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Expert Review

Inhaled Drug Delivery for Tuberculosis Therapy

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Abstract. One third of the world population is infected with tuberculosis (TB), and new infections occur at a rate of one per second. The recent increase in the emergence of drug-resistant strains of *Mycobacterium tuberculosis* and the dearth of anti-TB drugs is threatening the future containment of TB. New drugs or delivery systems that will stop the spread of TB and slow down or prevent the development of drug-resistant strains are urgently required. One of the reasons for the emergence of drug-resistant strains is the exposure of mycobacteria to sub-therapeutic levels of one or more antibiotics. Lung lesions containing large numbers of bacteria are poorly vascularized and are fortified with thick fibrous tissue; conventional therapy by the oral and parenteral routes may provide sub-therapeutic levels of anti-TB drugs to these highly sequestered organisms. Administering drugs by the pulmonary route to the lungs allows higher drug concentrations in the vicinity of these lesions. Supplementing conventional therapy with inhaled anti-TB therapy may allow therapeutic concentrations of drug to penetrate effectively into lung lesions and treat the resident mycobacteria.

KEY WORDS: alveolar macrophages; dendritic cells; inhalation delivery; microparticles; tuberculosis.

INTRODUCTION

It is likely that tuberculosis (TB) has beset mankind since its inception (1). For, certain, 6,000-year-old human remains exhibit characteristic pathologies of TB (2). Inhalation therapy has a 4,000 year history (3). It is surprising, from these observations, that a convergence of aerosol drug delivery and the disease therapy has only recently been considered. Early twentieth-century literature mentions the development and use of adrenaline nasal spray in asthma and as a decongestant in hay fever and rhinitis, followed by inhaled isoprenaline for asthma in 1951 (4). Aerosolized antibiotics delivered to the lungs were only evaluated in the 1950s and 1960s for treatment of pulmonary infectious diseases.

The respiratory tract represents one of the main routes of entry for infections caused by bacteria, fungus and viruses (5,6). A broad range of drugs including anti-inflammatory and anti-infective agents are available to prevent and treat lung diseases caused by these organisms. Conventional therapy for respiratory tract infections consists of administering antimicrobial agents by the oral or parenteral route. However, the amount of drug reaching the site of infection may be small due to poor pulmonary distribution of most systemically administered drugs. Delivering drugs by inhalation directly to the lungs results in local drug concentration far higher than that achievable by either oral or parenteral administration (7). A drug having narrow therapeutic window or requiring prolonged treatment regimen by conventional routes of administration, when administered by the inhalation route would have reduced systemic exposure and toxicity. Consequently, this approach may be advantageous where the patient's system is overburdened with the traditional range of chemotherapeutic agents employed to treat TB.

Aerosol delivery of drugs to the lungs has been used to treat various local infectious diseases described in Table I. In the past decade, approximately 75% of Cystic Fibrotic (CF) patients over 19 years of age have been treated with some form of aerosol therapy (8). Several well-written reviews have addressed the history and progress made in the field of inhaled antimicrobial therapies for the treatment and prophylaxis of various respiratory infections (9–12).

The following section presents TB, its continued prominence as a deadly disease, and various drugs and delivery systems used currently. Many of these therapeutic approaches have been ineffective in controlling TB due to the rising incidence of drug resistance, with rates as high as 35% in some parts of the world (13,14). Aerosol delivery to the lungs for TB therapy is in its infancy, but has been successful in decreasing the bacterial burden of the lungs compared to conventional therapy in animal models. A number of drugs

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ABBREVIATIONS: AFB, Acid-fast bacilli; AMΦ, Alveolar Macrophage; APC, Antigen presenting cells; BAL, Bronco-alveolar lavage; CF, Cystic fibrosis; CMI, Cell mediated immunity; DTH, Delayed-type hypersensitivity; EMB, Ethambutol; INH, Isoniazid; Inhl, Inhalation; IV, Intravenous; GI, Gastro-intestinal; MDR, Multi-drug resistant; Mtb, *Mycobacterium tuberculosis*; PD, Pharmacodynamic; PK, Pharmacokinetic; PLGA, Poly(lactic-co-glycolic acid); PNAPs, Porous nanoparticle aggregate particles; PZA, Pyrazinamide; RIF, Rifampicin; STR, Streptomycin; TB, Tuberculosis; XDR, Extremely-drug resistant.

Table I. Drugs Available Commercially and in Clinical Trials for Administration as Aerosols to the Lungs

Disease	Organism	Drugs used in humans	Device	Specified site of action in lung airways	References
Cystic fibrosis	*Pseudomonas aeruginosa*	Tobramycin	Nebulizer/ DPI	Upper and lower	(130,131)
		Gentamicin	Nebulizer		(132)
		Colomycin	Nebulizer		(133)
		Denufosol tetrasodium	Nebulizer		(134)
		Aztreonam lysine	Nebulizer		(135)
		Duramycin	Nebulizer		(136)
Pneumonia	*Pneumocystis jiroveci*	Pentamidine Isethionate	Nebulizer	Upper and lower	(129,137)
Influenza	*Influenza virus A&B*	Zanamivir	DPI	Lower	(138)
Respiratory tract infections	*Respiratory syncytial virus*	Ribavirin	Nebulizer	Upper and lower	(139)
Pulmonary Aspergillus	*Aspergillus fumigatus*	Amphotericin B	Nebulizer	Peripheral	(38)

and delivery systems have been examined *in vitro* and *in vivo*. Pharmacokinetic and pharmacodynamic studies have been performed to assess inhalation delivery of therapeutic agents for TB. A range of animal models and the bacterial strains have been employed. A comprehensive rationale for targeting drugs to the lungs leading to better clearance of mycobacteria is outlined.

THERAPY FOR TB

Epidemiology of Disease

TB causes substantial mortality and morbidity. Each year, it leads to approximately 14.6 million chronic active cases, 8.9 million new cases and 1.6 million deaths; new infections are occurring at a rate of one per second according to the latest WHO report (15). One-third of the world's population is infected with TB (16), of which 90% do not exhibit any symptoms, 5% of the infected individuals progress rapidly to primary disease, and 5% of those who initially suppress infection later reactivate leading to disease sometime during their lifetime (17). Drugs or vaccines have not been developed to rapidly prevent transmission to uninfected individuals or to treat apparently healthy, recently infected individuals, in part because of the requirement for early diagnosis of infection. These alarming figures have put TB in a category, with AIDS and Malaria, of diseases that urgently require attention to improve global public health.

The spread of HIV in conjunction with TB, the leading cause of death among HIV-positive individuals, has accelerated the global resurgence of this disease. The emergence of multi-drug resistant (MDR) and lately extremely-drug resistant (XDR) strains of TB is of particular concern. Treatment costs have increased by billions of dollars and some strains of TB that were treatable in the past have become untreatable, increasing speculation that they may spread uninhibited around the world. Since the end of the last century, mycobacteria have emerged with resistance patterns rendering most of the currently available antibiotics ineffective. The pharmaceutical industry has largely abandoned TB drug development; this may be attributed to the misconception that with few exceptions infectious disease is no longer a serious concern in the developed world. Of course, this overlooks not only the recent increase in infectious disease but also the facility with which individuals can travel. The latter observation negates, from a health perspective, the socio-economical and geographical distinction of developed and developing worlds. Over the past 45 years, new drug classes to treat TB have not been commercialized.

TB Pathogenesis

TB infects almost all organs of the human body, but the most common form is pulmonary TB. It is estimated that more than 80% of all disease originates as pulmonary TB (18). The lungs are the main port of entry for *Mycobacterium tuberculosis* (Mtb) and an important site of disease manifestation which spreads to other organs (16). Mtb, the causative agent for TB, is a successful intracellular pathogen that targets and inhabits the professional antigen presenting cells (APC's), the alveolar macrophage (AMΦ) and the dendritic cells during the early stages of infection (19). The microorganism enters the human lung through droplet inhalation of as few as two to three bacilli, sufficiently small (1–5 μm) to be deposited in the alveolar space (20). AMΦs and dendritic cells play an important role in the initiation and maintenance of immune response against these pathogens. Mtb has evolved to evade most of the host-defense mechanisms enabling not only their intracellular survival but also replication within phagosomes of AMΦs; Mtb-infected phagosomes do not fuse with the lysosomes, thus escaping degradation by hydrolytic enzymes (21,22). Once Mtb establishes a niche for itself inside the APCs, it multiplies and spreads to other parts of the body depending on the immune response generated by the host. TB is a systemic disease for which the most prominent extra-pulmonary sites of infection include liver, kidneys, spleen, uterus and bones.

