Tuberculosis 91 (2011) 71–81

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

Inhaled drug therapy for treatment of tuberculosis

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ARTICLE INFO

Article history:
Received 4 August 2010
Received in revised form
12 August 2010
Accepted 29 August 2010

Keywords:
Pulmonary drug delivery
Dry powder inhalation
Nebulization
Microspheres
Nanoparticles
Rifampicin
Isoniazid
Capreomycin

SUMMARY

The lungs have received attention as a portal for drug delivery in tuberculosis (TB) from researchers addressing diverse objectives. These include: (a) targeting alveolar macrophages that harbour TB bacilli; (b) maintaining high drug concentrations in lung tissue; (c) systemic delivery of potent or second-line anti-TB agents; and (d) delivering agents that may change the host-pathogen dialectic. Formulation design considerations for each of the above objectives differ in slight, but important ways. As distinct from vaccine delivery formulations, inhalations intended for drug delivery are presumed to require chronic and repeated administration of larger amounts of material. This review seeks to summarize the consensus on the ways and means available or under development, to deliver different anti-TB agents as aerosols for inhalation. These agents include drugs in current clinical use, singly or in combination, experimental chemical entities, siRNA against host molecules, and finally, drugs in clinical use for unrelated pharmacological action, as modifiers of the host-pathogen dialectic. The pharmacokinetics of drug bioavailability in the lung, the blood and other tissues following lung deposition of inhaled therapies are also addressed. Finally, considerations on efficacy studies of drugs administered through aerosol delivery are discussed.

1. The rationale for pulmonary drug delivery in tuberculosis

The lung is the primary, if not the sole, portal of entry for mycobacteria that cause TB. It has therefore been of interest since the 1950s to deliver drugs used in the management of TB through the same route.[1] Pulmonary drug delivery in TB was initially investigated in Russia, where issues ranging across drug selection, aerosol generation, clinical outcome and adverse effects were addressed.[2–8] However, that body of literature is not easily accessible. In recent years, there is renewed interest in formulating drugs for pulmonary delivery for reasons that remain significant. First, the lung mucosa represents a large surface from which drugs may be systemically absorbed into the bloodstream, without having to undergo hepatic first-pass.[9] During the process of systemic absorption from the lungs, drugs introduced into this organ are likely to provide early and high concentrations within it. This is advantageous if, as in pulmonary TB, the lungs are the intended target site of drug delivery. Second, lung macrophages are efficient at fulfilling their evolutionary role of phagocytosing material entering the lungs. It has long been established that macromolecular drugs[10] and particulate[11–13] or vesicular[14–16] drug delivery systems introduced into the deep lung are likely to be picked up by alveolar macrophages (AM).[17] Finally, it has been argued that uptake of drug delivery systems by infected macrophages effects rescue of the macrophage from 'alternative activation,'[18] enabling the elaboration of innate bactericidal responses that could help in killing or containing TB bacilli.[12,19,20]

2. Methods available for aerosol delivery of TB therapies

Aerosol generation and inhalation by human patients may be accomplished by any of three well-established methods in clinical use. The oldest method of aerosol delivery to the respiratory tract is by inhaling smoke, but anti-TB therapies do not lend themselves

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doi:10.1016/j.tube.2010.08.009

easily to this method. Nebulization, or the generation of a mist of droplets through the use of compressed air or oxygen has been in clinical practice for a long time, and is recently experiencing advances in technology.[21] This technique consists of dispersing solid or phase-separated drug delivery systems into droplets suspended in a small amount of medium. The patient is expected to inhale the mist through the nose and mouth (Figure 1A).

The most familiar technique of administering medication to the airways and lungs is the pressurized metered-dose inhaler (pMDI or MDI). Such a device uses an aerosol propellant and a dose-metering valve to regulate the egress of defined amounts of medication to the respiratory tract (Figure 1B).[22,23] The single major issue with pMDIs relates to the necessity of training the patient to coordinate inspiration and actuation of the device. Time-lags between emission of a spray of medicament from the device and the beginning of inhalation lead to wastage of a proportion of the emitted dose.[24] A spacer device that contains the spray and prevents it from dissipating, while allowing more time for the patient to inhale is often used to mitigate this loss.[25]

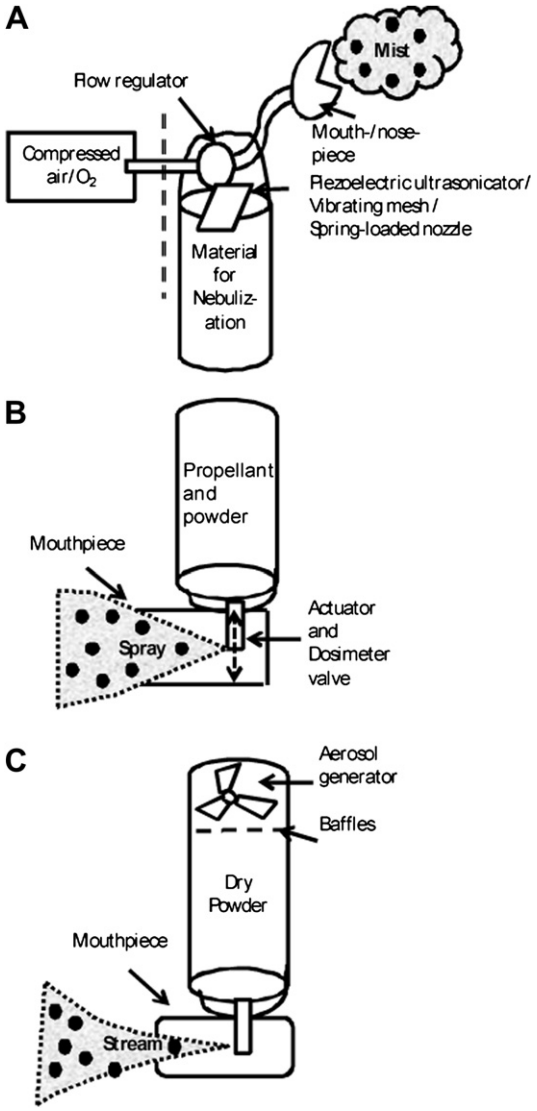

Figure 1. Schematic illustration of therapeutic aerosol delivery. (A) A nebulizer may achieve atomization of droplets by a stream of compressed air, or through piezoelectric sonication, or by mechanical means such as a vibrating mesh or spring-loaded nozzle. (B) A pMDI uses a dose-metering valve to deliver medicament suspended in a propellant spray. (C) A DPI delivers a more gentle stream of inhalant through indrawn breath.

Dry powder inhalers (DPI) represent an option that lies mid-way between nebulization of a medicament in an external aqueous or gaseous phase, and its delivery under positive pressure. DPI relies on the indrawn breath of the patient to pull in a dry powder (Figure 1C). The aerosol is generated by turbulence-creating devices actuated by the airstream of indrawn breath.[26]

In experimental animals, especially in studies on TB therapy, pulmonary delivery is often accomplished by intratracheal delivery. Various authors have reported on the instillation of small volumes of suspensions in the trachea of mice, rats and guinea pigs by endotracheal intubation. An increasingly-used approach for testing pulmonary delivery of drugs and vaccines in animal models is local/regional aerosol administration with a PennCentury MicroSprayer® (for liquids) or Dry Powder Insufflator™. These methods permit intratracheal administration of liquid aerosols or dry powders directly to the lungs. The tip of the device is inserted at the first bronchial bifurcation down the trachea of the anesthetized animal, bypassing the nose and throat and making it easier to precisely quantify the delivered dose. This is in contrast to MDI or DPI inhalers and/or nebulizers in animal studies, which filter the aerosol through the nasopharynx, are prone to inter subject variability and are not capable of targeting a desired, predetermined area of the lungs. However, studies using fluorescent particles as well as dyes have shown that the distribution of the thus applied formulations is mainly within the central lungs, with only a minor percentage reaching the alveolar space.[27–29]

Misra et al. have proposed the use of a “nose-only” exposure chamber that has been demonstrated to deliver reproducible doses in an animal biosafety level-3 setting.[30]

3. Design and process considerations for aerosol delivery systems

Aerosols are multicomponent systems, which exemplify the need for design approaches to facilitate rational development and guarantee their quality.[31] Multivariate statistical methods can be employed to design the process space for each of the major aerosol systems. Examples of input and output characteristics are shown in Figure 2 as they relate to the three major components of all pharmaceutical aerosol products, namely the formulation, metering and aerosol dispersion mechanism. Experiments can be conducted in which the variables in particle manufacture, for example, are evaluated.[32,33] The variables involved in particle manufacture can be adjusted to optimize the drug content, dissolution rate, particle size (mass median aerodynamic diameter, MMAD and geometric standard deviation, GSD), emitted dose (dose delivered from the device) and fine particle dose.[34] The fine particle dose is the proportion of the total dose below a defined particle size related to the cut-off for entry into the lungs.

The complexity of pharmaceutical aerosol products contributes to considerable regulatory oversight and compendial quality and performance standards. The foregoing considerations require products to meet release specifications reproducibly and at minimum expense in terms of time, resources and cost. These are important elements in the development of commercially viable products in general, and especially in the context of products for the treatment of diseases of poverty with regard to the ultimate viability of the product in the country of use.

