentration of Amb on initial dosing but maintain the BAL concentration for an extended period of time. This may also relieve the minor side effects that were observed. With the inherent complexities associated with AmB and appearance of resistant organisms, other antifungal agents are being evaluated for respiratory delivery [55,56].

4.2 Diabetes and insulin

No other aerosol product has been met with such public anticipation than the respiratory delivery of insulin. Its development had the promise to pioneer the pathway for the introduction of other inhalation products for the systemic delivery of drugs (**Table 5**). However, Exubera® was not an ideal system and was removed from the market. The failure was apparently a result of poor patient acceptance of the relatively large device. Exubera was intended for immediate release and therefore does not meet the selection rules for this review. However, maintaining low glucose levels in diabetic patients by prolonged delivery of insulin is an important goal that can potentially be achieved by inhalation. As reproducible deposition and the subsequent transport of insulin into the blood have been demonstrated, it remains to develop formulations that provide for the sustained release of insulin analogous to the commercially available parenteral formulations (zinc, protamine insulin formulations).

In considering the pharmacological effect of insulin, ideal delivery can not be achieved with a formulation that has a predictable, predetermined delivery rate. Under normal physiological conditions, insulin is released in response to elevated glucose levels, which in turn depend on the consumption of glucose. Although diabetic patients can constrain their diet/physical activities and administer insulin in anticipation of future needs, this is hardly an optimum situation. For ideal delivery, the availability of insulin should coincide with the needs of the patient, which requires a feedback mechanism. This represents a very significant challenge in the development of a suitable microparticulate formulation.

Two excellent reviews have been written on the subject; one covers the delivery of insulin to the lungs [1], and the other, recent developments in formulations [2]. Langer and co-workers [57] chose insulin as one of the first compounds to demonstrate the effectiveness of large porous particles. Such particles undergo the processes of fluidization and deagglomeration more readily than micronized powders. A larger fine particle fraction is obtained through a reduction

Table 5. Formulations with insulin.

Formulation	Ref.
Liposome	[131]
Porous part	[132]
Hyaluronate	[64]
PLGA microparticles	[60]
Oligosaccharide ester derivatives	[61]
Calcium phosphate/polyethylene glycol	[62]
Diketopiperazine derivative	[63]
Liposome	[66]
Agglomerated vesicles	[67]

PLGA: Poly(lactide-co-glycolide).

in the particle density, and a larger geometric size curtails macrophage engulfment following deposition. Nevertheless, the absorption is slow, and perhaps less than 6% of the dose reaches the systemic circulation with much of it lost by clearance and metabolism [2,58]. As noted, there is ample room for improvements in bioavailability [1].

The initial landmark investigation of porous aerosols was followed by a study where the release of insulin was sustained by incorporating protamine and/or zinc chloride into the powder along with lactose, albumin and DPPC [59]. The respirable fraction was determined to be 40% but was increased to 58 – 75% by using the Aero-Breather. Using a rat model, the plasma level of insulin was sustained for half a day, which is similar to that achieved with subcutaneous injection. The relative bioavailability was 80.5%, and the initial high peak concentration at 30 min was followed by relatively constant levels that fluctuated between 30 and 50 IU/ml for the remaining 24 h.

Kawashima *et al.* [60] loaded insulin into PLGA nanospheres that had weight mean diameters of 400 nm. *In vitro*, 85% was released in the initial burst, and the remaining 15% was released in the next few hours. *In vivo*, the nanospheres were administered to guinea pigs over a 20 min period at a dose of 3.9 IU/kg. The blood glucose level was reduced significantly, and hypoglycemia was prolonged over 48 h, compared to the 6 h observed with a nebulized aqueous solution. This result was attributed to the sustained release of insulin from the nanospheres deposited widely throughout the lung.

Blair *et al.* [61] used DPPC and an oligosaccharide ester derivative [Solidose®] as excipients for an insulin microsphere system. The absorption time in rats was shown to be increased over four fold in comparison to pure insulin delivered intratracheally. Garcia-Contreras *et al.* [62] combined insulin with calcium phosphate and polyethylene glycol (BioAir™) and achieved a 58% load with a controlled precipitation procedure. The formulation extended the duration of release by almost a factor of two compared with an SC injection of solution. Steiner *et al.* [63] combined insulin with a diketopiperazine derivative, which was claimed to provide rapid dissolution as well as enhanced tissue permeability. The bioavailability of insulin was increased to 15%.