Mycobacteria reach the alveoli (most often in the middle and lower lung zones), multiply intracellularly in AMΦs, and stealthily spread through the lymphatics to hilar or other lymph nodes, and then to the bloodstream to infect other organs (23). Mtb continues to grow in the lungs for 2 to 12 weeks until, in an immuno-competent host, cell-mediated immunity (CMI) develops and is recognized by a positive response to tuberculin skin test (20). At this time granulomas are formed and growth of Mtb is inhibited; the *Mycobacterium* can remain in the "dormant state" inside macrophages

in the granulomas for years (24). Its morphology in human beings is characterized by a central necrotic core surrounded by concentric layers of macrophages, T and B lymphocytes, neutrophils, epithelioid cells, foamy macrophages, multinucleated langhans giant cells and extracellular matrix components (Fig. 1 A) (24). The granulomas are also isolated from the outside environment due to a thick, calcified stratum surrounding them (Fig. 1 A).

Clinical disease can develop in 5% of immuno-competent patients at a time remote from the initial infection due to endogenous reactivation of a previously established dormant focus (20). The sites for re-activation are those of high oxygen tension like the superior lung segments, kidney, bones and CNS.

Each of the above steps is determined by the interaction between the host immune response and the bacterial virulence. Many of the detrimental symptoms of TB, including tissue damage that ultimately liquefies infected parts of the lungs are mediated by the host immune response towards Mtb rather than a direct manifestation of the bacterium itself (25). The liquefied material is an excellent growth medium for the bacilli to multiply and reach enormous numbers. AMΦs do not survive in liquefied caseous material; macrophages entering them are possibly killed by toxic fatty acids originating from host cells and/or the bacilli.

Conventional Therapy for TB

Oral antibiotic treatment is the standard means of controlling and treating most cases of TB. For drug-susceptible TB disease, the initial intensive phase consists of a minimum of three drugs administered concurrently to reduce the rapidly dividing bacillary load; a minimum of two drugs is used in the continuation phase aimed at sterilizing lesions containing fewer and slow-growing bacilli. The first-line therapy consists of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) in the United States. Streptomycin is sometimes substituted for EMB outside the US to reduce expense. INH and RIF are the primary agents in a combination therapy and act against the metabolically dynamic mycobacteria that multiply perpetually and rapidly, and also against the quasi-dormant bacilli. RIF has the added advantage of acting at a very early stage of bacillary propagation. The second-line class of drugs includes: aminoglycoside antibiotics, cycloserine, ethionamide and fluoroquinolones. Amikacin, kanamycin and streptomycin are the prominent aminoglycoside antibiotics; levofloxacin and moxifloxacin are the fluoroquinolone antibiotics, while capreomycin sulphate is a cyclic polypeptide antibiotic effective against Mtb (26). Second-line anti-TB drugs are employed only if the patient is not responding to the first-line

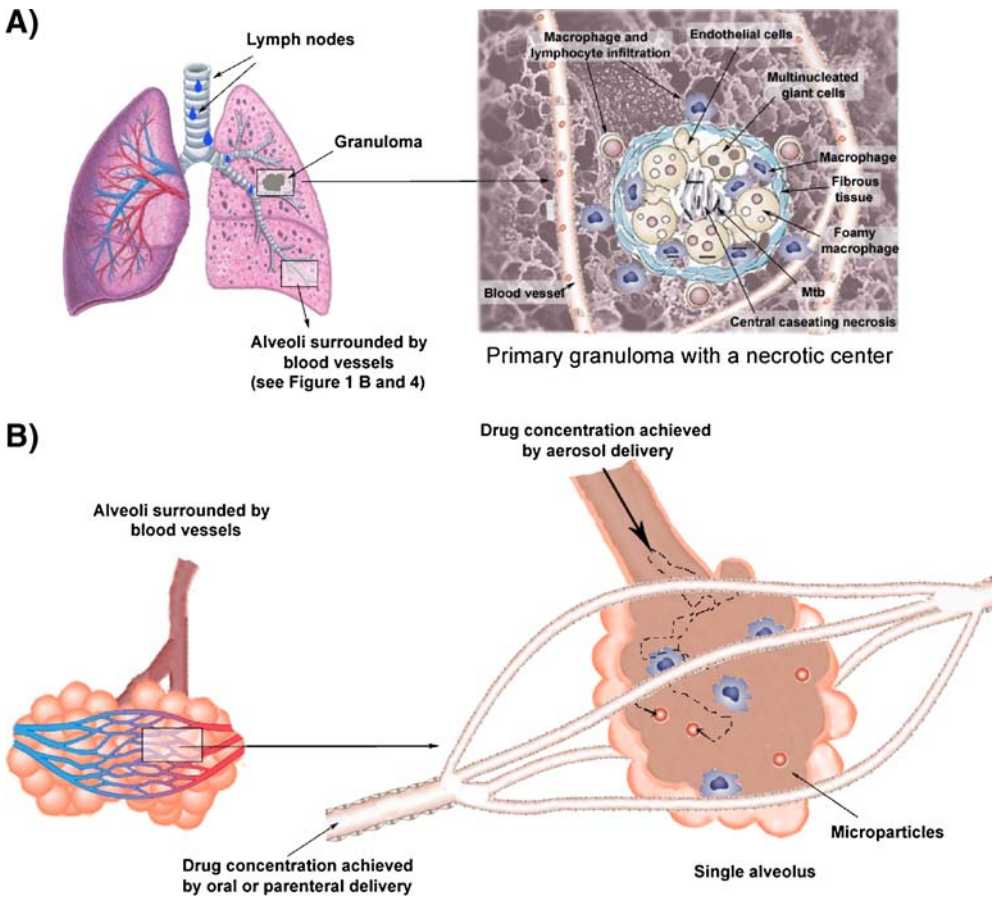

Fig. 1. Microparticulate drug delivery to the lung for TB therapy. **A** The location and morphology of a granuloma in a diseased lung. **B** Lung alveoli surrounded by blood vessels.

therapy and/or are believed to be infected with drug-resistant strains of Mtb; second-line agents are less effective and more toxic than the first-line drugs. The above antibiotics are sometimes administered by the parenteral route, but this is not preferred due to the associated or perceived discomfort. Blood-borne infections are also thought to originate from the re-use of needles in resource poor nations.

Challenges for the Conventional Therapy

Current TB therapy administered by the conventional routes, predominantly oral ingestion, in patients infected with drug-susceptible mycobacterial strains consist of a minimum of six to nine months of treatment regimen. Therapeutic drug concentrations are achieved in regions of the body having adequate blood circulation. In mice, approximately 99% of bacteria are killed within two weeks of drug treatment, but requires at least 3 more months of treatment to clear the remaining 1% bacteria (27–29). The poorly vascularized lesions, granulomas or tubercles, in the lungs harbor bacilli in microenvironments where hypoxic conditions confound treatment and extend therapy for many more months to be effective. Mycobacteria are protected in granulomas, and conventional therapy may not penetrate into them at therapeutic levels.

The standard therapy can be extended for up to two or more years if the patient fails to respond to the initial treatment based on sputum conversion, or if patients are believed to be infected with drug-resistant strains. Mycobacterial strains exhibiting resistance to one or more drugs are arising at an alarming rate, requiring incorporation of more drugs in the increasingly complex combination therapy and consideration of extension of the duration of therapy. Prolongation of therapy may lead to reduced patient compliance and further increase the chances of emergence of drug-resistant strains. The protracted duration of therapy is also limited by constraints on drug dosage, adverse drug reaction, which is exacerbated in HIV patients, and inadequate drug distribution at the sites of pathology (30). Conte *et al*. have shown that PZA achieves greater concentrations in the lungs than in the plasma in AIDS patients when administered orally and may be responsible for the effectiveness of this drug for treating pulmonary TB (31). Intrapulmonary concentration of oral INH was below the MIC for mycobacterial strains in AIDS patients and normal subjects and may explain the rapid emergence of INH-resistant organisms when used alone to treat TB (32).