4. Formulation

Numerous papers are available in the literature on formulating inhaled therapies for TB, but no anti-tubercular inhalable

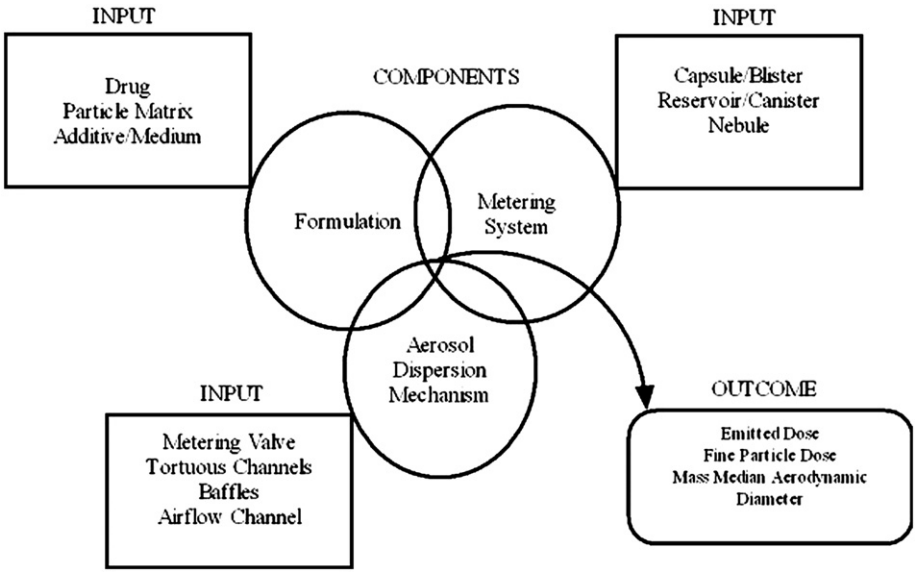

Figure 2. Important elements (inputs) of the major components of an aerosol product and their combined performance characteristics (outputs).

formulation is yet available in clinics.[35] The reason for this delay may reside in the complexity of engineering drugs in respirable formulations, using safe and accepted excipients and developing scalable processes.[36,37] Aerosolized solids or liquids need to possess proper particle sizes and morphology, as well as sufficient drug loading. For clinical relevance in topical or systemic inhalation treatments they must reach the deep airways and deliver a dose of therapeutic molecules suitable for the therapeutic purpose.[35]

Anti-TB drugs have been formulated into particles for pulmonary delivery by a number of researchers.[38–40] Incorporation of drugs into particles offers improved stability and protection of the molecule of interest against, e.g., enzymatic degradation, sustained drug action and release, enhancing bioavailability of poorly soluble therapeutics and drug targeting to specific organs, cells or receptors. In addition, the small size of nanoparticles (<200 nm) allows them to escape both phagocytic and mucociliary clearance mechanisms in the lung.[41,42]

4.1. Drug vesicles for nebulization

The first and most simple approach to deliver drug solutions directly to the lungs by nebulization was followed by the use of aerosolized liposome suspensions. Drug incorporation into liposomes had the main aims of solving solubility/pharmacokinetics issues and of reducing systemic toxicity as well as increasing lung penetration and residence time.[43]

Most of the liposomal anti-tubercular drugs proposed in the scientific literature were conceived for administration by injection. The most studied formulation, MiKasome®, is an injectable small unilamellar vesicle (SUV) suspension containing Amikacin (AMK), patented and produced by former NeXstar, now Gilead.[44,45]

Capreomycin (CPR) has been prepared as a liposomal formulation for pulmonary administration. Among others, distearoylphosphatidylcholine was shown to be the most suitable phospholipid with regard to vesicle stability and lipid–drug interaction. The vesicles had a narrow size distribution, with a mean diameter lower than 200 nm. They contained 10–13% by weight of the drug, and possessed a smooth surface and spherical/ellipsoidal morphology. These characteristics demonstrated their suitability for use in inhaled formulations.[46] By using both freeze-thawing technique and a response surface methodology, the drug content was further improved.[47] Isoniazid (INH) and rifampicin (RIF)-loaded multilamellar vesicles, when administered to guinea pigs as nebulized suspensions, yielded detectable plasma drug levels up to 48 h after administration, while nebulized free drugs were no longer detected after the first 24 h.[48] Recent lung deposition and clearance studies performed in human volunteers using gamma scintigraphy showed that 24 and 48 h after inhalation of AMK-loaded liposomal suspensions, 60.4% and 38.3% of radioactivity was still detected.[49]

Ligand-mediated targeting has been investigated as a supplementary approach to increase drug accumulation in AMs. Thus, aerosol administration of ligand-tagged liposomes showed preferential accumulation in lungs with a high drug concentration detectable even after 24 h. RIF-loaded maleylated bovine serum albumin or O-SAP (*O*-steroyl amylopectin) tagged liposomes limited the survival of *Mycobacterium smegmatis* inside macrophages to 7–11%. When free drug and conventional liposomes were administered, mycobacterial viability was as high as ~46% and 32%, respectively.[50]

In the specific case of liquid aerosols, whether free drug solutions or liposomal/nanoparticle suspensions, droplet size is the main determinant in lung deposition. Therefore, nebulizer features and efficacy are very important for clinical outcomes.[51,52] In addition, nebulization stress has been shown to alter liposomal drug properties.

A liposomal AMK formulation (Arikace™, registered trademark of Transave, Inc., USA), developed as inhaled treatment for gram-negative infections, has been tested after nebulization using an Andersen cascade impactor. When material collected on different stages of the impactor, representing fractions deposited in the different regions of the respiratory tract and lung was analyzed, some cause of concern emerged. Differences in lipid-to-drug ratio, AMK retention, and liposome size were found between stages 1–5 and stage 0, suggesting the possibility of heterogeneity in material reaching different parts of the respiratory tract.[53]

Liposomal formulations, due to the high biological safety of phospholipids, the natural lung surfactants, and the existence of different products for pediatric use in the market,[54] have a strong chance to reach the clinic in a short time.

4.2. Drug powders for inhalation

Another interesting and promising approach is the formulation of active pharmaceutical ingredients as respirable dry powders.

CPR has been formulated as dry powder by spray drying, by different investigators.[37,55,56] Using L—leucine as the main excipient, it was possible to obtain inhalable particles with drug contents from about 60 to 93%.[55,56] These formulations, assessed in guinea pigs, showed an absolute bioavailability of 59% with an increase in half-life of the drug. These results demonstrated the usefulness of inhalation therapy to reduce the overall administered dose. Inhalation, when compared to the conventional intramuscular route, was able to produce bioequivalent concentrations in the lungs at a lower dose. Inhaled CPR reduced inflammation, bacterial count and granulomas in the lungs. However, only i.m. administration reduced the bacterial count in the spleen, suggesting that inhalation therapies may not be sufficient for managing extra-pulmonary TB.[55,56] Other authors prepared respirable powders by ion-pairing CPR with sodium oleate. CPR oleate particles of size and shape suitable for inhalation were obtained by homogenization and spray drying.[37]

4.3. Microparticles, nanoparticles and composites

One of the most studied approaches in the last decade is the formulation of anti-tubercular drugs as respirable insoluble microparticles (MPs).[32,57,58] Besides targeted drug delivery to the lungs, or more specifically, lung macrophages, there are indications that MPs can activate bactericidal macrophage responses as discussed below. Studies by Hickey et al. demonstrated significantly higher efficacy of RIF in *poly*(lactic-co-glycolic acid) (PLGA) MPs.[32,57] Particle administration to infected guinea pigs led to a significant reduction of spleen inflammation when compared to the free drug.[57] Daily doses of RIF solutions over 10 or 20 days had a positive effect on pulmonary and splenic inflammation but not on the number of viable bacteria in the lungs, while a single administration of MPs or 20 days of dosing with free RIF equally decreased the bacteria population in the spleen. Besides, PLGA MPs increased drug residence time in the lungs.[59] These observations, too, highlight the issue of targeting pulmonary *versus* extra-pulmonary TB by inhaled therapies.

PLGA MPs have been used to deliver INH or a combination of INH and RIF.[58,60] This approach led to a higher drug accumulation in macrophages when compared to free drugs. Further studies showed that the administration of respirable *poly*(lactic acid) (PLA) MPs loaded with rifabutin (RFB) and INH, led to intracellular concentrations four-fold higher than the controls (drug solutions).[61]

Conventional and large porous PLGA MPs have been also proposed as carriers for CPR.[62,63] Labeled MPs of about 5 μm in diameter could be efficiently and completely taken up by murine AM, within <3 h[63]

Recently, RIF encapsulated in PLGA microspheres was administered to TB-infected rats.[64] This study showed that particles were not toxic since their phagocytosis did not produce any inflammatory mediators (i.e., TNF-α, NO) and could be considered as biosafe for the lungs. Moreover, particles were taken up very efficiently by macrophages inducing a potent bactericidal effect.[64]

RIF-loaded PLGA nanoparticles (NP) embedded in mannitol MS have been prepared using a four-fluid nozzle spray-dryer.[65] These composite particles possess the advantages of nanoparticles without their drawbacks (e.g., poor aerosolization). However, some doubts are expressed regarding the capability of NP to be taken up *in vivo* after mannitol solubilixation since the reported macrophage uptake was quite low ($<10\%$).[65]

5. Materials of construction (excipients) and inhalation safety

A limited number of substances are approved by drug regulatory agencies worldwide for use in inhalation therapy. In general, the choice is among the excipients used in injectable dosage forms. However, innovation in the field requires new solutions for new formulation problems. A recent review of excipients and formulation strategies addresses the relevant issues in detail.[66]

Excipients may be categorized according to the type of inhalation product. For nebulization solutions, excipients are typically the same as used in injectables or large-volume parenterals for pH and osmolarity control, preservation or solubilization and wettability.

