Chapter 5
Inhalation Therapy for Pulmonary Tuberculosis

Keiji Hirota, Keishiro Tomoda, Kimiko Makino, and Hiroshi Terada

5.1 Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) is one of the worst of infectious diseases. *Mtb* is transmitted from person to person via airborne droplets that arise from the one infected with the active *Mtb* organism, and thus this mode of transmission has the potential to cause a worldwide pandemic. In addition, the recent emergence of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) has become a major public health issue in both developing and developed countries. Although the development of new anti-TB drugs, such as TMC207, OPC-67683, and PA-824, is recently in progress throughout the world [1, 2], the establishment of an effective system for the delivery of such drugs as well as conventional anti-TB drugs also needed for the extermination of TB. In this chapter, essential medical treatment and promising formulations for the treatment of pulmonary TB through inhalation are described.

K. Hirota • K. Tomoda • K. Makino
Center for Drug Delivery Research, Faculty of Pharmaceutical Sciences, and Center for Physical Pharmaceutics, Research Institute for Science and Technology, Tokyo University of Science, Yamazaki, Noda, Chiba, Japan

H. Terada (✉)
Center for Drug Delivery Research, Faculty of Pharmaceutical Sciences, and Center for Physical Pharmaceutics, Research Institute for Science and Technology, Tokyo University of Science, Yamazaki, Noda, Chiba, Japan

Niigata University of Pharmacy and Applied Life Sciences, 265-1, Higashijima, Akiha-ku, Niigata City 956-8603, Japan
e-mail: teradah@rs.tus.ac.jp

P.V. Devarajan, S. Jain (eds.), *Targeted Drug Delivery: Concepts and Design*, Advances in Delivery Science and Technology, DOI 10.1007/978-3-319-11355-5_5

5.1.1 Epidemiology of TB

The Global Tuberculosis Report 2012, published by World Health Organization (WHO), stated that "the WHO declared TB a global public health emergency in 1993. Starting in the mid-1990s, efforts to improve TB care and control intensified at national and international levels" [3]. However, one-third of the world population has already been infected with *Mtb* [4, 5]; and the latest estimates by the WHO showed that 8.7 million people fell ill with TB, and 1.4 million people died from it, in 2011 [3].

In addition, the risk of TB pathogenesis in people infected with the human immunodeficiency virus (HIV) is 21–34 times greater than that for those without HIV infection [6]; and TB is the leading cause of death among HIV-positive individuals, being responsible for 22 % of HIV-related deaths. As people infected with both HIV and *Mtb* become a growing pool of the *Mtb*, treatment and prevention of TB need to be addressed in conjunction with acquired immunodeficiency syndrome (AIDS).

5.1.2 Pathology of TB

Mtb organisms infect the lung tissue of an individual through inhalation of the *Mtb-containing* airborne droplets that arise from coughing and conversing with TB patients. The initial infection with *Mtb* has a potential to occur in any of the lung lobes, which infection leads to the formation of small necrotic caseous lesions that contain *Mtb*s engulfed by macrophages and other recruited leukocytes. The onset of recognizable TB disease following the initial infection is evident in only a few individuals. In general, TB turns active and infectious when the bacteria have developed a secondary site of infection, which is usually the upper lobes of the lungs, though the mechanism remains unknown.

Alveolar macrophages patrol the alveoli and eliminate exogenous foreign substances by endocytic ingestion and subsequent exposure to reactive oxygen species (ROS) and digestive enzymes. As shown in Fig. 5.1, pathogens are first taken into