Novel drugs or delivery systems that are below a toxic threshold at the effective doses and act on the bacteria by a different mechanism are urgently needed to replace or supplement drugs that have been lost as therapies to drug resistance. Treatment regimens that are short and allow less frequent intake of drugs by patients would greatly benefit compliance.

Aerosol Therapy

Bennett used the pulmonary route for the "treatment" of TB four centuries ago (33). It is likely from the description of the use of "aromatic herbs" that this was a palliative rather than therapeutic treatment. Mtb establishes itself in humans following inhalation of droplet aerosols generated by infected individuals; the lungs, as a primary site of infection, are a logical target for therapy to treat TB. Exposing the mycobacteria in the lungs to high drug concentrations by aerosol therapy maximizes the chances of arresting the spread of infection from the lungs to other organs, within the individual, and dissemination to other individuals in the population (Fig. 2 A, B).

Several studies have addressed the physico-chemical characterization of formulations and delivery aspects of aerosol therapy to the lungs for TB. Aerosol delivery systems delivering large, reproducible doses of anti-TB drugs to the lungs have been evaluated in healthy animals. The true efficacy of drugs and their delivery systems for TB requires demonstration in a realistic experimental design of delivery, after infection has occurred. Few studies regarding the efficacy of drugs delivered to the lungs by the pulmonary route in Mtb-infected animal models have been accomplished. To date, no drug or formulation for delivery of anti-

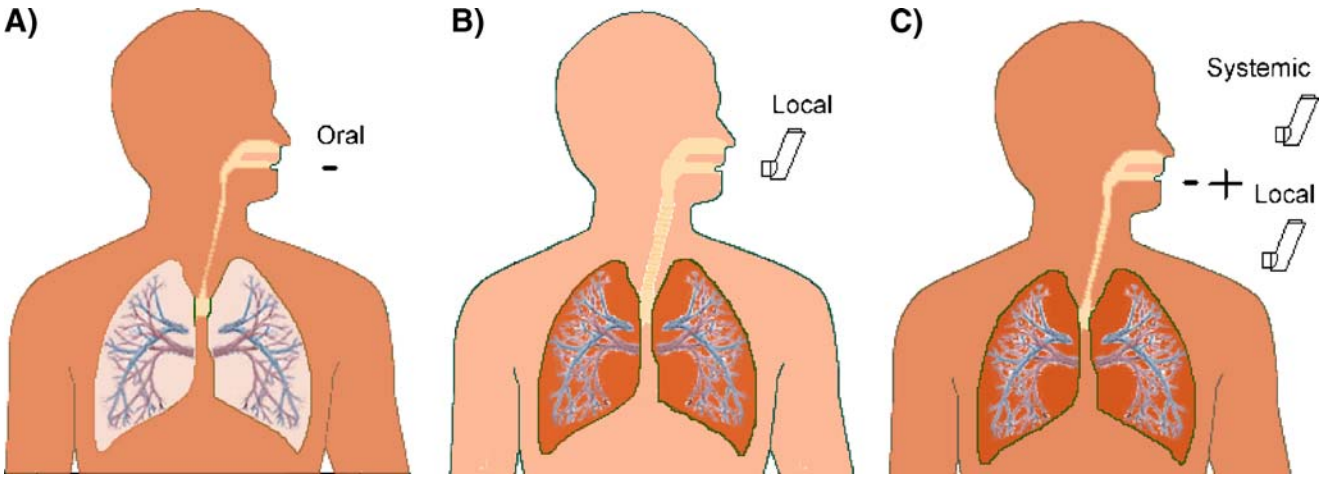

Fig. 2. Schematic representation of drug distribution in the lungs and body when administered by **A** Orally, more drug in the systemic circulation compared to lungs, **B** Inhalation, more drug in the lung compared to systemic circulation, **C** Combined oral and inhalation, drug equilibrium in the lungs and the systemic circulation (color intensity qualitative indicator).

TB drugs by inhalation to humans has been commercialized (Fig. 3). Drug delivery systems that can sterilize Mtb-infected animals with a modest dosage regimen and a shorter duration of therapy are desirable. The efficacy of anti-TB drugs delivered by the pulmonary route to Mtb-infected guinea pigs has been demonstrated in our laboratory (34–36). Other investigators have also delivered anti-TB drugs by the inhalation route using various formulations in animal models. Studies of the manufacture, characterization, pharmacokinetic and efficacy of drugs delivered by inhalation in infected animal models are summarized in the following section.

Manufacture/Characterization

TB therapy by inhalation may benefit from drug delivery to AMΦs in the periphery of the lungs (Fig. 1 B), AMΦs being the initial host cells for Mtb. Aerosolizing anti-TB drugs may not be adequate to target Mtb residing inside AMΦs; drugs need to be formulated into delivery systems that can simultaneously maneuver as a function of their aerodynamic properties into the deep lung and be endocytosed by AMΦs (Fig. 1 B). Particles having aerodynamic diameters in the 1–3 μm range are suitable for deep lung delivery (37). Various delivery systems have been engineered to incorporate anti-TB compounds to target AMΦs in the lung. These include liposomes, microparticles, nanoparticles (aggregated), and solid lipid nanoparticles, which are discussed briefly.

Liposomes are composite vesicles made of phospholipids that can encapsulate different types of drugs. Liposomes containing amphotericin B have been successfully administered as aerosols to the lungs of patients to treat fungal infections (38). Liposomal systems offer advantages, such as flexibility to modulate drug load, control size and physico-chemical properties and parameters that define the kinetics of drug release (39). Liposomes act as drug reservoirs providing slow and sustained release of the drug for TB therapy inside AMΦs. They may be engineered with respect to lipid composition and charge characteristics to influence their uptake by AMΦs. Drug-containing liposomes bearing specific markers on their surface (maleylated bovine serum albumin and O-steroyl amylopectin) have been effectively targeted to AMΦs of rats (40).

Nanoparticle technology for drug delivery became commercially available as recently as 2005; Abraxane®, an injectable suspension of albumin bound with paclitaxel for cancer therapy was the first nano-particulate delivery system. Drug-containing nanoparticles range in size from 10 to 1,000 nm and are usually defined as colloidal systems. They may be made from biocompatible and biodegradable materials such as polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., poly-lactides, poly-alkylcyanoacrylates), or solid lipids. The release of drug from nanoparticles takes place by diffusion, swelling, erosion, degradation or by adsorbing/attaching the active substance on the surface. They have the ability to achieve high drug loading, cross permeability barriers, incorporate both hydrophilic and hydrophobic drugs and evoke a better therapeutic response compared to other particulate delivery systems. Some of the methods used to prepare nanoparticles include spray drying, solvent evaporation, nano-precipitation and multiple emulsions techniques (41,42). Nanoparticles have been surface-modified to target AMΦs and extend drug release, e.g., nanoparticle surface functionalized with wheat germ agglutinin, thus enhancing interaction with lectin receptors on the alveolar epithelium (43). Deposition of individual nanoparticles in the deep lung is difficult to achieve due to exhalation of the majority of the dose (44). Novel formulations containing nanoparticles in micron-scale structures have been designed, referred to as 'porous nanoparticle-aggregate particles' (PNAPs), to overcome this disadvantage (45). The aggregated nanoparticle approach can be delivered efficiently to the lungs without the disadvantage associated with discrete nanoparticles being exhaled. The matrix of PNAPs consists of either nanoparticles alone or additional inert excipients, such as amino acids, sugars or phospholipids. Upon being deposited in the deep lung, these loosely aggregated nanoparticles would disperse, thus, promptly releasing and dissolving the nanoparticles in the deep lung. Nanoparticles approaching 500 nm or