DPI often avoid excipients if the shape and size of dry particles is sufficient for ensuring respirability of the formulation. In some cases, sugars, amino acids or soluble polymers are used as particle shapers. These excipients are employed for constructing respirable particles with techniques such as spray drying. Sustained-release polymers, such as PLA/PLGA or other polyesters, polyacrylates, albumin, chitosan or alginates are studied for extending the lung residence time of compounds. The goal is to increase efficacy and allow safer low doses to be effective. Biocompatible/biodegradable polymers are considered in particular for escaping or targeting macrophage uptake. In general, particles made up of polymers that are hydrophilic are taken up less efficiently by macrophages. For highly potent anti-TB agents (e.g. siRNA), when the dose is very low, lactose may be used as a 'carrier' for constructing ordered mixtures. It is important to stress that lactose intended for use in pulmonary delivery is a large-sized powder used for flow and packing improvement. During aerosolization, micronized drug particles are detached and lactose particles are deposited in the mouth.

MDI require the use of propellant. Currently, hydrofluoroalkanes (HFA) are used as propellants, since the elimination of chlorofluorocarbons (CFC). However, there are formulation and processing issues associated with the use of HFA.[67] The search for suspension aids to prepare high-quality suspensions or solubilizers to enable solution formulations at high doses continues. Lipids, phospholipids or surfactants are often present in the MDI formulations.

The formulation of inhaled therapies outlined above does not cite a variety of materials used in their preparation, since the introduction of a never-used-before adjuvant requires safety demonstration before it is accepted. Inhalation safety and toxicity are important considerations, especially in the light of reports indicating that clinicians, too, might overlook this crucial aspect. For instance, the anti-influenza drug Relanza® is formulated as a dry powder for inhalation. It was reported that the inhalation product was removed from its FDA-approved packaging and administered by nebulization to a patient on ventilator support. This led to clogging of the ventilator by lactose present in the formulation, and the death of the patient in consequence (http://www.fda.gov/safety/medwatch/safetyinformation/safetyalertsforhumanmedicalproducts/ucm186081.htm). Additionally, although concerns generated by reports of altered lung function as a result of inhaled insulin (Exubera®) were unfounded as these complications were not due to inflammation of the lungs or airways,[68] development of inhaled TB therapies needs to pay attention to the immunological status of the tubercular lung.

Thus, hypoallergenic materials such as lactose, mannitol, sodium citrate dihydrate, glycine, lysine, leucine, etc. described above, are generally used in inhalation products, including Exubra®. Such material is 'generally regarded as safe' and is not known to modify immune response to the inhalant in the lung.[69]

Biodegradable polymers, mainly the PLA-PLGA series, too, enjoy a long record of clinical safety in applications including injected drug delivery systems and surgical sutures. However, concerns have been voiced regarding continued administration of polymeric microparticles or nanoparticles over a time period of several months.

Some concerns, however, accompany the use of long-chain polymers that are not biodegradable, such as *poly*(ethylene glycol), and lipids. These concerns arise from incomplete knowledge of how lung tissue might respond to the continued presence of foreign material. However, innovative use of lipid surfactants can offer two advantages. Lipids such as dipalmitoylphosphatidylcholine (DPPC) can be used to formulate liposomes containing 1–3 first-line anti-TB drugs.[70] These liposomes have the potential to interact with endogenous lung surfactant to reduce alveolar atelectasis induced by *Mtb* products,[71] while also increasing the supply of drugs available at the alveolar surface.[72]

6. Targeting alveolar macrophages

There are two objectives of targeting AMs using inhaled therapies. First, such targeting can deliver extremely large amounts of the anti-TB drugs to the macrophage cytosol, even potentially sufficient to overcome drug resistance. Second, uptake of particles by macrophages has the potential to activate infected macrophages. A recent paper on RIF-loaded PLGA MPs reported that infected macrophages possess an enhanced phagocytic activity with respect to uninfected cells. This is a very interesting finding since it demonstrates that phagocytic activity, during infection, is not only maintained but even increased.[73]

6.1. Size, shape and surface characteristics relevant to targeting

Pulmonary delivery formulations intended for macrophage targeting should be easily recognized and phagocytosed by infected AM. For this purpose, they should possess appropriate physical dimensions and surface characteristics. Thus, although there is *in vitro* evidence of activation of human macrophages with particles as large as 90 μm,[74] the size amenable to phagocytosis is 1–10 μm, with an optimum of 3 μm.[75]

Similarly, the surface characteristics are important for both phagocytosis and macrophage activation. The biocompatible and biodegradable carrier PLGA is widely used in inhaled therapies. Makino et al. have developed a formulation for inhaled therapy with RIF.[64,73,75,76] These authors demonstrated that phagocytosis of particles made up of *poly*(styrene), an extremely hydrophobic material, is less efficient than that of PLGA particles of equivalent size and shape but of lower hydrophobicity. Misra et al.[58,61] have employed an extremely hydrophobic biodegradable polymer, high molecular weight *poly*(L-lactic acid), but still report efficient phagocytosis. This may be due to the fact that their formulations contain an unusually high drug payload, which could modify the surface characteristics of the particles by virtue of high surface density of drug molecules. Hickey et al. demonstrated the advantage of particles with an elliptical geometry for macrophage uptake.[77] However, surface and shape can also affect lung deposition of inhaled therapies.

6.2. Deposition in the airways and alveoli

Medication using DPI can result in high local levels of drug in epithelial lining fluid of the airways and lower respiratory tract. Drugs administered topically to the lungs, *via* aerosol are attractive in that they achieve higher levels in the lungs with fewer systemic side effects. Particles with aerodynamic diameter between 2 and 5 μm can effectively reach deep in the lungs. The 'fine particle fraction' (FPF), defined as % particulate matter below a threshold size, can be used to predict lung deposition.[78] In the airways, the humidity is very high, at almost 100%. Therefore, the drug carrier has to resist wetting and subsequent aggregation in the respiratory tract. It can thus be appreciated that the twin objectives of lung deposition and macrophage targeting will depend strongly on size, shape and surface properties of drug carrier systems.

7. Inhaled formulations of second-line anti-TB medications

Whereas first-line medications for TB seem to be amenable to uptake *via* the lung as nebulized agents, the question is whether there is really a compelling need for altering the conventional route of administration for agents that are adequately absorbed and distributed *via* the oral route. A point of consideration might be that adjunctive therapy by the pulmonary route of a drug that is also orally dosed, in order to achieve higher concentrations at the site of infection, might hold distinct advantages. For example, current clinical studies with rifampicin indicate that higher doses might be desirable to avoid relapse of disease. Given firmly established WHO and other agency protocols for treatment of TB worldwide by predefined dosage forms of fixed-dose combination oral drugs, and the large current market for FDCs, realigning policies and the manufacturing industry towards altered FDC dosage forms would be difficult and slow. Addition of drug such as rifampicin in a unique formulation would make sense under such a scenario. Similarly, new compounds dosed to the lung as adjunct therapy would have the same advantages.[6]

Second-line (reserve) drugs for TB pose special challenges for delivery. Most of the current drugs used for the treatment of MDR-TB are moderately potent, show restrictions with absorption or oral bioavailability, and have toxicity profiles that make patient management difficult. These drugs could be primary targets for development as inhalable agents. In 2001, Sacks et al. published a study of inhaled aminoglycosides (kanamycin and/or gentamicin) as salvage therapy for patients in South Africa that had remained culture positive for 2 months or more despite appropriate conventional drugs.[79] The population included 7 patients with drug susceptible and 12 with drug resistant TB, including 6 who were HIV-positive. These were all patients with unusually persistent positive sputum smears and cultures, with average smear positivity of 96 days, but ranging up to 439 days. Control subjects included only drug susceptible patients. Despite selecting for persistent culture positivity, among patients with drug susceptible isolates, 2 of 3 with cultures examined were negative within the first month of inhaled treatment. Among the 12 patients with drug resistant isolates, only 3 had cultures sent within 3 months of starting inhaled aminoglycoside therapy and all were negative. Overall, 68% of the patients converted their sputa from smear positive to negative while on therapy, including 6 of the 7 with drug susceptible and 7 of the 12 drug resistant TB. Among the 13 who converted their sputum, the median time to conversion was 23 days, but the authors note that shorter times to conversion might have been observed had sputum samples been obtained earlier in the course of treatment.

A few second-line anti-TB drugs have been formulated in dry microparticles for pulmonary delivery, including capreomycin[55,56] and para-aminosalicylic acid.[80] Results indicated that direct delivery to the lungs indeed results in high local concentrations and reduced bacterial burden compared to the same treatments delivered *via* other routes, offering the possibility of reduced doses and systemic side effects.