More recently, Surendrakumar *et al.* [64] prepared a dry powder of recombinant human insulin with hyaluronic acid and tested the delivery system in beagle dogs. Excess zinc ions and hydroxypropyl cellulose were used to modulate the release rate. In a similar study [65], cyclodextrins were used to produce large porous particles of insulin. By modulating the formulation components and processing conditions the release rate could be controlled.

Huang and Wang [66] encapsulated insulin in liposomes and used an ultrasonic nebulizer to deliver the formulation to animals. The transfer rate of insulin to the plasma was reduced when compared to controls. Finally, Karathanasis *et al.* [67] examined the use of the agglomerated vesicle technology for the respiratory delivery of insulin. In this system, liposomes were loaded with insulin and cross-linked via chemical bridges that are susceptible to cysteine. Certainly the idea of triggering the release of insulin would offer an advantage over a fixed release rate, but having the trigger depend on cysteine rather than glucose is obviously less desirable.

4.3 Organ transplantation and cyclosporin A

Lung transplantation is used in patients with advanced lung diseases, such as chronic obstructive pulmonary disease (COPD), CF, and interstitial lung diseases. The number of lung transplants performed in the United States is small (1100 in 2004) [68], and rejection of the transplanted organ remains a problem. There are two forms of rejection, acute and chronic. The acute form can often be reversed, whereas chronic rejection is the leading cause of death after lung transplantation. Cyclosporin A (CyA) is an immunosuppressive agent that can prevent rejection, but also predisposes patients to infections that are the second leading cause of death in this population [68]. In addition, patients have a higher risk of other complications including neoplasms, renal failure and diabetes mellitus.

CyA (Neoral®) is administered orally, but the bioavailability is extremely variable and ranges from less than 10% to almost 90% depending on disease state [43]. An emulsion based dosage form (Sandimmune®) was introduced but remains suboptimal for chronic rejection. For Neoral, the time to peak concentration is 1.5 – 2.0 h. Only about 6% of the drug is eliminated unchanged in the urine due to the extensive metabolism and biliary excretion. The terminal half-life is 8.4 h with a range of 5 – 18 h.

Because of the variability in the oral absorption, the traditional CyA was dosed based on the measurement of the trough level drug concentrations. The recommended doses are 9, 8 and 7 mg/kg/d for renal, liver and heart transplantation, respectively [43]. In *de novo* renal and liver transplant patients who were administered 7.95 and 6.89 mg/kg/d, the

Table 6. Formulations with cyclosporine.

Liposome	[133]
Liposome	[134]
Liposome	[127]
Liposome	[135]

corresponding trough levels were 361 and 268 ng/ml. To achieve a more accurate CyA dosing regimen, measurement of AUC for various durations (2 – 12 h) was assessed and although superior, was found to be impracticable [69]. Despite these efforts, side effects are not avoided, and long-term allograft loss is mainly due to CyA toxicity. Thus, inhalation has the potential to provide therapeutic concentrations of drug in the lungs while minimizing the side effects associated with high systemic concentrations [68]. As it also appears that the drug must be maintained above a minimum concentration, a sustained delivery formulation may provide benefits to lung transplant patients.

The early animal studies in a canine allograft model [70] and rat model of lung transplantation [71] demonstrated efficacy along with low blood levels. In the rat study [71,72], animals treated with intramuscular cyclosporine had reduced rates of rejection compared with controls but had a rate of pneumonia of 50%. In contrast, animals treated with aerosol cyclosporine demonstrated lower rates of rejection with no incidence of pneumonia. CyA was nebulized with either a propylene glycol (PG) or an ethanol based formulation (**Table 6**). It would seem that the liposomal formulation developed for treatment of pulmonary metastases [73] as is discussed below would provide an advantage over the use of organic solvents.

In humans, a technetium [Tc-99 m] tag was used to reveal the deposition patterns of CyA in the lung. The earliest of these studies included five subjects with chronic lung transplant rejection where a two-fold difference in the lung dose was observed [74]. A later study enrolled 7 subjects, who received a 300 mg nebulized dose of the PG-based form of aerosolized cyclosporine [75]. Deposited doses in the individual transplanted lungs varied from 6.8 to 24.8 mg. Over a 4-month period, a highly non-linear relationship between the deposited aerosol dose and improvement in pulmonary function was noted [75].