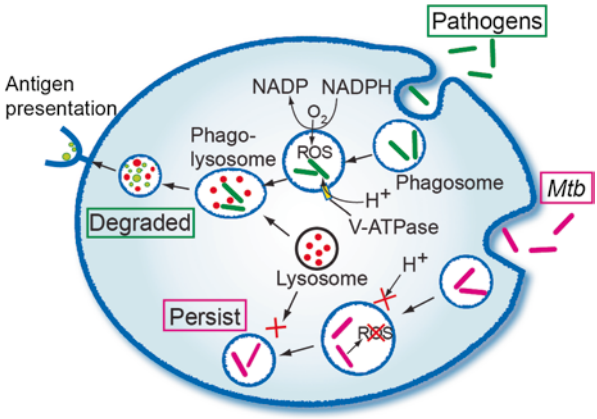

Fig. 5.1 Survival of *Mtb* in alveolar macrophage

phagosomes in macrophages through phagocytic pathways. Then, in general the pathogens are exposed to ROS generated via NADPH oxidase and to lysosomal enzymes after the fusion of the phagosomes with lysosomes to form phagolysosomes; and finally they undergo full digestion. However, *Mtb* organisms can escape from the clearance process by inhibiting this fusion of the phagosomes with the lysosomes by blocking V-ATPase expression and increasing the accumulation of TACO (tryptophan-aspartate-containing coat protein) around the phagosome membrane [7–9]. In addition, *Mtbs* become resistant to attack by ROS in the macrophages by generating catalases, peroxidases, and superoxide dismutases [10, 11]. These mechanisms allow *Mtbs* to persist in the macrophages.

The macrophages infected with *Mtbs* subsequently generate inflammatory responses that lead to the recruitment of neutrophils, monocytes, other macrophages, T cells, and B cells from neighboring blood vessels and lymph nodes. Recruited macrophages are transformed into several cell types such as epithelioid cells and Langhans giant cells (generated by fusion of epithelioid cells) during the prolonged inflammatory response, and these cells and the recruited lymphocytes form a granuloma that encloses the bacteria, a process taking approx. 2 years [12–14]. However, the *Mtbs* exploit this granuloma as a shelter to escape from monitoring by immune cells and attacks by antibiotics and thus persist in the lungs.

Mtb grows in the lungs by utilizing host-derived lipids as a carbon source [15, 16]. During the growth, caseation and cavitation gradually progress at the core of the granuloma. Then, the organisms that have proliferated exploit macrophages as well as neutrophils to be carried as a cargo and to move into the upper airways with the sputum [17]. The presence of *Mtbs* in the sputum indicates the infectious state. As *Mtb* latently infects individuals, a relapse of TB is associated with a depression of immune activity due to aging, a surgical operation, anti-TNF-α therapy or HIV infection.

5.2 TB Therapy

5.2.1 Current Treatment

Current treatment of TB is performed by oral intake of multiple anti-TB drugs according to the internationally accepted first-line treatment regimen [18]. The drugs used in the first-line treatment are isoniazid (INH), rifampicin (RFP), pyrazinamide (PZA), and ethambutol (EB). Sometimes streptomycin (SM) is employed instead of EB. These therapeutic drugs, except EB, possess mycobactericidal activities. The second-line anti-TB drugs include aminoglycosides, such as amikacin (AMK) and kanamycin (KM); capreomycin (CPM); fluoroquinolones; and *p*-aminosalicylic acid (PAS). These drugs are mainly employed in the treatment of MDR/XDR-TB due to their weaker anti-TB activities and greater toxicity than those used in the first-line treatment.

The standard regimen is effective to kill 99 % of TB pathogens within 1 month, but elimination of the remaining 1 % takes 3 months [19]. In addition, an additional

dose for 2 months after the disappearance of the bacterial burden in the lungs is needed to prevent a relapse of TB. The current therapy thus has a long duration of more than 6 months for a complete recovery from TB [19].

However, the continuous long-term dosing often ends in failure in TB treatment and consequently causes the emergence of drug-resistant organisms. To prevent the failure of TB therapy, the WHO has proposed the treatment of directly observed therapy, short course (DOTS), which has contributed to high drug compliance. Although the introduction of DOTS has resulted in increases in successful treatment outcomes [20], novel anti-TB drugs acting based on mechanisms other than those by conventional anti-TB drugs, and optimum therapeutic protocols to eliminate *Mtb* over the short term of less than 6 months, are needed for complete treatment of TB. In addition, overcoming MDR/XDR-TB is also a major clinical issue to be addressed.

5.2.2 Inhalation TB Therapy

The *Mtb* that has invaded into the lungs easily infects alveolar macrophages, in which it proliferates by inhibiting phagosome-lysosome fusion by several mechanisms previously described in Sect. 1.2. Hence, the infected alveolar macrophages should be a primal target for TB therapy, because the organisms accumulate in these cells [21]. Comparison between inhalation therapy and conventional oral dosing for TB treatment is summarized in Table 5.1.