Ofloxacin (OFX) has been studied for lung delivery in hyaluronan microspheres.[65] These particles were characterized by good aerosolization efficiency when combined with lactose used as carrier and were able to efficiently deliver the drug to the lungs. Even though hyaluronan forms a mucoadhesive gel in contact with broncheoalveolar fluids, OFX uptake by macrophages was higher when formulated in this fashion as compared to the drug alone. Intratracheal administration of OFX-loaded hyaluronan particles

resulted in 50% lower serum bioavailability with respect to intravenous or oral OFX. This observation supports the view that inhaled MPs may reduce systemic side effects, but also suggests that extrapulmonary TB may not be addressed by inhaled therapies alone.[65]

Capreomycin (CM) has been dosed by intravenous (IV) and intramuscular (IM) routes of administration for several decades in the treatment of TB; therefore its safety, side effect profile and tolerability by these routes are well understood and characterized in humans. It is expected that doses of CM delivered by the inhaled route will produce much lower systemic levels than CM given by the IM or IV routes.

8. "Stimulating the phagocytes"—macrophage activation induced by phagocytosis of inhaled formulations

Phagocytosed *Mtb* is resistant to phagosomal digestion and proliferates in AMs by making use of them as incubators[81,82] and inducing 'inappropriate' or 'alternative' activation.[18] It is expected that delivery of microspheres (MS) to AMs may induce classical, bactericidal activation upon phagocytosis.[20,83,84] According to this strategy, considerable attention has been paid to the development of anti-tuberculosis agent-containing respirable MS. First of all, the MS should be inhalable to reach AMs, but the MS should also meet at least 3 requirements; MS should be 1) well phagocytosed by *Mtb*-infected AMs,[73,75,76] and not inhibited by lung surfactant[85,86]; 2) effective in killing *Mtb* through payload delivery,[64] macrophage activation,[12] or a combination of both[87]; and 3) non-toxic to AMs.[64] Figure 3 illustrates the difference in intracellular drug concentrations achieved by administering either MS or drugs in solution to cultured macrophages infected *in vitro*.

A variety of pathways may be induced in infected AMs following phagocytosis of MS. It has been proposed that natural ligands of Toll-like receptors (TLRs) such as alginate may be used as a material of construction of MS for oral delivery.[88] Such MS may induce proinflammatory cascades conducive to killing intracellular bacteria. Chitosan is a similar material, and has been used for pulmonary delivery of a candidate TB vaccine.[28] Some authors argue that just the event of phagocytosis by an *Mtb*-infected AM may be sufficient to induce classical activation. Thus, production of reactive oxygen species (ROS) and nitrogen intermediates (RNI)[19]; secretion of proinflammatory cytokines like TNF, IL-6 and IL-12,[12,89] induction of apoptosis rather than necrosis of infected macrophages,[90] have all been demonstrated to result from exposure of infected AM to drug-containing and blank MS.

PLGA has been commonly used for the base of MS, because it is biodegradable and biocompatible. Phagocytosis of MS increases with increase in the particle number of MS, and optimum size for phagocytosis is between 3 and 6 μm.[75,76,91,92] In addition, phagocytosis of PLGA MS stimulates the phagocytic activity in such a way that it increases both the population of phagocytic AM and the number of MS that has been phagocytosed. The concentration of intracellular rifampicin taken up by AMs in a form of PLGA MS is about 20 times greater than that administered in a form of solution, causing potent anti-tubercular activity. Phagocytosis of rifampicin-loaded PLGA MS does not generate the toxic humoral factors to AMs, such as TNF-α and NO, and the phagocytosis does not affect the viability of AMs, showing that, PLGA MS are not toxic to AMs.[64] From these results PLGA MS loaded with anti-tubercular agent meet the minimum requirements described above. Hence, delivery of antibiotic-incorporated PLGA MS is promising for respirable formulation targeting to AM that are infected with *Mtb*.

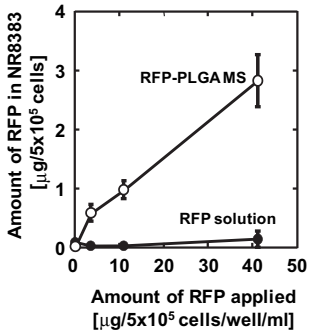

Figure 3. Effective uptake of rifampicin in a form of PLGA MS by the alveolar macrophage cell line NR8383.[24] Results after incubation for 12 h. Average diameter of MS was 2 μm.

9. Novel payloads for inhaled TB therapies

Inhaled drug delivery offers opportunities to administer anti-TB agents or modulators of innate immune function that might not be amenable to conventional methods of drug delivery through oral or injectable routes. For instance, Sukhanov et al. recently demonstrated benefits in resolution of lung lesions in human patients administered inhalations of cycloferon as an immunomodulator for 5 weeks.[93] The nitroimidazopyran antibiotic PA-824 discussed above is a novel payload, equally suited to pulmonary and oral delivery through appropriate formulation. Aerosolized streptomycin in combination with steroids has been demonstrated as safe and efficacious in endobronchial stenosis due to TB.[94–96]

The use of a host molecule, γ-interferon (IFN-γ), rather than a conventional anti-TB drug was demonstrated clinically to offer benefits in MDR-TB.[97–100] Thus, 500 μg doses of nebulized IFN-γ were deposited within alveoli and led to sputum conversion and general improvement in all clinical parameters after 12 doses spread over 1 month.[97] The argument spelling out the rationale of these studies was that IFN-γ is a potent effector molecule that may induce activation of several metabolic pathways in the infected macrophage. Hence, production of interleukin (IL)-1, upregulation of *NOS* or induction of *iNOS*, *NOX*, *PHOX* and similar pathways of oxidative/nitrosative radical generation, upregulation of MHC-I in addition to MHC-II and consequent enhancement of intracellular antigen presentation could be some of the pathways induced by IFN-γ. All of these would be inimical to the survival and proliferation of TB bacilli. Similarly, IFN-α has been used clinically with the objective of not only inducing macrophage activation, but also of cross-regulating the cytokine environment developing as a result of inappropriate macrophage activation and immunopathology.[101] The authors reported reduction of sputum counts of *Mtb*, improvement in clinical parameters, and a significant change in the cytokine milieu of patients administered IFN-α. Thus, patients receiving 3 MU/dose of IFN-α, 3 times a week for 2 months showed highly significant downregulation of tumor necrosis factor (TNF)-α, IL-1 and IL-6 in the BAL fluid, while the control group (on conventional therapy alone) displayed a much slower rate of depletion of these cytokines. These results argue that inhaled IFN-α can speed up the process of disease resolution.

Rather than the effector host molecule itself, inhaled therapies incorporating chemical inducers of the relevant molecules would be easier to prepare, store and distribute. Thus, muramyl dipeptide (MDP), a generic ligand of the crucial Nod-like receptor family,[102,103] was investigated in rats and guinea pigs as a potential payload for inhaled TB therapy.[104] Macrophages exposed to inhaled MDP showed upregulation of enzymes and dose-dependent increase in NO production, indicating that relevant benefits might be derived through the administration of MDP. Recent work on inhalable microparticles incorporating US-FDA-approved NO donors such as

isosorbide mononitrate and sodium nitroprusside, or agents such S-nitroso thiols (S-nitrosoacetyl penicillamine, SNAP), has shown encouraging results *in vitro* and in guinea pigs infected with *Mtb* (Verma et al., unpublished). This work is based on a conjecture that a deficiency in the ability of an infected macrophage to produce bactericidal amounts of NO might be a contributing factor to establishment of infection, and that providing the effector molecule in the macrophage cytosol might contribute to bactericidal activity.

Rather than potentiate innate responses, a section of the patient population might benefit from the administration of inhaled steroidal anti-inflammatory agents.[105] This approach argues that a certain set of patients with persistent TB-associated fever may be treated with inhaled prednisone or budenoside.[106] Reports of inhaled phytochemicals, e.g., from *Eucalyptus globulus*, helping in clinical management of TB may also be related to similar phenomena.[107]

Yet another novel approach to inhaled TB therapies is embodied in the delivery of surfactant to the lung.[108] The authors demonstrated in a cohort of 70 patients compared with age, stage and gender-matched controls that a cumulative dose of 700 mg of a surfactant designated as "Surfactant-BL" over 4 weeks resulted in rapidly falling sputum counts in 70% of cases, complete sputum conversion in 83% in 2 months, and highly improved resolution of lesions. This approach is similar to that of Chimote et al. discussed above.[70–72,109]

The latest entrant among candidates that may be used in inhaled TB therapies is siRNA targeting the host chemokine XCL-1 or lymphotactin, discussed in greater detail elsewhere in this volume.[110] The most encouraging finding with reference to pulmonary drug delivery is the observation that out of 10 μg of a naked, 19-base RNA sequence delivered to the lungs of mice, sufficient amounts were taken up by mouse AM, and continued to exhibit up to 50% pull-down of the target RNA up to 3 days after administration. Clearly, there are aspects of uptake and/or phagosome escape of macromolecules that require further study, but as far as macromolecular drug delivery to AM is concerned, there is room for optimism.

10. Drug disposition and pharmacokinetics following aerosol delivery

Pharmacokinetics (PK) of anti-TB agents or immunomodulators following inhalation of aerosols depend very strongly on aerosol characteristics. The foremost requirement of inhaled therapies is that the drug reaches the deep lungs with minimal loss in the oropharynx and airways. Figure 4 below summarizes the path of the drug from orally-inhaled aerosols.