IV and inhalation administration of cyclosporine was compared, in which the aerosol consisted of 300 mg of the PG formulation, and the IV formulation was given at a dose of 1 mg/kg [76]. The values for C_{max} for the aerosol ranged from 119 to 402 ng/ml with a mean of 206 ng/ml. The AUC ranged from 716 to 1565 ng/ml h with a mean of 1034 ng/ml h. The AUC following IV was higher at 3580 ng/ml h. Interestingly, the half-life was extended from 6.5 h with IV to over 40 h following inhalation. This suggests that the lung sequesters CyA and may account for the low AUC.

A study was also conducted to evaluate aerosol CyA for the prophylaxis of rejection [77]. Subjects received either 200 or 300 mg of aerosol cyclosporine in the PG formulation. Eight of nine single lung transplant recipients had preferentially deposition in the transplanted lung with doses ranging 2.2 – 9.2% of the loaded dose, whereas double lung recipients had 3.3 – 7.1% deposition of the loaded dose per transplanted lung. Linear relationships between deposited aerosol dose in the transplanted lung and improvement in lung function were demonstrated at all six time points considered during the 2 year trial. The peripheral transplanted lung deposited dose was better correlated to the change in lung function than the total transplanted lung dose, which allowed the therapeutic peripheral lung dose of 5 mg to be established. At this dose, subjects tended to experience improved lung function, less high grade acute rejection, and less chronic rejection than subjects in the placebo arm of the trial [77].

Nine subjects with chronic rejection were treated with aerosolized cyclosporine [78]. Seven of these subjects demonstrated improvements in rejection histology, and pulmonary function stabilized in the treated subjects when compared to contemporary controls. Four of these subjects had measurable blood levels of cyclosporine measured immediately after delivery and still at 24 h later. Levels immediately post treatment ranged from 59 to 148 ng/ml and from 14 to 48 ng/ml at 24 h. In a later study, a multi-variable analysis model of survival with aerosol cyclosporine use was 4.5 years compared with 2.4 years for local controls and 2.3 years for multi-center controls [79].

4.4 Asthma and bronchodilators and steroids

The most common respiratory disease in the US is asthma [2]. Bronchodilators are used for symptomatic relief of the bronchoconstriction during a so-called "attack". Steroids are also used, and their mechanism of action is related to the anti-inflammatory effect. Beginning with bronchodilators, it may seem that sustaining the delivery of these short acting agents would be of benefit. However, clinical studies have not distinguished the preferred dosing strategy. In principle, one could argue that maintaining a constant level would provide a therapeutic advantage by improving compliance and reducing side effects. However, maintaining constant drug levels also has the risk of inducing tolerance.

A number of investigators have developed microparticulate systems for prolonging the delivery of bronchodilators (**Table 7**). One of early reports by Lai *et al.* [80] involved the use of PLGA to encapsulate isoprenaline. This was administered to rats by tracheal instillation at a dose of 0.1 mg/kg, and serotonin-induced brochoconstriction was prevented for more than 12 h. Control experiments failed to provide even 30 min of protection, consistent with the rapid clearance of drug from the lung. As noted by Sakagami and Byron [2], there was a disconnect between the

Table 7. Formulations with bronchodilators and steroids for asthma.

Agent	Formulation	Ref.
Bronchodilators		
Albuterol	Liposome	
Pranlukast hydrate	Surface-modified powder	[136]
Metaproterenol	Liposome	[137]
Metaproterenol	Liposome	[138]
β-2-Adrenergic agonists	Liposome	[139,140]
Sodium cromoglycate	Liposome	[141]
Terbutaline	Liposome	[142]
Terbutaline	Porous particles	[81]
Steroids		
Budesonide	Lipid dry powder	[143]
BDP	Liposome	[134]
Glucocorticoids	Liposome	[144]
BDP	Liposome	[83]
Beclomethasone dipropionate	Liposome	[145]
Budesonide	Liposome	[74]
Corticosteroid	Liposome	[146]

BDP: Beclomethasone dipropionate.

expected lung levels based on *in vitro* release studies and therapeutic levels.