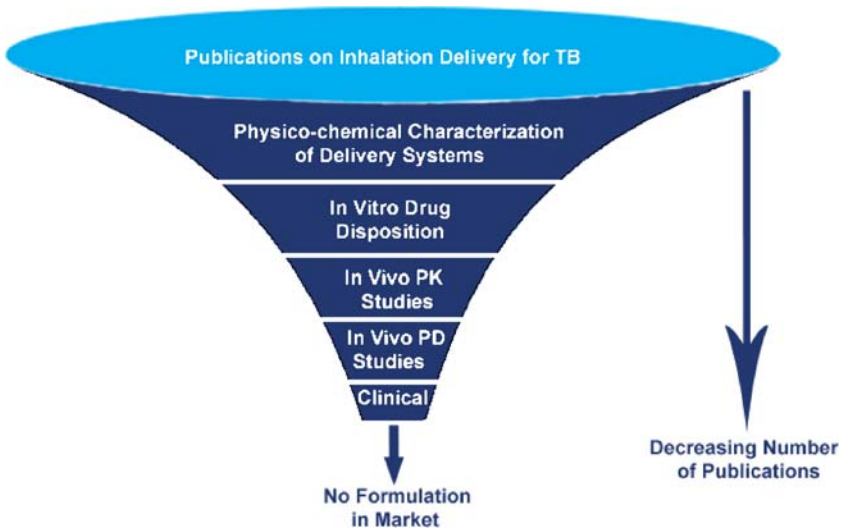

Fig. 3. Publications in the field indicating the larger number of formulation manufacture and characterization on inhaled therapy for TB compared to PD studies in animal models. It is important to note that currently there is no anti-TB aerosol drug product approved for human use.

above in size can be captured by AMΦs; sizes less than that may be amenable for uptake by dendritic cells but to a lesser extent than AMΦs (Fig. 4) (46,47). Since the majority of dendritic cells are present in the epithelial side of the basal membrane, the mechanism by which dendritic cells reach the particles deposited in the alveolar space is not well-understood (48). Some recent dendritic cell observations include an ability to open tight junctions, extend processes, sample antigens and bacteria, and migrate to local lymphoid tissues (Fig. 4) (49,50). PNAP constructs acquire the merits of both nano- and microparticulate delivery systems.

Microparticles used as a delivery system for anti-TB drugs by injectable, oral and pulmonary routes have been reported by several investigators (51). They are prepared using polymers and techniques similar to those for the preparation of nanoparticles. Microparticles in the appropriate size range (1–3 μm) for deep lung delivery can be easily targeted to AMΦs. Poly(lactic-co-glycolic acid) (PLGA) microparticles containing RIF for inhaled therapy was achieved in our laboratory by adopting a solvent evaporation and spray-drying technique (52). Various authors have prepared microparticles incorporating more than one anti-TB drug (Table II). They are usually a heterogeneous population in size and may contain nanoparticles that may lead to rapid dissolution and diffusion of drug, thus maintaining a high concentration gradient. A number of techniques have been used to prepare aerosol formulations capable of efficiently delivering drugs to AMΦs. Novel particle manufacturing technologies have recently emerged, consisting of micronization and powder blending, controlled solvent crystallization, spray drying, spray freeze drying, particle formation from liquid dispersion systems, supercritical fluid processing and particle coating. These techniques have been discussed elsewhere in detail (53–56) and are beyond the scope of this review.

In Vitro Assessment

Mtb is a facultative intracellular pathogen that can survive and replicate within the host macrophage; a key phase of the early life cycle of Mtb is spent within the human macrophage. To study the efficacy of drug delivery systems, several *in vitro* cell culture models have been established; infections of these cells with various mycobacterial strains and treatment with different drugs and delivery systems have been evaluated. Macrophage from humans, mice, rats, rabbits

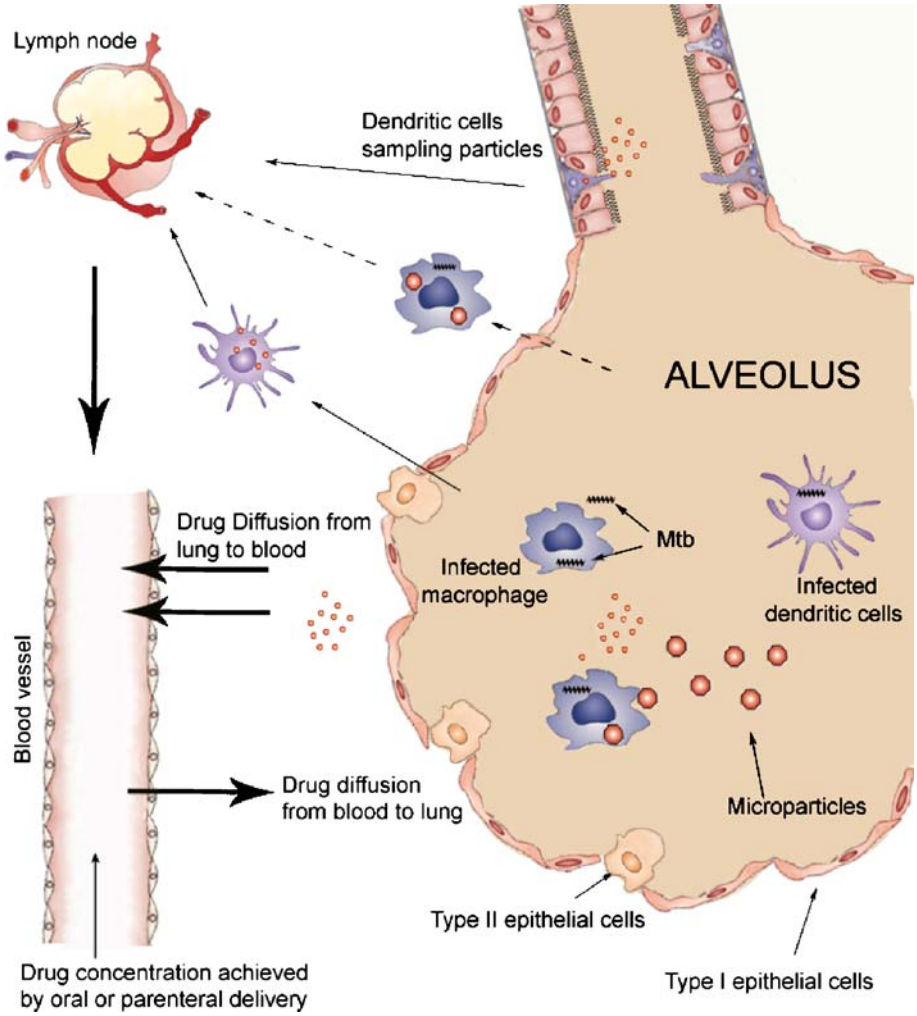

Fig. 4. Drug concentration achieved in the lung alveolus after administering anti-TB drugs by the conventional route and inhaled route (currently debated mechanism– – – – –▶, accepted mechanism ——▶).

and guinea pigs have been employed. These may be either immortalized cell lines or primary AMΦs recovered from animal species. Although macrophages originate from bone marrow, they have a wide tissue distribution and show regional heterogeneity based on function, morphology and phenotype. Primary AMΦs from humans obtained by broncho-alveolar lavage (BAL) are the most relevant for *in vitro* studies; other sources include monocyte-derived macrophages and the macrophage-like cells represented by THP-1 cell line. Cell lines are immortal and offer homogeneity in their population, do not require animal sacrifice, and are subject to experimental control that leads to reproducibility in results. THP-1 cells closely model the behavior of primary human AMΦs in various respects (57). Primary cells withdrawn by BAL from a human source reflect the relevant *in vivo* situation better than cell lines, although variability may exist due to a heterogeneous population. Various strains of *Mycobacterium* are used to infect cells *in vitro* and are discussed in a later section. Flexibility in the study design can be achieved when evaluating the efficacy of drug delivery systems *in vitro*, such as the use of frozen (dormant or slow replicating) or actively growing bacteria, the multiplicity of infection (the ratio of bacteria to the number of macrophages), time allowed for phagocytosis to occur, and death of macrophages during the culture period. Caution should be exercised when comparing results between slightly different study designs employing cell culture models.