Deposition of inhaled drug delivery aerosols has been extensively reviewed,[111–113] and is not revisited here. The key concepts regarding deposition of orally-inhaled aerosols are:

- Aerodynamic rather than merely physical dimensions of inhaled material direct its entrapment in the oropharynx and upper airways; deposition in deep lung; or loss in expired air.
- Material deposited on the alveolar surface first encounters lung surfactant, and is rapidly (1–5 min) phagocytosed by AM unless it dissolves and permeates through lung epithelium.
- Poorly-aerated regions of the lungs receive lesser amounts of inhaled material, and vice versa. Better perfused regions of the lungs are depleted more rapidly of soluble material.

With specific regard to inhaled TB therapies, there are some studies that highlight interesting aspects of pulmonary delivery PK.

Radio-iodinated IFN-γ nebulized to healthy volunteers rapidly saturated the lungs, regardless of the nebulizer load, and equally rapidly decayed in concentration with bi-exponential kinetics.[114] The relative contributions macrophage uptake and degradation and of clearance from the lung through blood circulation could not be established in this study. Capreomycin PK following inhalation of large porous particles showed dose-dependent AUC and C_{max}, but similar bioavailability (41–59%) at the 3 dose levels tested in guinea pigs.[55] This study also demonstrated near-complete uptake of the drug presented to the lungs, such that BAL fluid recovered 12 h after dosing did not contain detectable capreomycin. Further,

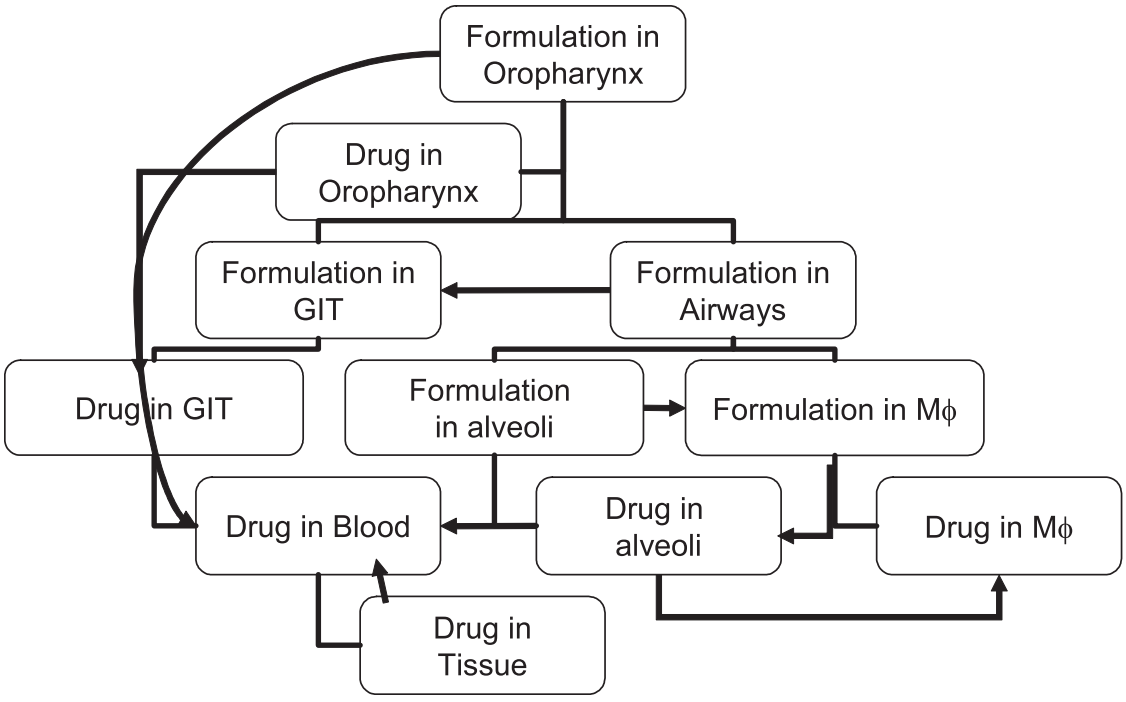

Figure 4. Overview of drug distribution rate processes affecting pharmacokinetics of inhaled formulations.

pulmonary delivery smoothened out biphasic elimination kinetics observed on parenteral dosing, reduced clearance (CL), and thereby enhanced $t_{1/2}$. These results provide strong support to the objective of using the lung as a portal of systemic delivery of anti-TB agents.

Large, porous particles of *para*-aminosalicylic acid (PAS) formulated with dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) designed to avoid macrophage uptake showed additional interesting results in a limited study on rats.[80] The particles (5 mg) were administered by intratracheal insufflation. Blood, BAL and lung tissue was sampled over 6 h. Rapid rise of blood levels, followed by rapid (biphasic?) decline was observed over a 3 h period. In BAL fluid, however, no PAS was detectable after the first 15 min. Thus, the phenomenon sometimes termed "lung capacitance"[115] or "depot",[116] which refers to the establishment of a reservoir of the drug in the lungs or airways following airway delivery, was not observed in this study.

PK resulting from administration of controlled-release preparations is more complex. As evident from Figure 4, if drug release from the formulation is itself a rate process, PK will reflect the effect of the controlled drug release. Evidence to this effect is available from studies on particles containing isoniazid (INH) and rifabutin (RFB) in a fixed-dose combination.[87] Serum concentration *versus* time plots of the two agents indicated distinct absorption and elimination phases after pulmonary delivery. In comparison to intravenous administration of solutions containing equivalent amounts of the two drugs, inhaled microparticles delivered longer-lasting blood levels. Tissue distribution following inhalation of microparticles was tilted very favorably towards accumulation in lungs, and more specifically, lung macrophages. The liver and kidney received a much lower amount in comparison to intravenous administration of solutions. The limitations of currently-available PK mathematical models for controlled delivery to the lungs was brought out during this study — available non-compartment models best fitting the data calculated an impossibly large difference between AUC observed upon intravenous administration of INH ($\sim$17 μg/ml/h) and the same amount in inhaled microparticles ($\sim$55 μg/ml/h). However, this study did not support the observations of Khuller et al. who have repeatedly observed sustained drug levels in the lungs for periods of 5–15 days after a single nebulization.[38,40,117]

In monkeys (Verma et al., unpublished), blood levels, intracellular concentrations in lung macrophages, and concentrations in lung tissue, liver and kidney resembled the results observed in mice.[87] The use of the INH-RFB combination is proposed to give rise to a situation of pharmacokinetic synergy, wherein the superior early bactericidal activity of INH combines with sustained bactericidal concentrations of RFB.

In studying PK following pulmonary delivery, it is important to distinguish between concentrations achieved in generalized lung tissue, granulomata and caseous lesions, and also within the cytosol of AM. Mouton has strongly questioned the relevance of analyzing tissue homogenates for antimicrobial drug concentrations.[118] The need to establish PK at 'the site of action' deserves greater attention when inhaled TB therapies are being designed.

11. Efficacy of inhaled therapies in animal studies

Unlike other dosage forms intended for oral or parenteral delivery, aerosol product performance depends on a transient, non-equilibrium phenomenon: aerosol generation. Droplet or particle deposition in the lungs is known to be species dependent largely because of anatomical and physiological differences. In addition, the extent to which the pathogenesis of TB is replicated in animals with respect to man varies. Consequently, a number of serious considerations are required in the selection of an appropriate species in which to conduct efficacy studies.

Important factors in the selection of an animal model include tidal volume, breathing frequency and particle size cut-off for penetration into the lungs. The first two of these factors have been described in terms of allometric scaling where $Y = aM^b$ and for tidal volume (mL) a = 7.69, b = 1.04 and breathing frequency (min^{-1}), a = 53.5 and b = −0.26.[119] The important features of the aerosol employed is its mass median aerodynamic diameter, the measure of central tendency, and geometric standard deviation, the measure of distribution, these parameters define the dose in a size range suitable for penetration into the lungs.[111] Ultimately the efficiency of delivery of aerosol particles/droplets can be measured in pharmacokinetic studies in both normal and infected animals to estimate the dose deposited in the lungs by broncheoalveolar lavage and systemic disposition.[120]

Several species have been used to study tuberculosis including, mice, guinea pigs, rabbits and non-human primates.[121] The pathogenesis of disease in humans includes low dose aerosol infection followed by primary granuloma formation, bacillemia, secondary granuloma formation, cavity formation with necrosis and caseation. Of the species mentioned non-human primates are thought to be most relevant models for humans. In contrast, mice require large inocula to become infected where guinea pigs and rabbits are susceptible to low dose infection. Mice and guinea pigs exhibit granuloma formation but there is little evidence for cavity and caseation. Rabbits in contrast to mice and guinea pigs exhibit cavities and caseation. With respect to aerosol delivery the larger the animal the greater the proportion of the aerosol dose delivered in the shortest time. Methods of particle/aerosol delivery include direct intubation followed by insufflation/instillation; nose-only exposure and whole body exposure with passive inhalation by the animals.