Ben-Jebria *et al.* [81] prepared porous particles containing salbutamol sulfate with DPPC, human serum albumin and lactose. Using a carbachol-induced bronchoconstriction guinea pig model, prevention was provided for greater than 16 h, which compares favorably with the 5 h duration of action found with the free drug. Dellamary *et al.* [82] used PulmoSpheres to encapsulate cromolyn sodium, albuterol sulfate and formoterol fumarate. These were not tested *in vivo*, but a fine particle fraction of 70% was achieved.

Steroids are commonly given to asthmatic patients, although it is often not until one to two weeks after initiation of therapy that benefits are seen [2]. Long acting steroids are given to reduce the dosing frequency and thereby reduce the side effects of oropharyngeal candidiasis and adrenal cortex suppression. A number of studies have been carried out in an effort to develop prolonged delivery system of steroids. Vidgren *et al.* [83] incorporated beclomethasone dipropionate (BDP) in dilauryl phosphatidylcholine (DLPC) liposomes and administered them by nebulization to normal humans. The lipids were tagged with ^{99m}Tc to allow the extent of deposition to be evaluated. Of the amount inhaled, about 15% was deposited depending on nebulizer and between 82 and 93% of the deposited dose was retained in the lung at 3 h.

Huang *et al.* [84] encapsulated betamethasone dipropionate in chitosan, type-A gelatin and ethylene oxide-propylene oxide block copolymer (Pluronic F68). The *in vitro* release showed a dose dependent burst followed by a slower release phase. No *in vivo* work was carried out. Sakagami *et al.* [85] prepared mucoadhesive microparticles with BDP using hydroxyl propyl cellulose (HPC). This represents perhaps the only study in which a formulation was developed to affect the rate of mucociliary clearance. Between 80 and 90% of BDP remained in the lung at 3 h in comparison to the 20% for controls, which consisted of inhalation of crystalline BDP. The delivery appeared to achieve the promised constant rate, but concerns of safety have prevented clinical testing of this promising system. Finally, Bandi *et al.* [86] incorporated budesonide into PLGA microparticles for the purpose of lung cancer prevention, although this may have an application for asthma therapy. A maximum of 94% loading efficiency and a release profile extending for 21 d were obtained *in vitro*.

4.5 Lung cancer

4.5.1 Introduction

Lung cancer now represents a leading cause of cancer death in humans is the US and the world [87]. Treatment of lung cancer by local delivery would appear to provide a great benefit. First, the survival rate of patients with lung cancer has not appreciably changed after 40 years of effort with oral and IV therapy. Second, the well known problem with chemotherapy is the life-threatening side effects arising from the agents that can effectively destroy cancerous cells. Thus, delivery of the agents directly to the site of cancer should provide an immediate benefit of a higher local concentration and lower peripheral/systemic concentration that in principle will increase the efficacy and reduce the toxicity.

However, the high mortality associated with lung cancer is primarily due to the symptomless development. In fact, treatment of lung cancer, whether it be by surgical resection, radiation or systemic delivery of chemotherapeutic agents, as a localized disease in its early stages, I or II, has a relatively good prognosis. The fundamental problem is that the vast majority (85%) of lung cancer is detected only after it has reached stage III or worse. In these cases, the cancer has infiltrated surrounding tissues or metastasized to distant organs, such as the liver or brain, and the prognosis is very poor. At this point, it no longer is a localized disease, and the justification for localized therapy appears lost.

An alternative approach is prevention. Specifically, chemoprevention, that is the administration of a chemical entity to prevent the occurrence of cancer, has been shown to be an effective strategy for many cancers including lung cancer [88]. Moreover, with 90% of the cause of cancer attributable to smoking cigarettes, prevention becomes even more attractive, as it appears that susceptible individuals can be readily identified. Nevertheless, the probability of getting

lung cancer even with heavy smoking is perhaps only one in ten. Thus, unless there is a better means of predicting the incidence of lung cancer, chemoprevention of lung cancer does not have a good cost-benefit ratio.

Despite this somewhat negative introduction, respiratory delivery of microparticles for chemotherapy may provide a therapeutic benefit. Anderson and Kubitz have systematically developed a liposomal interleukin-2 (IL-2) aerosol formulation that has been translated into the clinic [89]. Knight and his co-workers [90-93] have explored the inhalation delivery of camptothecins and paclitaxel incorporated into liposomes for chemotherapy, and Hitzman *et al.* [90-93] has examined the use of 5-FU encapsulated into lipid coated nanoparticles for the treatment of lung cancer. As expected, none of these groups has developed an ideal delivery system, but important progress has been made (**Table 8**).