Various markers are employed to assess the efficacy of drugs and their delivery systems in AMΦs *in vitro*. The role of apoptosis in killing intracellular Mtb after treatment with drugs in the soluble and microparticulate forms has been studied. Drug-containing microparticles were more efficient than soluble drugs in eliciting a host defense response to Mtb in THP-1 cells (58). THP-1 intracellular drug concentrations achieved with respect to time after exposure were higher when drug was delivered in microparticulate forms rather than in solution (59). This may be explained by the greater concentration of drug in particles in which the macrophage is predisposed to phagocytose, as distinct from the more dilute nature of molecularly dispersed drug in solution placed in the general environment of the macrophage. *In vitro* experiments serve as useful tools to estimate residence times and sampling intervals of relevance to intracellular drug levels anticipated *in vivo*. Primary mouse macrophages were infected with a non-pathogenic strain of Mtb and treated with soluble and microparticulate anti-TB drugs. The release of reactive oxygen and nitrogen species and various cytokines were evaluated (60). THP-1 cell activation based on cell morphology and various biochemical assays has been demonstrated when these cells were exposed to different microparticulate adjuvants (61). Assays for various enzymes, including alkaline phosphatase, N-acetyl-glucosamine and lactate dehydrogenase, and cell viability, were conducted. The choice of the source of macrophage depends on the type of experiment to be performed and the result output to be anticipated. Cytokine release studies after treatment with anti-TB drugs have been studied in J774.1 cell lines from BALB/c mice; these cell lines have been reported to show the same cytokine profile as primary human macrophages.

In Vivo Pharmacokinetic Assessment

To design and evaluate controlled drug delivery systems and have a positive therapeutic outcome *in vivo*, it is important to have a basic understanding of the bio-pharmaceutic and pharmacokinetic (PK) parameters. Branching of

Table II. Drugs Used Experimentally to Treat TB by the Pulmonary Route

Drug used	Formulation	Delivery method	Dosing regimen	Model	TB strain	Route of infection	Adjunct therapy	Year	References
Capreomycin sulfate	Dry powder aerosol	Insufflation	Single	Guinea pigs	None	N/A	N/A	2008	(140)
PA-824	Dry powder aerosol	Insufflation	Single	Guinea pigs	None	N/A	N/A	2009	(63)
Capreomycin sulfate	Dry powder aerosol	Insufflation		Guinea pigs	Mtb H37Rv	Pulmonary	No	2007	(34)
Rifampicin	Microsphere suspension	Nebulization	Multiple	Guinea pigs	Mtb H37Rv	Pulmonary	No	2006	(36)
Rifampicin	Microparticles	Insufflation/ Nebulization	Double	Guinea pigs	Mtb H37Rv	Pulmonary	No	2001	(35,83)
Para-aminosalicylic acid	Large porous particles	Insufflation	Single	Rats	None	N/A	N/A	2003	(141)
IFN-γ	Solution	Nebulization	Multiple	Humans	Mtb	N/A	Yes	2003	(117)
IFN-γ	Particles	Nebulization	Multiple	Humans	Mtb	N/A	Yes	2004	(118)
Aminoglycosides	Solution	Nebulization	Multiple	Humans	Mtb	N/A	Yes	2001	(30)
Rifampicin and isoniazid	Microparticles	Passive inhalation	Single	Rats	None	N/A	N/A	2001	(142)
Rifampicin, isoniazid and pyrazinamide	Nanoparticles	Nebulization	Single	Guinea pigs	Mtb H37Rv	IM	No	2003	(43,143)
Rifampicin, isoniazid and pyrazinamide	Solid lipid particles	Nebulization	Multiple	Guinea pigs	Mtb H37Rv	IM	No	2005	(144)
Rifampicin and isoniazid	Liposomes	Nebulization	Single	Guinea pigs	None	N/A	N/A	2004	(145)
Rifampicin	Liposomes	Nebulization	Single	Rats	None		N/A	2004	(40)
Rifampicin	Liposomes	Nebulization	Multiple	Mice	None	N/A	N/A	1995	(146)
Rifampicin	Lipid microspheres	Intra-tracheal	Single	Rats	None	N/A	N/A	2008	(147)
Rifampicin	Microspheres	Intra-tracheal	Multiple	Rats	Mtb Kurono	Intra-tracheal	N/A	2006	(148)
Rifabutin and isoniazid	Microparticles	Passive inhalation	Single	Mice	None	N/A	N/A	2008	(59)
Rifabutin and isoniazid	Microparticles	Passive inhalation	Single	Mice	None	N/A	N/A	2007	(149)
Rifabutin and isoniazid	Microparticles	Passive inhalation	Single	Mice	Mtb H37Rv	Intra-venous	Yes	2007	(150)

IM Intramuscularly; *N/A* not applicable

the airways makes approximation of drug bioavailability following lung deposition complex. Deposition of aerosols is generally below 100% of the total dose due to the natural filtration mechanism of the upper respiratory tract; this may not be significant for locally acting drugs, as absorption is not a prerequisite for therapeutic effect, but may play a major role for systemic targeted drugs. Apart from filtration, lung bioavailability is influenced by the enzymatic degradation, dissolution kinetics, membrane permeability, diffusion, tissue extraction, mucociliary and AMΦ clearance mechanism. Plasma and urine analysis can be used to estimate the PKs of pulmonary-delivered drugs.

Certain aspects of drug absorption are unique to lung delivery compared to the oral or intravenous (IV)-administered drugs. Rate or extent of drug absorption from the lungs may be determined by model-dependent or model-independent methods. Compartmental models, linear system analysis, and observational methods are currently being used for evaluation of drug absorption from the lung (62). Compartmental models assume that absorption occurs by a specific rate process; linear system analysis allows amount or percentage of drug absorbed as a function of time to be measured; and observational methods are the most common for assessing the rate and extent of absorption. The traditional PK parameters consisting of the area under the curve (AUC), elimination rate constant (K), mean residence time (MRT), half-life ($t_{1/2}$), and bioavailability (F) can also be obtained for inhaled drugs using non-compartmental or compartmental methods.

PK parameters of drugs administered by inhalation in guinea pigs in our laboratory are discussed in Table III. Drugs delivered directly to the lungs increase the local tissue concentrations for prolonged periods in a dose-dependent manner. Likewise, plasma drug concentrations are at detectable levels for extended periods when administered by pulmonary route to the lungs compared to oral delivery; serious side-effects can be diminished due to low plasma drug concentration (63). Elimination rate (K) was lower when drug was administered as aerosols to the lungs, correlating with a significantly higher half-life and mean residence time (Table III). Thus, the delivery of drug directly to the lungs confers the advantage of increasing the tissue concentration at the primary site of Mtb infection for longer periods than delivered by other routes. Drugs delivered as sustained release formulations (microparticles and nanoparticles) by the pulmonary route may reach the periphery and act as depot releasing drug which would diffuse to the nearest blood vessel over prolonged periods. This depot phenomenon may be complicated by particle uptake by phagocytic cells, notably (AMΦs) (Figs. 1 B and 4).

One of the methods of assessing the PK properties of inhaled drug consists of exposure of isolated cells in culture. Cell models to evaluate drug transport include airway and alveolar epithelial cells. Also, isolated lung perfusion models have been used for pulmonary dissolution, absorption, lung tissue binding, transport phenomenon and metabolism while maintaining the physiological properties of the lung. Pulmonary clearance through mucociliary transport occurs in the upper portion of the lung but represents a mechanism of movement of particles from peripheral ciliated regions, thereby depleting drug at that location.

In Vivo Pharmacodynamic Assessment

The efficacy of novel inhaled drug delivery systems is assessed in infected animal models, where the primary endpoint is the burden of bacteria following treatment. The infection in animal models can be established by intravenous or the pulmonary route with virulent Mtb strains. IV infection of Mtb in animal models will ultimately lead to the haematogenous seeding of the lung, but it does not represent the extrapulmonary dissemination associated with the pathogenesis of disease in humans following respiratory infection. The pulmonary route of infection in animal model leads to deposition of Mtb in the alveolar spaces of the lungs. This is achieved using aerosol generation devices calibrated to deliver small numbers of bacilli. Aerosol infection with virulent strains of Mtb requires biosafety level-3 (BSL-3) facility that is available to a small number of laboratories

Table III. Pharmacokinetic Parameters of Drugs Used by the Inhalation Route Compared to Other Routes in Guinea Pigs