The Glasscol and Madison exposure chambers are classical infection methods employed for all animals except non-human primates. These are whole body exposure chambers where animals with modest restraint are exposed to nebulized suspensions of *Mycobacterium tuberculosis*. The baffle systems employed are intended to reduce exposure to large droplets which results in very dilute aerosols in sizes suitable for inhalation by the laboratory animals. Inoculum doses can be modulated by changing the concentration of the suspended micro-organisms. In the case of mice, hundreds of colony-forming units (CFUs) are required to establish the infection, whereas in guinea pigs and rabbits individual or tens of CFUs are sufficient to achieve this objective.

Drug delivery requires an accurate knowledge of the dose delivered. Consequently, the way in which animals receive aerosol particles or droplets is important to the investigation of subsequent disposition or effect.[122] Whole body exposure does not offer sufficient control and allows animals to ingest drug deposited on their exterior by grooming, which may confound interpretation of the data. Consequently, the convention for the assessment of aerosol drug effects has become initial studies using direct insufflation or spray instillation to allow the pharmacokinetics of disposition to be established followed by nose-only exposure studies in which a true aerosol is delivered and the dose can be calculated with reference to the insufflation/instillation studies.

Efficacy in response to aerosols delivered to the lungs of infected animals has been recently reviewed elsewhere.[35] A variety of drugs including rifampicin, rifabutin, rifapentine, isoniazid, ethambutol, ethionamide and capreomycin have been studied as aerosols to treat TB in laboratory animals. None has progressed to a treatment trial at this point. In most cases a significant benefit, in terms of reduction in bacterial burden in the lungs, and in some cases systemically, were noted. These observations lead to the prospect of

the use of aerosols to supplement therapy administered by other routes and the possibility of rapid reduction in lung burden of bacteria. Indeed, a recent patent indicates that complete sterilization (no organisms cultured from lungs or spleens) was achieved in mice following pulmonary delivery anti-tubercular drugs.[123] Work continues on new[124,125] and abandoned[126,127] drugs attempting to open new opportunities for therapy.

References

1. Berishvili TA. [PAS inhalation therapy of respiratory tuberculosis in the sanatorium LIBANI; preliminary communication.]. *Probl Tuberk* 1954; **4**:69–70.
2. Kulik NM. [Inhalation of cycloserine in the complex therapy of patients with pulmonary tuberculosis]. *Probl Tuberk* 1967;**45**:38–40.
3. Kulik NM. [Inhalation therapy of tuberculosis patients with antitubercular agents of the first and second line]. *Probl Tuberk*; 1974:34–7.
4. Kulik NM. [Treatment of pulmonary tuberculosis by the inhalation of aerosols of antibacterial preparations and pathogenetic agents]. *Probl Tuberk*; 1980:40–2.
5. Pilipchuk NS, Protsiuk RG. [Effect of inhalation of aerosols of antitubercular preparations on pulmonary surfactant]. *Vrach Delo*; 1986:21–5.
6. Protsiuk RG. [Effect of the inhalation of tuberculostatic aerosols on the dynamics of bacterial excretion in pulmonary tuberculosis patients]. *Probl Tuberk*; 1983:38–42.
7. Protsiuk RG. [Effect of the inhalation of drug aerosols on external respiration in pulmonary tuberculosis patients]. *Klin Med (Mosk)* 1985;**63**:50–5.
8. Semenova EV. [Basis for the administration of streptomycin and isoniazid in ultrasonic aerosols in the treatment of intrathoracic tuberculosis]. *Antibiotiki* 1977;**22**. p. 469–471.
9. Mathias NR, Hussain MA. Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. *J Pharm Sci* 2010; **99**:1–20.
10. Lombry C, Edwards DA, Preat V, Vanbever R. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. *Am J Physiol Lung Cell Mol Physiol* 2004;**286**:L1002–8.
11. Moller W, Barth W, Kohlhaufl M, Haussinger K, Stahlhofen W, Heyder J. Human alveolar long-term clearance of ferromagnetic iron oxide microparticles in healthy and diseased subjects. *Exp Lung Res* 2001;**27**:547–68.
12. Yadav AB, Sharma R, Muttil P, Singh AK, Verma RK, Mohan M, et al. Inhalable microparticles containing isoniazid and rifabutin target macrophages and 'stimulate the phagocyte' to achieve high efficacy. *Indian J Exp Biol* 2009;**47**:469–74.
13. Evora C, Soriano I, Rogers RA, Shakesheff KN, Hanes J, Langer R. Relating the phagocytosis of microparticles by alveolar macrophages to surface chemistry: the effect of 1,2-dipalmitoylphosphatidylcholine. *J Control Release* 1998; **51**:143–52.
14. Myers MA, Thomas DA, Straub L, Soucy DW, Niven RW, Kaltenbach M, et al. Pulmonary effects of chronic exposure to liposome aerosols in mice. *Exp Lung Res* 1993;**19**:1–19.
15. Shephard EG, Joubert JR, Finkelstein MC, Kuhn SH. Phagocytosis of liposomes by human alveolar macrophages. *Life Sci* 1981;**29**:2691–8.
16. Forsgren P, Modig J, Gerdin B, Axelsson B, Dahlback M. Intrapulmonary deposition of aerosolized Evans blue dye and liposomes in an experimental porcine model of early ARDS. *Ups J Med Sci* 1990;**95**:117–36.
17. Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 1990;**141**:471–501.
18. Kahnert A, Seiler P, Stein M, Bandermann S, Hahnke K, Mollenkopf H, et al. Alternative activation deprives macrophages of a coordinated defense program to Mycobacterium tuberculosis. *Eur J Immunol* 2006;**36**:631–47.
19. Sharma R, Muttil P, Yadav AB, Rath SK, Bajpai VK, Mani U, et al. Uptake of inhalable microparticles affects defence responses of macrophages infected with Mycobacterium tuberculosis H37Ra. *J Antimicrob Chemother* 2007; **59**:499–506.
20. Terada H, Makino K, Soma G-I. Remedy. *PCT Int Pat Appl*; 2004. WO/2004/019982.
21. Lange CF, Finlay WH. Liquid atomizing: nebulizing and other methods of producing aerosols. *J Aerosol Med* 2006;**19**:28–35.
22. Berger W. Aerosol devices and asthma therapy. *Curr Drug Deliv* 2009;**6**:38–49.
23. Fink JB. Metered-dose inhalers, dry powder inhalers, and transitions. *Respir Care* 2000;**45**:623–35.
24. Rau JL. Practical problems with aerosol therapy in COPD. *Respir Care* 2006;**51**:158–72.
25. Newman SP. Spacer devices for metered dose inhalers. *Clin Pharmacokinet* 2004;**43**:349–60.
26. Onoue S, Misaka S, Kawabata Y, Yamada S. New treatments for chronic obstructive pulmonary disease and viable formulation/device options for inhalation therapy. *Expert Opin Drug Deliv* 2009;**6**:793–811.
27. Century T. Personal communication, 2010.
28. Bivas-Benita M, van Meijgaarden KE, Franken KL, Junginger HE, Borchard G, Ottenhoff TH, et al. Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of Mycobacterium tuberculosis. *Vaccine* 2004;**22**:1609–15.
29. Bivas-Benita M, Zwier R, Junginger HE, Borchard G. Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. *Eur J Pharm Biopharm* 2005;**61**:214–8.
30. Kaur J, Muttil P, Verma RK, Kumar K, Yadav AB, Sharma R, et al. A hand-held apparatus for "nose-only" exposure of mice to inhalable microparticles as a dry powder inhalation targeting lung and airway macrophages. *Eur J Pharm Sci* 2008;**34**:56–65.
31. Hickey AJ, Ganderton D. *Pharmaceutical process engineering*. New York: Informa Healthcare; 2010.
32. O'Hara P, Hickey AJ. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. *Pharm Res* 2000;**17**:955–61.
33. Taylor MK, Hickey AJ, VanOort M. Manufacture, characterization, and pharmacodynamic evaluation of engineered ipratropium bromide particles. *Pharm Dev Technol* 2006;**11**:321–36.
34. Hickey AJ, Mansour HM. Delivery of drugs by the pulmonary route. In: Florence AT, Siepman J, editors. *Modern pharmaceutics: informa healthcare*; 2009.
35. Muttil P, Wang C, Hickey AJ. Inhaled drug delivery for tuberculosis therapy. *Pharm Res*; 2009.
36. Vehring R. Pharmaceutical particle engineering via spray drying. *Pharm Res* 2008;**25**:999–1022.
37. Schoubben A, Blasi P, Giovagnoli S, Ricci M, Rossi C. Simple and scalable method for peptide inhalable powder production. *Eur J Pharm Sci* 2010;**39**:53–8.
38. Pandey R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B. Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. *J Antimicrob Chemother* 2003;**52**:981–6.
39. Sharma A, Sharma S, Khuller GK. Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. *J Antimicrob Chemother* 2004;**54**:761–6.
40. Zahoor A, Sharma S, Khuller GK. Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. *Int J Antimicrob Agents* 2005;**26**:298–303.
41. Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. *Trends Biotechnol* 2007;**25**:563–70.
42. Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards DA. Trojan particles: large porous carriers of nanoparticles for drug delivery. *Proc Natl Acad Sci U S A* 2002;**99**:12001–5.
43. Gilbert BE. Liposomal aerosols in the management of pulmonary infections. *J Aerosol Med* 1996;**9**:111–22.
44. Whitehead TC, Lovering AM, Cropley IM, Wade P, Davidson RN. Kinetics and toxicity of liposomal and conventional amikacin in a patient with multidrug-resistant tuberculosis. *Eur J Clin Microbiol Infect Dis* 1998;**17**:794–7.
45. Fielding RM, Lewis RO, Moon-McDermott L. Altered tissue distribution and elimination of amikacin encapsulated in unilamellar, low-clearance liposomes (MiKasome). *Pharm Res* 1998;**15**:1775–81.
46. Giovagnoli S, Blasi P, Vescovi C, Fardella G, Chiappini I, Perioli L, et al. Unilamellar vesicles as potential capreomycin sulfate carriers: preparation and physicochemical characterization. *AAPS PharmSci Tech* 2005;**4**:E69.
47. Ricci M, Giovagnoli S, Blasi P, Schoubben A, Perioli L, Rossi C. Development of liposomal capreomycin sulfate formulations: effects of formulation variables on peptide encapsulation. *Int J Pharm* 2006;**311**:172–81.
48. Pandey R, Sharma S, Khuller GK. Nebulization of liposome encapsulated antitubercular drugs in guinea pigs. *Int J Antimicrob Agents* 2004;**24**:93–4.
49. Weers J, Metzheiser B, Taylor G, Warren S, Meers P, Perkins WR. A gamma scintigraphy study to investigate lung deposition and clearance of inhaled amikacin-loaded liposomes in healthy male volunteers. *J Aerosol Med Pulm Drug Deliv* 2009;**22**:131–8.
50. Vyas SP, Kannan ME, Jain S, Mishra V, Singh P. Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. *Int J Pharm* 2004;**269**:37–49.
51. Labiris NR, Dolovich MB. Pulmonary drug delivery. Part II: the role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. *Br J Clin Pharmacol* 2003;**56**:600–12.
52. Le Brun PP, de Boer AH, Heijerman HG, Frijlink HW. A review of the technical aspects of drug nebulization. *Pharm World Sci* 2000;**22**:75–81.
53. Li Z, Zhang Y, Wurtz W, Lee JK, Malinin VS, Durwas-Krishnan S, et al. Characterization of nebulized liposomal amikacin (Arikace) as a function of droplet size. *J Aerosol Med Pulm Drug Deliv* 2008;**21**:245–54.
54. Jobe A, Ikegami M, Nathanielsz PW. The labelling of pulmonary surfactant phosphatidylcholine in 19–31-days-old lambs. *J Dev Physiol* 1979;**1**:245–59.
55. Garcia-Contreras L, Fiegel J, Telko MJ, Elbert K, Hawi A, Thomas M, et al. Inhaled large porous particles of capreomycin for treatment of tuberculosis in a guinea pig model. *Antimicrob Agents Chemother* 2007;**51**:2830–6.
56. Fiegel J, Garcia-Contreras L, Thomas M, VerBerkmoes J, Elbert K, Hickey A, et al. Preparation and in vivo evaluation of a dry powder for inhalation of capreomycin. *Pharm Res* 2008;**25**:805–11.
57. Suarez S, O'Hara P, Kazantseva M, Newcomer CE, Hopfer R, McMurray DN, et al. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model. *Pharm Res* 2001; **18**:1315–9.