4.5.2 *Interleukin 2*

Anderson and co-workers began their investigations with the use of IL-2 in the 1990s, and their efforts culminated in clinical testing of a dimyristoyl phosphatidylcholine (DMPC)-IL2 liposomal formulation for the treatment of cancer. Mice with pulmonary micrometastases were treated once daily with free cytokine, and intrathoracic administration was shown to be superior to intraperitoneal or subcutaneous routes [94]. In terms of survival and numbers of pulmonary metastases, an even greater effect was seen when IL-2 liposomes were administered by the local intrathoracic route. Minimal toxicity was observed.

Khanna *et al.* [95] explored the activity of inhaled IL-2 in dogs by assessing the immunological activation. Free IL-2 resulted in an increase in the peripheral blood mononuclear cell activation as compared with saline control-treated dogs. IL-2 liposomes also caused a significant increase in BAL effector activation in a dose response manner as compared with empty liposome controls. The BAL leukocyte cell count was also increased significantly after inhalation of IL-2 liposomes compared with inhalation of free IL-2. BAL effector populations included a greater proportion and total number of lymphocytes and eosinophils after treatment with IL-2 liposomes.

In the subsequent study [96], the physical and biological characteristics of nebulized IL-2 liposomes were assessed. The aerosol droplet size distribution and the physical stability of the IL-2 liposomes were examined, and the distribution following inhalation was determined in dogs. The aerosols were deposited evenly throughout the lung with a central-to-peripheral lung deposition ratio of 1.12 ± 0.03, and IL-2 liposomes were retained in the lung for 24 h.

The efficacy of IL-2/liposomes was then invested in dogs with pulmonary metastases and primary lung carcinoma [97]. Two of four dogs with metastatic pulmonary osteosarcoma had complete regression of metastases. This regression remained stable for more than 12 and more than 20 months, respectively. One of two dogs with lung carcinoma had stabilization of disease for more than 8 months, whereas the last dog had disease progression. There were minor side effects which included an increase in BAL cell numbers by more than fourfold and greater proportions and total numbers of eosinophils and lymphocytes. Mean BAL effector lytic activity was significantly greater after 15 days of IL-2 liposome inhalation compared with pretreatment activity, but the effect was reversed after 30 days. No allergic reactions were associated with inhaled IL-2 liposome therapy, and canine antibodies against human IL-2 and HSA were detected in all dogs.

In a final study, a Phase I clinical study was undertaken to test the feasibility and toxicity of administering IL-2 liposomes by aerosol to patients with pulmonary metastases [98]. The IL-2 liposomes were administered by nebulization for about 20 min in nine patients in three cohorts of three patients at 1.5, 3.0 and 6.0×10^6 IU of IL-2 3 times a day and subsequently, in a larger cohort of patients with hepatitis C [99]. The study lasted 12 weeks, and no changes in chest X-ray or pulmonary function were seen. It appears that the delivery of IL-2 liposomes by inhalation is well tolerated even in those with primary immune deficiency (N = 15).

4.5.3 *Camptothecins*

Knight *et al.* [92] evaluated the efficacy of 9-nitrocamptothecin encapsulated in DLPC liposomes in mice. Human cancer (CLO: infiltrating duct carcinoma type of breast cancer, SPA adenocarcinoma type of lung cancer, and SQU moderately differentiated adenocarcinoma of colon cancer) were implanted over the right dorsal chest region, and the drug was administered daily, five times a week. Tumor growth was greatly reduced and in some cases was undetectable after several weeks of treatment. Not surprisingly, oral therapy was found to be significantly less effective, and even the response with intramuscular delivery was found to be equally poor.

In the companion paper, the distribution of drug was examined following inhalation and intramuscular delivery [100]. High concentrations of drug were found in the lung following aerosol administration with levels of 181 and 179 ng/g at 15 and 30 min after completion of a 30 min inhalation. The AUC was also higher in the lung at 7,551 ng.min/g compared with 3,433, 1,514, 1,444 and 1,392 ng.min/g for the liver, kidneys, blood and tumor. In contrast, the hydrophobic camptothecin remained at the site of intramuscular injection with 60% of the drug still present at the site 1 h after injection. These studies demonstrate the importance of distribution in determining the efficacy of the drug. That is, camptothecin appears to be an effective chemotherapeutic agent but must be delivered in an appropriate manner in order to derive the beneficial effects.