Drug	Formulation	Route of admin.	Dose (mg/kg)	PK parameters							Reference and year
				K (h^{-1})	C_{max} μg/ml	T_{max} (h)	AUC (μgh/ml)	F (0-inf)	$t_{1/2}$ (h)	MRT (h)	
PA-824	PP	INHL	40	0.17±0.05	3.42±1.14	3.25±2.09	32.34±16.79	0.63±0.32	4.38±1.06	7.52±1.91	(63), 2009
	Solution	IV	20	0.37±0.04	9.19±1.54	0.11±0.07	26.54±2.20	–	1.91±0.24	2.69±0.31	
	Micelle suspension	Oral	40	0.30±0.05	4.14±0.78	4.00±0.63	25.77±6.40	0.56±0.12	2.43±0.56	5.37±0.53	
RIF	PP	INHL	2.5	0.46±0.28	0.67±0.18	0.19±0.14	1.06±0.38	0.57±0.21	2.26±1.63	1.50±0.50	(151), 2009
	$PNAP_{80}$	INHL	2.5	0.50±0.26	0.50±0.11	0.21±0.15	0.98±0.34	0.53±0.18	1.78±0.95	1.37±0.39	
	Solution	IV	10	0.56±0.05	3.88±0.58	0.12±0.09	7.44±1.06	–	1.24±0.12	1.81±0.20	
	Micelle suspension	Oral	2.5	0.31±0.15	0.08±0.02	1.00±0.50	0.31±0.09	0.17±0.05	2.63±1.16	1.31±0.46	
CAP	PP	INHL	14.5	0.48±0.1	6.7±1.3	–	17.0±3.9	–	1.5±0.4	1.8±0.1	(140), 2008
	Solution	IV	20	0.92±0.1	53.6±3.9	–	49.3±10.7	–	0.8±0.1	0.9±0.1	
	Solution	IM	20	0.68±0.18	32.3±3.8	–	58.2±11.0	–	1.1±0.3	1.4±0.3	

INHL Administered by the inhalation route, *IV* intravenous, *AUC* total area under the curve (time zero to infinity), *K* elimination rate constant, $t_{1/2}$ half-life, *MRT* mean residence time, C_{max} maximum concentration, T_{max} time at which C_{max} occurs, *F* bioavailability (time zero to infinity), *PNAP* porous nanoparticle-aggregate particle, *PP* porous particles, *RIF* rifampicin, *CAP* capreomycin

around the world for a variety of technical and financial reasons.

The biochemical and physiological effects of drugs on various organs after treatment are important parameters to evaluate for the safety and efficacy of various delivery systems. Conventional protocols require sacrificing animals four to six weeks after infection with untreated controls prior to the start of treatment to determine the initial bacterial load and at the end of the treatment period. Six weeks after infection complete hematogenous dissemination of bacilli and the reseeding of the lung has occurred (64). The experimental endpoints with respect to efficacy of novel drugs and formulations following virulent Mtb challenge in animal models includes assessment of clinical parameters (e.g. weight loss, fever, respiratory distress), quantitative estimation of the bacillary loads (colony forming units, cfu), number and size of lesions and degree of histopathology (including size enlargement), in the lungs, spleen, lymph nodes and liver, at the time of necropsy or mortality. Wet tissue weights are used as quantitative measures indirectly related to degree of inflammation (65). The larger organ weight is considered evidence of inflammation due to Mtb infection. Histopathological studies are performed to examine the degree of involvement of infection in various tissues and the pathology of lesion formation. Granulomatous involvement of lung lobes, necrosis and lymphocytic infiltration have been observed to a lesser extent in aerosol-treated animals compared with positive controls. Guinea pigs are susceptible to the development of severe lymphadenopathy of the mediastinal lymph nodes after a few days after infection with Mtb (66). Bacterial infection in the lymph nodes is severe and clearly evident compared to that in lungs and spleen; this makes the outcome of drug efficacy studies more pronounced in the lymph nodes than in any other organ. Table II depicts the administration of various drug formulations in infected animals and humans for TB by inhalation using different delivery methods. In all cases the evidence for therapeutic potential is clear as indicated by the reduction of bacterial burden in various organs.

In the following sections some of the mycobacterial strains and the animal models used to study efficacy of drugs and their formulations are discussed.

Strains of Mtb Used for Experimental Infection

The majority of the more than 50 species that comprise the genus *Mycobacterium* are non-pathogenic environmental mycobacteria, related to the soil bacteria *Streptomyces* and *Actinomyces*. A few of these species are highly successful pathogens, including Mtb, *M. leprae*, and *M. ulcerans*, the causative agents of TB, leprosy, and Buruli ulcer, respectively. Their success can be attributed to their ability to infect and proliferate inside host macrophages, the very cells responsible for providing protection against infectious organisms (67). The Mtb complex consists of Mtb, *M. africanum*, *M. canettii*, *M. bovis*, *M. microti*, *M. caprae* and *M. pinnipedii*, which are closely related organisms (68). However, they differ significantly in morphology, biochemistry, host range, and disease patterns in experimental animals (69). Mycobacterial species can also vary in their ability to be transmitted and cause infection, elicit cytokine responses (host immunity), and grow in animals and macrophages (70–72). *M. bovis* and *M. marinum* have been studied in cattle and fish/amphibians, respectively, which are their natural hosts and act as surrogates for Mtb infection in humans. The *M. avium* complex (MAC) is a group of related environmental mycobacteria, including *M. avium* subspecies *avium*, *paratuberculosis*, and *M. intracellulare*. MAC is resistant and less susceptible to most anti-TB drugs than other species; this may be explained by a decrease in the organism's permeability to these drugs (73). Different mechanisms exist for transport of drug across the hydrophobic cell wall barrier for different strains of mycobacteria. A thorough analysis of the outer membrane, the intervening cell wall region and the inner bacterial cell membrane may yield valuable information on the uptake and efflux mechanisms of various anti-TB drugs. Variations in the strain have implications for the development of novel drugs and vaccines based on the fitness and transmission dynamics of resistant mycobacteria. Some strains of Mtb used for research purposes have become adapted for growth in the laboratory and are poor representative organisms for those encountered in actual clinical settings (74). Mycobacterial strains used in the laboratory include H37Ra (non-pathogenic strain), H37Rv, Erdman and Beijing and more recently, the clinical strain CDC1551. These are all pathogenic strains. Different strain lineages may also be associated with particular geographic regions (75). There is strong evidence that various Mtb strains differ in virulence in different animal models (76).

ANIMAL MODELS IN TB

Several animals have contributed substantially to our understanding of TB, including pathogenesis and immunology of this disease. Early TB pathogenesis can be studied only in animal models, as samples of human lesions are usually unavailable. Existing animal models have not been able to mimic progression of the disease in a heterogeneous human population; each animal species represents more or less accurately aspects of the human disease and may have a fundamental influence on the outcome. Drug disposition and metabolism in animal species also vary in more than one parameter. An understanding of such differences is extremely important to establishing the efficacy of new drugs and delivery systems. Mice, guinea pigs and rabbits are small animal models that approximate the human TB. Animal models for pulmonary TB have been described in an extensive literature (77) and allow the interpretation of new experimental results in the context of proposed relevance to humans. Large animal models, such as primates, are considered more relevant than small animals, as they most closely parallel human disease. However, primates are not commonly used because of their expense, ethical issues and limited availability.

Mice

Mice have been the most extensively studied animal model and have contributed significantly to our understanding of the mechanism of resistance, immune response, and evaluation of drugs and vaccines. They have some similarities in the pathophysiology of the disease with respect to humans, though differences exist. The granuloma that form in

response to infection is quite different between mice and humans. The human lungs contain an array of granuloma types, from solid to necrotic to caseous to cavitary, containing varying numbers of bacteria, which is not the case for mice. Mice are more resistant to infection with Mtb, and the disease progression differs from human TB. Mice are able to sustain extremely high levels of bacillary loads in their lungs without the manifestation of disease, unlike infected humans. Caseation, necrosis and liquefaction (cavitation), which are hallmarks of human TB, do not occur in mice. Mice have been manipulated to induce latent TB infection, which is known as the Cornell model. Variants of the Cornell model exist and mainly consist of drug-induced and low-dose chronic infected models (78). Genetically altered, immunodeficient and transgenic mouse models, including knockouts, to screen new drugs and delivery systems also exist. The murine immune system is well characterized compared to other animal species and, consequently, a large number of reagents are commercially available. They have a clear advantage with regards to the required space for housing and performance of experiments in a BSL-3 facility. Many more mice can be housed compared to other larger animal species, and the cost of purchasing, housing and husbandry is smaller.