58. Sharma R, Saxena D, Dwivedi AK, Misra A. Inhalable microparticles containing drug combinations to target alveolar macrophages for treatment of pulmonary tuberculosis. *Pharm Res* 2001;**18**:1405–10.
59. Garcia-Contreras L, Sethuraman V, Kazantseva M, Godfrey V, Hickey AJ. Evaluation of dosing regimen of respirable rifampicin biodegradable microspheres in the treatment of tuberculosis in the guinea pig. *J Antimicrob Chemother* 2006;**58**:980–6.
60. Zhou H, Zhang Y, Biggs DL, Manning MC, Randolph TW, Christians U, et al. Microparticle-based lung delivery of INH decreases INH metabolism and targets alveolar macrophages. *J Control Release* 2005;**107**:288–99.
61. Muttil P, Kaur J, Kumar K, Yadav AB, Sharma R, Misra A. Inhalable microparticles containing large payload of anti-tuberculosis drugs. *Eur J Pharm Sci* 2007;**32**:140–50.
62. Giovagnoli S, Sonoara D, Blasi P, Ricci M, Perioli L, Rossi C. Preparation and characterization of respirable biodegradable microspheres for capreomycin pulmonary delivery. In: *12th International Pharmaceutical Technology Symposium*, 2004.
63. Blasi P, Schoubben A, Giovagnoli S, Rossi C, Ricci M. Fighting tuberculosis: old drugs, new formulations. *Expert Opin Drug Deliv* 2009;**6**:977–93.
64. Hirota K, Hasegawa T, Nakajima T, Inagawa H, Kohchi C, Soma G, et al. Delivery of rifampicin-PLGA microspheres into alveolar macrophages is promising for treatment of tuberculosis. *J Control Release* 2010;**142**:339–46.
65. Hwang SM, Kim DD, Chung SJ, Shim CK. Delivery of ofloxacin to the lung and alveolar macrophages via hyaluronan microspheres for the treatment of tuberculosis. *J Control Release* 2008;**129**:100–6.
66. Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *Int J Pharm* 2010;**392**:1–19.
67. Smyth HD. The influence of formulation variables on the performance of alternative propellant-driven metered dose inhalers. *Adv Drug Deliv Rev* 2003;**55**:807–28.
68. Liu MC, Riese RJ, Van Gundy K, Norwood P, Sullivan BE, Schwartz PF, et al. Effects of inhaled human insulin on airway lining fluid composition in adults with diabetes. *Eur Respir J* 2008;**32**:180–8.
69. Fineberg SE, Kawabata T, Finco-Kent D, Liu C, Krasner A. Antibody response to inhaled insulin in patients with type 1 or type 2 diabetes. An analysis of initial phase II and III inhaled insulin (Exubera) trials and a two-year extension trial. *J Clin Endocrinol Metab* 2005;**90**:3287–94.
70. Chimote G, Banerjee R. Evaluation of antitubercular drug-loaded surfactants as inhalable drug-delivery systems for pulmonary tuberculosis. *J Biomed Mater Res A* 2009;**89**:281–92.
71. Chimote G, Banerjee R. Effect of mycobacterial lipids on surface properties of Curosurf: implications for lung surfactant dysfunction in tuberculosis. *Respir Physiol Neurobiol* 2008;**162**:73–9.
72. Chimote G, Banerjee R. In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis. *J Biomed Mater Res B Appl Biomater* 2010;**94**:1–10.
73. Hirota K, Tomoda K, Inagawa H, Kohchi C, Soma G, Makino K, et al. Stimulation of phagocytic activity of alveolar macrophages toward artificial microspheres by infection with mycobacteria. *Pharm Res* 2008;**25**:1420–30.
74. Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA, et al. MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and *Mycobacterium tuberculosis*. *PLoS Pathog* 2009;**5**:e1000474.
75. Hirota K, Hasegawa T, Hinata H, Ito F, Inagawa H, Kochi C, et al. Optimum conditions for efficient phagocytosis of rifampicin-loaded PLGA microspheres by alveolar macrophages. *J Control Release* 2007;**119**:69–76.
76. Hasegawa T, Hirota K, Tomoda K, Ito F, Inagawa H, Kochi C, et al. Phagocytic activity of alveolar macrophages toward polystyrene latex microspheres and PLGA microspheres loaded with anti-tuberculosis agent. *Colloids Surf B Biointerfaces* 2007;**60**:221–8.
77. Garcia-Contreras L, Wong YL, Muttil P, Padilla D, Sadoff J, Derousse J, et al. Immunization by a bacterial aerosol. *Proc Natl Acad Sci U S A* 2008;**105**:4656–60.
78. Hickey AJ, Martonen TB, Yang Y. Theoretical relationship of lung deposition to the fine particle fraction of inhalation aerosols. *Pharm Acta Helv* 1996;**71**:185–90.
79. Sacks LV, Pendle S, Orlovic D, Andre M, Popara M, Moore G, et al. Adjunctive salvage therapy with inhaled aminoglycosides for patients with persistent smear-positive pulmonary tuberculosis. *Clin Infect Dis* 2001;**32**:44–9.
80. Tsapis N, Bennett D, O'Driscoll K, Shea K, Lipp MM, Fu K, et al. Direct lung delivery of para-aminosalicylic acid by aerosol particles. *Tuberculosis (Edinb)* 2003;**83**:379–85.
81. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, et al. Lack of acidification in *Mycobacterium phagosomes* produced by exclusion of the vesicular proton-ATPase. *Science* 1994;**263**:678–81.
82. Ferrari G, Langen H, Naito M, Pieters J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* 1999;**97**:435–47.
83. Prior S, Gander B, Blarer N, Merkle HP, Subira ML, Irache JM, et al. In vitro phagocytosis and monocyte-macrophage activation with poly(lactide) and poly(lactide-co-glycolide) microspheres. *Eur J Pharm Sci* 2002;**15**:197–207.
84. Torche AM, Le Corre P, Albina E, Jestin A, Le Verge R. PLGA microspheres phagocytosis by pig alveolar macrophages: influence of poly(vinyl alcohol) concentration, nature of loaded-protein and copolymer nature. *J Drug Target* 2000;**7**:343–54.
85. Jones BG, Dickinson PA, Gumbleton M, Kellaway IW. Lung surfactant phospholipids inhibit the uptake of respirable microspheres by the alveolar macrophage NR8383. *J Pharm Pharmacol* 2002;**54**:1065–72.
86. Jones BG, Dickinson PA, Gumbleton M, Kellaway IW. The inhibition of phagocytosis of respirable microspheres by alveolar and peritoneal macrophages. *Int J Pharm* 2002;**236**:65–79.
87. Verma RK, Kaur J, Kumar K, Yadav AB, Misra A. Intracellular time course, pharmacokinetics, and biodistribution of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to mice. *Antimicrob Agents Chemother* 2008;**52**:3195–201.
88. Ahmad Z, Pandey R, Sharma S, Khuller GK. Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. *Indian J Chest Dis Allied Sci* 2006;**48**:171–6.
89. Yadav AB, Muttil P, Singh AK, Verma RK, Mohan M, Agrawal AK, et al. Microparticles induce variable levels of activation in macrophages infected with *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* 2010;**90**:188–96.
90. Yadav AB, Misra A. Enhancement of apoptosis of THP-1 cells infected with Mycobacterium tuberculosis by inhalable microparticles and relevance to bactericidal activity. *Antimicrob Agents Chemother* 2007;**51**:3740–2.
91. Brandhonneur N, Chevanne F, Vie V, Frisch B, Primault R, Le Potier MF, et al. Specific and non-specific phagocytosis of ligand-grafted PLGA microspheres by macrophages. *Eur J Pharm Sci* 2009;**36**:474–85.
92. Yoshida A, Matumoto M, Hshizume H, Oba Y, Tomishige T, Inagawa H, et al. Selective delivery of rifampicin incorporated into poly(DL-lactic-co-glycolic) acid microspheres after phagocytotic uptake by alveolar macrophages, and the killing effect against intracellular *Mycobacterium bovis* Calmette-Guerin. *Microbes Infect* 2006;**8**:2484–91.
93. Sukhanov DS, Ivanov AK, Kovalenko AL, Dziuba EG, Andreeva MN, Romantsov MG. [Pathogenetic therapy of tuberculosis of respiratory organs during sanatorium-and-spa treatment]. *Vopr Kurortol Fizioter Lech Fiz Kult*; 2009:34–7.
94. Rikimaru T, Oizumi K. [Aerosolized therapy with streptomycin and steroids in treatment of bronchial stenosis due to endobronchial tuberculosis]. *Kekkaku* 1999;**74**:879–83.
95. Rikimaru T. [Evaluation of aerosol therapy of streptomycin for tracheobronchial tuberculosis]. *Kekkaku* 1994;**69**:733–8.
96. Rikimaru T, Tanaka Y, Higuchi E, Katsuki M, Ichikawa Y, Oizumi K. [Evaluation of aerosol therapy of streptomycin for tracheobronchial and pulmonary tuberculosis]. *Kansenshogaku Zasshi* 1992;**66**:206–11.
97. Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. *Lancet* 1997;**349**:1513–5.
98. Condos R, Hull FP, Schluger NW, Rom WN, Smaldone GC. Regional deposition of aerosolized interferon-gamma in pulmonary tuberculosis. *Chest* 2004;**125**:2146–55.
99. Koh WJ, Kwon OJ, Suh GY, Chung MP, Kim H, Lee NY, et al. Six-month therapy with aerosolized interferon-gamma for refractory multidrug-resistant pulmonary tuberculosis. *J Korean Med Sci* 2004;**19**:167–71.
100. Grahmann PR, Braun RK. A new protocol for multiple inhalation of IFN-gamma successfully treats MDR-TB: a case study. *Int J Tuberc Lung Dis* 2008;**12**:636–44.
101. Giosue S, Casarini M, Alemanno L, Galluccio G, Mattia P, Pedicelli G, et al. Effects of aerosolized interferon-alpha in patients with pulmonary tuberculosis. *Am J Respir Crit Care Med* 1998;**158**:1156–62.
102. Netea M, van de Veerdonk FL, Kullberg BJ, Van der Meer JW, Joosten LA. The role of NLRs and TLRs in the activation of the inflammasome. *Expert Opin Biol Ther* 2008;**8**:1867–72.
103. Brodsky I, Monack D. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. *Semin Immunol* 2009;**21**:199–207.
104. Pettis RJ, Hall I, Costa D, Hickey AJ. Aerosol delivery of muramyl dipeptide to rodent lungs. *AAPS PharmSci* 2000;**2**:E25.
105. Muthuswamy P, Hu TC, Carasso B, Antonio M, Dandamudi N. Prednisone as adjunctive therapy in the management of pulmonary tuberculosis. Report of 12 cases and review of the literature. *Chest* 1995;**107**:1621–30.
106. Yew WW, Lee J, Chau CH. Role of inhaled budesonide in the treatment of tuberculous pyrexia. *Chest* 2000;**118**:567.
107. Sherry E, Reynolds M, Sivananthan S, Mainawalala S, Warnke PH. Inhalational phytochemicals as possible treatment for pulmonary tuberculosis: two case reports. *Am J Infect Control* 2004;**32**:369–70.
108. Lovacheva OV, Erokhin VV, Chernichenko NV, Evgushchenko GV, Lepekha LN, Rozenberg OA. [Results of use of surfactant in complex therapy of patients with destructive pulmonary tuberculosis]. *Probl Tuberk Bolezn Legk*; 2006:12–7.
109. Chimote G, Banerjee R. Lung surfactant dysfunction in tuberculosis: effect of mycobacterial tubercular lipids on dipalmitoylphosphatidylcholine surface activity. *Colloids Surf B Biointerfaces* 2005;**45**:215–23.
110. Rosas-Taraco AG, Higgins DM, Sanchez-Campillo J, Lee EJ, Orme IM, Gonzalez-Juarrero M. Intrapulmonary delivery of XCL1-targeting small interfering RNA in mice chronically infected with *Mycobacterium tuberculosis*. *Am J Respir Cell Mol Biol* 2009;**41**:136–45.
111. Hickey AJ. *Pharmaceutical inhalation aerosol technology*. New York: Marcel Dekker; 2004.
112. Siekmeier R, Scheuch G. Systemic treatment by inhalation of macromolecules–principles, problems, and examples. *J Physiol Pharmacol* 2008;**59** (Suppl. 6):53–79.