4.5.4 *Paclitaxel*

The efficacy of the aerosol delivery of paclitaxel in a DLPC liposome for inhibiting spontaneously arising primary and metastatic lung cancers in dogs was examined [101]. In the

Table 8. Microparticulate formulations of agents to treat lung cancer.

Agent	Formulation	Ref.
β-Cytosine arabinoside	Liposome	[147,148]
Muramul dipeptide	Liposome	[149]
Interleukin 2	Liposome	[94,150,151,40, 98,152,97]
Interleukin 2	Liposome	[95]
Interleukin 2	Liposome	[153]
Paclitaxel	Liposome	[100,102,73,91]
Camptothecins	Liposome	[154,92,90,155, 90,156,157,102]
5-FU	Liposomes, microparticles, LNP	[93,103,104,105]
All-*trans*-retinoic acid	Liposomes	[158]
Budesonide	PLGA microparticles	[86]
Farnesol	Liposome	[159]
Urocanic acid	Chitosan	[160]
Doxorubicin	Nanoparticles	[161]
Doxorubicin	Microparticles	[162]
SLIT cisplatin	SLIT	[163]
Γ-Interferon	Liposomal	[164]
CT X-ray contrast agent	Particles	[107]

5-FU: 5-fluorouracil; PLGA: Poly(lactide-co-glycolide).

24 dogs, there were 6 complete and partial responses with no systemic toxicities noted. As with the camptothecins, the distribution was examined in mice. High concentrations were found in the lung, and lower concentrations were detected in the liver, spleen, kidneys, blood and brain. Knight and his coworkers [102] also examined the efficacy of aerosol delivery of paclitaxel in a DLPC liposome for inhibiting pulmonary metastases in a murine renal carcinoma model. The most effective schedule involved inhalation of the drug for 30 min, 3 days per week, and there was a significant reduction in lung weights and reduced number of visible tumor foci. The survival was also prolonged.

Cyclosporine [91,102] was used to modulate the distribution of paclitaxel based on the presumed inhibition of efflux transporters. In administering both drugs as liposome aerosols, the paclitaxel alone and CyA/paclitaxel groups were found to be significantly more effective compared to controls as determined by lung weights and tumor area. This represents the first time demonstration of improved efficacy in lung cancer with the use of efflux transport inhibitors.

4.5.5 5-Flurouracil

Hitzman *et al.* [93,103-105] carried out a series of studies in an effort to develop a respirable microparticulate delivery system for 5-fluorouracil (5-FU), which was shown to be active in humans with upper respiratory squamous cell carcinoma [106]. The initial study involved the use of microdialysis to determine the release rate of 5-FU from liposomes, PLGA microparticles and solid lipid nanoparticles. Most significant, microdialysis was validated as a useful tool for the measurement of the release rate, as it provides excellent time resolution in an automated manner for these microparticulate dosage forms [105]. In the next paper, these approaches for formulation were evaluated in terms of the potential to provide a zero order delivery rate that would be appropriate for the reversible inhibition of DNA synthesis [103,104]. Although the solid lipid nanoparticles did not provide a zero order release rate as expected, they were selected as the optimal formulation among those tested. A model for the release profile, which was based on the polydispersity of the cores and shell coatings, was found to be self-consistent with the data set.

The final contribution involved the delivery of the 5-FU particles to the hamster animal model and subsequently measuring the time dependent concentrations in the lung, trachea, larynx, esophagus and blood [93]. Total drug and total particle concentrations were measured, from which it was possible to fit the data to a model and thereby estimate the concentrations of free drug. Therapeutic effective concentrations in the lung, larynx and trachea were prolonged for a 12-h period, which is significant when compared to the 30 min duration of free drug administered by aerosol. Serum concentrations were very low and thereby would not be expected to cause side effects. A 30-day safety study was conducted involving the daily administration of the particles and microscopic foci were observed indicating very slight inflammation occurred. Measurement of lung levels of particles and 5-FU indicated minor accumulation (< 10%) of particles occurred in this time period.