Guinea Pigs

The guinea pig has been a prominent model in TB research for more than a century. Apart from being Koch's classical animal model in the late 19th century, guinea pigs have become the "gold standard" for potency testing and biological standardization of tuberculins for use in human skin testing (79). They are sensitive to skin testing and demonstrate a delayed-type hypersensitivity reaction. The distinct similarities to humans in the progression of granuloma pathology have made guinea pigs important for TB vaccine testing (80,81) and drug evaluation (66,82,83). They are similar to humans in being susceptible to very low doses of virulent bacteria, and virtually all infected guinea pigs succumb to the disease (84). Once infected with a low dose aerosol infection, they develop progressive pulmonary granulomas that mimic human granulomas in every important aspect except cavitation, which rarely occurs in this species (77,85). The morphology of the necrotic granulomas in infected guinea pigs and their burden of bacteria are similar to human lesions (24,86). Guinea pig primary granulomas are characterized by a lack of vascularity leading to decreased oxygen tension or hypoxia, which are hallmarks of human tissues (87–89). Hypoxia transitions mycobacteria into a non-replicating state, thereby affecting their response to drugs (90); hypoxia is absent in the mouse model of TB (91). Naïve guinea pigs develop primary lesions similar to humans after initial infection that differ in morphology compared to secondary lesions originating from hematogenous dissemination after activation of CMI (86,92). The extrapulmonary dissemination of Mtb from the draining lymph nodes to the blood stream and secondary granuloma formation has been well-characterized in this model. Like humans, guinea pigs rapidly develop progressing granulomatous and necrotizing lymphadenitis in lymph nodes that ultimately drain the primary lung lesion (93,94). Bacterial burden in lymph nodes and their inflammation can measure the bacterial clearance in lymphoid organs, thus increasing the relevance of guinea pigs as an animal model for TB. Though guinea pigs do not demonstrate cavitary TB, latent models have been recently proposed (unpublished report, McMurray D.N). Several reagents have recently been developed to study the immunological determinants to TB in guinea pigs, though not as common as in mice. Compared to larger animal models, guinea pigs are comparatively inexpensive and easy to house under BSL-3 conditions.

Rabbits

The rabbit has been used as a model for TB for many decades (95). Although rabbits are naturally immune to Mtb infection and susceptible only to infection with virulent *M. Bovis*, they reliably reproduce the range of pathological events seen in human TB. They are the only small animal model in which softening and liquefaction of caseous granulomas occur, ultimately leading to cavitation and release of massive numbers of bacilli that can disseminate by droplet nuclei. Therefore, the rabbit is a valuable animal to study Mtb infection in cavitary lesions and to test new compounds and formulations. Dermal hypersensitivity reaction to mycobacterial antigens similar to responses in infected humans is shown reproducibly by rabbits. The disadvantage of using the rabbit is their cost (purchase, housing and husbandry) and the limitations of biosafety space required to conduct experiments. Some might argue that their lack of susceptibility to Mtb is a drawback but this has not substantially impeded their use in understanding mycobacterial disease.

Non-human Primates

Non-human primates have been studied for TB pathology for many years, and the recent renewed interest in them as an animal model may be beneficial for vaccine and drug efficacy studies. Different species of primates (notably Rhesus and Cynomolgus) are known to be infected with various strains of mycobacteria under natural conditions. Primates infected with low dose virulent Mtb exhibit similar susceptibility and disease pattern to humans infected by the natural (pulmonary) route (96). The disease in monkeys usually spreads rapidly to pulmonary infection with both haematogenous and bronchial spread of the bacilli. Extensive caseous necrosis along with cavity formation has been observed (97). In 1996, Walsh *et al*. published data demonstrating that inoculation of macaques with low-dose Mtb did not cause severe disease, raising the possibility that they may be an excellent model of human TB (98). The disadvantage of using primates is that they are very expensive, much less accessible and costly to maintain under BSL-3 facility. Thus, performing TB research in primates is generally costly. Beyond screening studies, monkeys potentially play a significant role in mechanistic research and late-stage preclinical testing of new drugs and formulations for safety and efficacy.

Other Species

Recently, other non-mammalian species that cover certain aspects of TB pathology have been used to study drug efficacy. They include amphibians (frogs) and fish

(Zebra fish) that are infected with their natural mycobacterial pathogen, in this case *M. marinum*. Zebra fish are an attractive model, as they are genetically amenable and have both innate and adaptive immunity, and because the embryos are transparent, which allows for real-time imaging of infection dissemination (99). The main advantages of using these models are their low cost of purchase and upkeep, relatively rapid growth of the organism, thus shortening the experimental time, ease of working with large number of organisms and increasing the statistical confidence of the results, and ability to carry out infection studies in the absence of BSL-3 facility.

Critical reviews on animal models of TB are published elsewhere (80,100,101).

Comprehensive Rationale for Aerosol Therapy

Local

The goal of any infectious disease therapy is to deliver sterilizing doses of drug to microorganisms present anywhere in the body. When administered by the inhalation route, a high drug concentration is achieved in regions of the lungs that may not be accessible by the circulatory system. Drugs can be delivered to the lungs as solutions, suspensions or dry particulate forms. Drug is released from particulate carriers initially by the removal of surface-bound/adsorbed drug and later by rapid dissolution and diffusion. Microparticles and nanoparticle aggregates in the appropriate size range are deposited in the deep lung and ingested by AMΦs and dendritic cells; these immune cells are transported from there to the nearest lymph nodes. Migration of AMΦs to the lymph node after phagocytosis was first observed at the end of the last century (102,103), although recent observations appear to demonstrate that dendritic cells are the only APCs that can migrate to the lymph nodes (49). Regardless of mechanism, particles targeted to the deep lungs have been reported to be present in the lymph nodes (Fig. 4) (104). They may remain there for long periods or may migrate to the vicinity of granulomas (Fig. 1 A). Dendritic cells help T-cells acquire the capacity to produce powerful cytokines like interferon-gamma to activate macrophages to prevent infection by microbes (105), or interleukins to mobilize phagocytes at body surfaces to resist extracellular bacilli (106).

Lenaerts *et al.* have suggested the presence of a physical barrier surrounding granulomas that might prevent the penetration of drug to less-accessible regions of the lungs (89). The central region of a granuloma may become devoid of blood vessels leading to a hypoxic environment; this results in less drug diffusion into the granulomas from blood vessels in the vicinity. Lung granulomas may be located proximal to an airway branching, blood and lymphatic vessels; anti-TB drugs could reach these granulomas by any of these routes. The bacteria may be present in the central region or inside the macrophages in the peripheral part of the granuloma (Fig. 1 A). Drug delivery systems that can permeate through the biological barriers associated with a granuloma should be designed. To complicate this phenomenon, granulomas vary in size, which presents a more-or-less significant barrier to drug penetration.

A fundamental question that remains is whether adequate drug is present in the pulmonary blood vessels proximal to the granuloma, so that therapeutic levels of drug may travel the distance and diffuse into the fortified granuloma to prevent microbial growth (Fig. 1 A). High EMB and macrolide concentrations were achieved in BAL cells relative to plasma when these drugs were administered orally to human subjects (107–109); whereas, rifapentine achieves substantially lower concentrations in the lungs than observed in plasma. Patients being treated for pulmonary TB may have suboptimal drug concentration intracellularly, thus predisposing them to development of drug-resistant strains of micro-organism (110). The advantage of aerosol delivery of microparticulate drugs is their uptake by AMΦs. These AMΦs may migrate to the periphery of lung granulomas leading to increased drug penetration (since the driving force of concentration gradient, an important element of Fick's Law, will be larger) (Fig. 1 A). Oral or parenteral anti-TB drugs cannot elevate drug at the periphery of granuloma beyond circulating concentration. In order to mimic the inhaled drug delivery and effectively penetrate the granulomas, large and potentially toxic systemic doses would be required by oral or parenteral delivery. Conversely, conventional oral and systemic doses may be insufficient to penetrate all granulomas at therapeutic levels, thereby leaving viable bacteria that may be selected for antimicrobial drug resistance.

Patients with diseases such as CF, asthma and COPD have airway obstruction and may exhibit variation in lung deposition following aerosol drug delivery. Many of the anatomical features of the lungs are preserved in TB patients, and only regions containing granulomas or cavities may be obstructed. Drug delivered as an aerosol may be absorbed from the airways into the circulation in one region of the lungs and then re-absorbed elsewhere in the tissue before exiting to other regions of the body; lesions in other regions may benefit from the potential for drug re-absorption.