113. Finlay WH, Martin AR. Recent advances in predictive understanding of respiratory tract deposition. *J Aerosol Med*; 2008.
114. Smith-Jones PM, Linkesch W, Virgolini I. Interferon-gamma for respiratory diseases. *Lancet* 1997;**350**:524.
115. Upton RN, Doolette DJ. Kinetic aspects of drug disposition in the lungs. *Clin Exp Pharmacol Physiol* 1999;**26**:381–91.
116. Che MM, Conti M, Boylan M, Sciuto AM, Gordon RK, Nambiar MP. Blood and bronchoalveolar lavage fluid acetylcholinesterase levels following micro-instillation inhalation exposure to sarin in Guinea pigs. *Inhal Toxicol* 2008;**20**:821–8.
117. Pandey R, Khuller GK. Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. *Tuberculosis (Edinb)* 2005;**85**:227–34.
118. Mouton JW, Theuretzbacher U, Craig WA, Tulkens PM, Derendorf H, Cars O. Tissue concentrations: do we ever learn? *J Antimicrob Chemother* 2008; **61**:235–7.
119. Derelanko MJ, Hollinger MA, editors. *Handbook of toxicology*. Boca Raton: CRC Press; 2002.
120. Suarez S, Hickey AJ. Pharmacokinetic and pharmacodynamic aspects of inhaled therapeutic agents. In: Martonen TB, editor. *Medical applications of computer modelling: the respiratory system*. Southampton, UK: WIT Press; 2002.
121. McMurray DN, Collins FM, Dannenberg Jr AM, Smith DW. Pathogenesis of experimental tuberculosis in animal models. *Curr Top Microbiol Immunol* 1996;**215**:157–79.
122. Cryan SA, Sivadas N, Garcia-Contreras L. In vivo animal models for drug delivery across the lung mucosal barrier. *Adv Drug Deliv Rev* 2007; **59**:1133–51.
123. Sen H, Jayanthi S, Sinha R, Sharma R, Muttil P. Microsphere of two anti-tubercular drugs and a biodegradable polymer administered by inhalation alone or combined with oral route, and process thereof. *PCT Int Pat Appl*; 2005. WO/2005/039529.
124. Sung JC, Garcia-Contreras L, Verberkmoes JL, Peloquin CA, Elbert KJ, Hickey AJ, et al. Dry powder nitroimidazopyran antibiotic PA-824 aerosol for inhalation. *Antimicrob Agents Chemother* 2009;**53**:1338–43.
125. Garcia-Contreras L, Sung JC, Muttil P, Padilla D, Telko M, Verberkmoes JL, et al. Dry powder PA-824 aerosols for treatment of tuberculosis in guinea pigs. *Antimicrob Agents Chemother* 2010;**54**:1436–42.
126. Wang C, Hickey AJ. Isoxyl particles for pulmonary delivery: in vitro cytotoxicity and potency. *Int J Pharm*; 2010.
127. Wang C, Hickey AJ. Isoxyl aerosols for tuberculosis treatment: preparation and characterization of particles. *AAPS PharmSci Tech* 2010; **11**:538–49.