4.5.6 Granulocyte macrophate-colony stimulating factor

A Phase I dose escalation study was carried out in which granulocyte macrophate-colony stimulating factor (GM-CSF) was delivered to patients by nebulization twice-a-day for 7 days [89]. Six of seven patients were successfully dose escalated from 120 ug/d to 240 ug/d and ultimately to 480 ug/d × 7 days. No toxicity was seen. Comparison of day 0 and day 7 blood counts showed no significant increases in either the number of leukocytes or percentage of neutrophils. Pulmonary functions test changes were minor, and no significant change in forced vital capacity, FEV1, peak flow, or FEF 25-75 related to either time or dose level was observed. Although one patient's lung metastases progressed, the other five patients received an additional 2 – 6 months of intermittent aerosol GM-CSF at dose level 3 without side effects. One patient with Ewing's sarcoma had a complete response, and a patient with

Table 9. Microparticulate formulations of other agents.

Agent	Formulation	Ref.
99mTc_DTPA	Liposomes	[165]
DTPA	Porous particles	[166]
Estradiol	Porous particles	[167]
Almon calcitonin	Gelatin microspheres	[168]
Betamethasone	Chitosan microparticles	[84]
Deslorelin	Porous PLGA particles and HPβCD complexes	[169]
Immunoglobulin	Microparticles	[170,171]
Immunoglobulin		[172]
Interferon	Liposome	[173]
Catalase	Liposome	[174]
Glutathione	Liposome	[175,176]
Pentamidine	Liposome	[177]
Enviroxime	Liposome	[178]

PLGA: Poly(lactic-co-glycolic acid).

melanoma had a partial response; the other three had stabilization of pulmonary metastases for 2 – 6 months.

4.6 Other agents

A number of other studies involving inhalation of drugs for the diagnosis [107] and treatment of diseases, which are not covered here but are cited in **Table 9** for completeness.

5. Conclusion

In development of microparticulate formulations for inhalation in the lung, there is a need for a greater understanding of the disease state. In addition, the pharmacological properties of the drugs need to be understood, as this is the key to revealing the optimal concentration time profile for the specific mechanism of action. The pharmacokinetic properties of the pure drug are also critical and should be determined by IV administration, as well as by inhalation to establish a baseline from which the controlled release formulation can be evaluated. *In vitro* release studies will allow better identification of the best dosing strategy to be used in efficacy and safety studies. If the therapeutic levels can be estimated with reasonable certainty, it is perhaps best to carry out pharmacokinetic studies with the controlled release formulation prior to efficacy studies. In contrast, when the effective concentrations are poorly understood, it is often better to carry out a dose-escalation study for safety and efficacy prior to the pharmacokinetic study.

6. Expert opinion

Inhalational delivery provides an extremely effective means of achieving a high concentration of drug in the lung and reducing systemic exposure. Moreover, the technology to encapsulate drugs within a formulation that provides for a controlled delivery is rapidly developing. In the disease states that have been examined, tobramycin has been identified as a concentration dependent antibiotic. That is, the microorganism death rate is correlated to the concentration that exceeds the MIC. Encapsulating tobramycin in a slow release formulation under conditions when the MIC may never be achieved will not only be ineffective but also has the potential of inducing bacterial resistance to tobramycin. In fact, the more desirable approach would be to give a bolus dose periodically [108] and focus on increasing the deposition efficiency and reproducibility. In addition, the strategy should also involve a means of reducing the clearance rate of drug from the lung without reducing the free concentration.

For TB, it appears that impressive advances have been made in preclinical studies involving microparticles and liposomes to deliver anti-TB drugs. Nevertheless, there do not appear to be any completed clinical studies, and it is difficult to identify which system would be the most advantageous to test in humans. Each formulation provided an extended duration of encapsulated drug in the serum, but the deposition fractions and free concentration of drug were often not determined. Moreover, as the *in vitro* release rates were not provided, the free concentration of drug can not even be estimated. In most studies, the formulations were found effective in reducing the bacterial counts to a minimum detectable level. Although encouraging, it prevents a comparison of the formulations based on a dose response. Thus, despite the large number of studies of many different formulations, critical information is missing to allow a clinical study to be initiated. Additionally, comparative formulation studies as well as more detailed investigations with the optimal formulation are needed, rather than repetitive studies with different formulations.