Complimentary With Oral and IV

A study conducted from 1982–1986, showed resistance to one or more anti-TB drugs in 8.8% and 23% of previously untreated and treated TB patients, respectively (111). This demonstrates that the emergence of resistant strains of mycobacteria is greater in patients treated with anti-TB drugs than in untreated patients (112). Drug resistance in untreated patients may be due to random mutations occurring in high bacterial loads, whereas the significantly higher resistant strains observed in treated patients may be explained by poor drug penetration into certain areas of the lungs that harbor high bacterial loads and/or poor patient compliance. Patients being treated for six or more months under the DOTS program had a 5% possibility of relapse (113). Thus, emergence of drug-resistant strains is not explained simply by lack of patient compliance. Resistant strains can emerge in regions of high bacillary burden of the lungs due to poor penetration of the dose. Therefore, sub-therapeutic concentration of drug inside granulomas containing high bacterial burden may give rise to resistant strains. Treatment failure due to resistant bacilli may result in severely damaged lungs containing a large number of bacilli and may necessitate pulmonary resection (114). Surgical resection is indicated for patients having MDR or XDR TB; it

may be required in certain areas of the lung that may not be accessible to conventional therapy. Apart from evaluating new classes of drugs for treating MDR-TB, novel methods of delivery including aerosolization of cytokines and drugs to the lungs have been recommended (115).

Delayed sputum smear conversion in patients with pulmonary TB after starting anti-TB therapy has been observed. This may be due to either previously existing drug-resistant strains or the emergence of resistant strains due to the failure of systemic drugs to reach extensive cavitary disease in the lungs that contain numerous acid fast bacilli (AFB) (116). The same authors have shown that HIV-positive individuals converted to smear negative at a faster rate than HIV-negative patients. Cavitary lesions are known to be absent in HIV-positive patients, and, hence, there seems to be no barrier for the systemic drug to reach sites in the lung that harbor Mtb. Administration of a combination of anti-TB drugs by the conventional route may result in sub-therapeutic levels of one or more drugs, leading to the emergence of drug-resistant Mtb strains. Inhaled aminoglycosides in conjunction with conventional therapy have led to faster sputum sterilization in drug-susceptible and resistant patients and resulted in decreased transmission (30).

In HIV-positive patients, TB leads to immune response generation and increased HIV-1 replication in AMΦs. IFN-γ, a cytokine produced mainly by CD 4 T lymphocytes can activate AMΦs to be effective phagocytic cells against Mtb. High IFN-γ levels in the lungs act as adjuvants to enhance the local immune response. TB patients co-infected with HIV when receiving recombinant IFN-γ as nebulized aerosols to the lungs with standard oral or parenteral anti-TB therapy showed improved symptoms and chest radiographs. This treatment also led to reduced AMΦ inflammatory cytokines and HIV-1 virus loads in BAL fluid (117). Patients on conventional anti-TB therapy receiving IFN-γ particles by inhalation showed significant deposition in the lower respiratory tract (118). IFN-γ administration by subcutaneous route results in systemic toxicity, limiting its use.

Systemic

The alveolar surface area of the lung is enormous, approximately 100 m^2 in an adult human, containing 250 million to 350 million alveoli and an extensive blood supply, thus offering an ideal route for drug delivery for systemic diseases (119,120). The ratio of capillary surface area to the surface area of the alveolar air space in most mammalian species is slightly less than 1, and the mean distance between the surface of the alveolar space and the blood surface of the capillaries located in the inter-alveolar septa is only 0.6 μm in humans (Fig. 4) (121). Delivery devices and formulations can be fabricated that can deliver drug to either the local lung milieu or from there to the systemic circulation (122,123). As depicted in Fig. 2 C, topical administration of drugs to the deep lung may lead to substantial diffusion to the systemic circulation, in a drug-dependent fashion. The figure (color intensity not to scale) shows that the lowest drug concentrations are achieved in the systemic circulation. This might be improved by consideration of important physicochemical characteristics of the drug and the formulation that dictates bioavailability. In this respect, consideration of Lipinski's rule is a starting point for prediction of the potential for systemic availability.

The lungs can be used as a portal of entry for low molecular weight drugs and macromolecules into the systemic circulation. Commercially available inhaled drugs for treatment of local lung diseases, such as asthma, are absorbed into the circulation, with some of them having very high systemic bioavailability. Drugs administered by inhalation must first dissolve in the lung fluid to be absorbed into the systemic circulation. The bioavailability and permeability of drugs and macromolecules into the systemic circulation from the lungs are more than any other non-invasive route of drug delivery. No other non-invasive route of drug delivery provides a faster therapeutic action than the inhaled route (124). The lungs are also more permeable to small molecules than the gastrointestinal tract (125). Small hydrophobic and hydrophilic molecules are absorbed into the systemic circulation in a matter of seconds and minutes, respectively, after inhalation delivery. Lipophilic molecules are absorbed over a wide concentration range and seem to be nonsaturable. They are thought to be absorbed by integrating with the lipid bilayer surrounding the cells, whereas lipid-insoluble compounds most likely are absorbed by passing through aqueous pores in the intracellular tight junctions (126). A number of compounds like disodium cromoglycate are absorbed by active or "carried-mediated" transport (127).

Most of the present-day inhalers deliver drugs that deposit in different regions of the respiratory tract, from the trachea to the alveoli in the deep lung. Drugs are absorbed at different rates throughout the length of the respiratory tract based on the thickness of the epithelia, which is thicker in the upper region leading to less absorption (128). Thus, the plasma profiles resulting from inhaled drugs are usually due to their absorption across markedly different epithelia. As the termini of the lungs, the alveoli act as a reservoir for drug molecules that prolongs their stay; the presence of lung fluid in the alveoli further aids in drug absorption. This is not the case with absorption of drug across GI tract or nasal passage, where absorption has to take place when the drug molecules are in apparent transition. Drugs can also be formulated to have a slow absorption from the lungs into the systemic circulation. Drugs having a rapid rate of dissolution and absorption can be encapsulated in delivery systems such as microparticles, nanoparticles and liposomes, since drug release, rather than absorption, is the rate-limiting step across the lung-blood barrier.

Limitations of Inhaled Therapy

Inhaled therapies have most frequently been employed for lung diseases, notably those affecting pulmonary function, such as asthma and COPD. Even antimicrobial agents have been delivered for diseases with a strong local manifestation, such as cystic fibrosis. Reproducible delivery of drugs to the lungs presents one of the most challenging design tasks in aerosol delivery. One of the main challenges in inhalation therapy for CF is to selectively deliver drugs in the heavily obstructed regions of the lung, rather than the better ventilated healthier regions where drugs are more likely to deposit. Patients treated with inhaled drugs have been diagnosed with upper lung infection rather than the

typical diffuse whole lung disease showcasing the failure of inhaled therapy (129). This indicates the non-uniform distribution of inhaled drug in the lungs leading to their sub-therapeutic levels, and consequently, the emergence of drug-resistant strains. Thus, it is imperative to have delivery systems that can deliver drugs uniformly to the lungs. Successful delivery of active medications to the lungs is determined by many factors, including the efficiency of the delivery device to generate respirable-sized particles and patient-dependent factors. Inhaled delivery of antibiotics against specific micro-organisms may sometimes result in their extrapulmonary dissemination due to poor systemic bioavailability of the drug through the lungs. This may necessitate the use of adjunct oral and parenteral therapy to maintain sufficient systemic drug concentration and prevent extra pulmonary infection and development of drug-resistant strains.

Since there are no approved anti-TB aerosol therapies, learning from the limitations to the treatment of other diseases with aerosols is the basis from which to further the development of this therapeutic strategy.

CONCLUSION

The urgency to treat TB may be addressed with approaches consisting of newer drugs and delivery systems. Drugs delivered in various aerosol delivery systems to the lungs in conjunction with those administered by conventional routes holds promise in reducing the problem of drug-resistant strains of TB. Various drug delivery systems such as microparticles, nanoparticles and liposomes can be delivered to the periphery of the lungs for instantaneous and targeted or controlled release. This may lead to therapeutically effective drug concentrations in regions of the lungs containing large burdens of bacteria. Aerosol delivery to the lungs as an adjunct to conventional therapy has the potential to decrease the dosing frequency and the duration of therapy. This may ultimately lead to less severe side-effects, improved patient compliance and reduced emergence of drug-resistant strains of TB.

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