In addition, the localization in the lung was initially cited as a justification for respiratory delivery as it should be. However, 15% of the cases of TB involve extrathoracic disease, and it is doubtful whether aerosol formulations will achieve sufficient therapeutic levels in the serum. As it is difficult to identify those patients with extrathoracic infections, aerosol delivery will be effective in 85% of the cases but have a 15% failure rate. This is unacceptably high. Concurrent oral or parenteral administration is possible, but then much of the advantage of local delivery is lost. The development of specialized delivery systems is certainly warranted but currently impractical and cost-prohibitive in developing countries where the vast majority of TB cases occur. There may be a benefit for the respiratory delivery in developed countries such as the US, but here drug resistance is a major issue. As such, application of specialized formulations of one first line drug may be of limited value, because multiple drugs or second line drugs are required to overcome bacterial resistance.

Fungal infections often occur secondary to inhalation of spores that germinate in the ideal conditions of the lung lining, and respiratory delivery will result in a high local concentration at this site perhaps in a preventive capacity. By contrast, active diseases are associated with a very poor prognosis, and because the organism has often disseminated throughout the body, local delivery does not provide an advantage. For prevention, a troubling aspect is that fungal infections commonly occur in the lung with the target patients, but just as in the case of TB, are not limited to the lung. Because of the low water solubility and complicated self association of amphotericin B, it is difficult to distinguish the pharmacokinetic factors from the pharmacological factors that determine efficacy. As such, painstaking clinical studies are being carried out to determine the optimal formulation and dosing regimen (dose and frequency) based on variable clinical response. Thus, the arduous path is laid out, and aerosol delivery will likely remain in an adjunctive role where it is combined with IV therapy, which can be used at a lower dose.

For diabetes, there is an immediate need to increase the availability of insulin and even more importantly increase the reproducibility of delivery. There is also a need for a smaller device for better patient acceptance. A feedback mechanism would be highly desirable but extremely difficult to develop. A more realistic goal would be to focus efforts on developing prolonged release formulations that can be used as a substitute for those that are currently given by subcutaneous injection.

Cyclosporine has been shown to be effective by aerosol administration in reducing the incidence of transplant rejection. The doses used for aerosol administration were high, 200 – 300 mg and nebulized from an organic solvent. The high dose appears to be limiting in terms of the excessive mass that would need to be inhaled. Thus, first and foremost, the delivery would benefit by increasing the deposited fraction. Second, the use of a liposome formulation may be desirable to replace the organic solvents but would not seem to provide sufficient drug. However, if indeed the efficacy is correlated with trough levels, then a large dose is not necessary. Rather, it would be appear that a better strategy would be to maintain a lower, but effective, free drug concentration over an extended time period. This may not be difficult to achieve, as the half-life of elimination from the lung is very long as reflected in the long half-life of CyA given by inhalation. This observation is also consistent with the existence of efflux transporters in the lung that limit the clearance drug [73]. Thus, it may be possible to reduce the mass of CyA in the formulation by excipients that further extend the duration of release.

For bronchodilators, the motivation for preparing a prolonged release dosage form has not been provided in clinical studies. On the other hand, no system has been developed that provides a stable, constant delivery. In fact, large burst effects followed by slow release with rapid clearance are the norm. Given that oral controlled release products of bronchodilators have found acceptance in the medical community, it would seem there is a role for the development of a good sustained release dosage form of bronchodilators that can be given by inhalation. In contrast, there does not appear to be a good understanding between steroid lung levels and therapeutic efficacy. Moreover, the steroids are inherently long acting and therefore typical aerosol particles may not provide significant benefit if they are largely deposited in the upper airways.

In principle, respiratory delivery of chemotherapeutic agents should provide enhanced efficacy and reduced toxicity for patients that are diagnosed with stage I or II lung cancer, but due to the relatively good cure rates, it may be difficult to show a statistically significant improvement. For most lung cancer patients that are diagnosed and are in stage III or IV, local delivery of chemotherapeutic agents does not appear reasonable for a non-localized disease. Nevertheless, there may be some opportunities in the form of adjuvant or neoadjuvant aerosol delivery that can prevent recurrence or enhance the success of surgical/radiological interventions.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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