The amount of inhaled anti-TB drug in alveolar macrophages is expected to be greater than that administered by oral dosing, if an inhalable formulation that incorporates an anti-TB drug and is easily taken up by alveolar macrophages is available. It is reported that hydrophilic aminoglycosides in a form of an aerosol can sterilize *Mtbs* in human sputum and reduce the burden of *Mtb* in the mouse lung [22, 23]. In addition, anti-TB drugs formulated into microparticles having a diameter of 3 μm, which is suitable for ingestion by alveolar macrophages [24], should contribute to their efficient deposition in the lungs. The delivery of anti-TB drugs directly into the lungs also decreases their concentration in the circulation system and thus avoids adverse effects such as hepatic dysfunction and neurologic disorders. Hence, inhalation therapy is promising for complete treatment of TB.

Table 5.1 Comparisons between inhalation therapy and oral treatment for TB

	Inhalation therapy	Oral treatment
Drug distribution	Local	Systemic
Dose	Milligram order	Gram order
Medication	Inhalation using special devices	Intake with water
Formulations	Liquid, suspension, dry powder	Tablet, capsule
Adverse effects	Unknown, but expected to be nontoxic	Hepatic dysfunction, neurologic disorders, allergic response

5.3 Preparation of Inhalable Particles Containing Anti-TB Drugs

The human airway becomes narrower after the bifurcation forming the bronchi, decreasing in diameter from 1.8 cm at the entry of the trachea to 0.041 cm at the distal airway [25]. As the alveolar macrophages reside in the deepest region of the lungs, there is a size limitation of the particles reaching there. In addition, an effective particle size in the airflow is different from its geometric size due to the emergence of a drag force dependent on the velocity; and this effective size is defined as the aerodynamic diameter (D_{aer}), as shown in the equation below:

$$D_{aer} = D_{geo} \times \sqrt{\frac{\rho}{\chi}},$$

where D_{geo} is the geometric diameter, ρ is the particle density, χ is the shape factor (a sphere gives 1; but elongated particles, such as fibers and needles, are greater than 1) [26]. This aerodynamic diameter is useful to estimate where particles will become deposited in the respiratory system. From the mathematical model based on the experimental data, it has been shown that particles with a D_{aer} between 1 and 5 μm are efficiently deposited in the alveolar pulmonary region [27]. Most of the larger particles of D_{aer} >10 μm are trapped at the oropharynx, whereas smaller particles of D_{aer} <1 μm reach the alveoli; however, most of them are exhaled without settlement in the lungs [28]. Methods for the preparation of inhalable particles for TB therapy are summarized in the following sections:

5.3.1 Emulsion/Solvent Evaporation

There are a considerable number of reports on the formulation of microparticles and nanoparticles of various biomaterials for the delivery of anti-TB drugs. Biodegradable materials are advantageous for the formulation of particles used for long-lasting drug release [29]. One of the most frequently employed methods to formulate the particles is based on the emulsion/solvent evaporation method. The emulsification is conducted by mixing a dispersed oil phase into a continuous aqueous phase. For preparation of the particles, hydrophobic polymers, such as poly(lactic-co-glycolic) acid (PLGA) as bases, and hydrophobic anti-TB drugs, such as RFP, as active ingredients, are dissolved in an organic solvent such as dichloromethane and emulsified in an aqueous solution, in which both the base polymer and the drug are insoluble in the presence of emulsifiers, such as glycerol, poly vinyl alcohol and surfactants, under continuous agitation [30, 31]. The dispersed particles that have encapsulated the drugs are obtained after evaporation of the organic solvent. The hydrophilic INH and hydrophobic RFP have been encapsulated into particles successfully obtained by use of suitable solvents [32]. Particle size and its distribution depend on mixing methods, agitation speed, and concentration of the solutes. Extrusion of the

dispersed phase into the aqueous phase through a Shirasu porous glass membrane under pressurization using inactive N_2 gas allows the formulation of particles with a narrow size distribution [33]. The main limitation of the emulsion/solvent evaporation method is the small batch size for preparation of the particles.

5.3.2 Spray-Drying

Spray-drying is one of the most commonly employed techniques to formulate dry powders in sizes from nanometers through micrometers. In general, active pharmaceutical ingredients (API) and excipients are dissolved in an aqueous or organic solvent, and the liquid is sprayed through a narrow atomization nozzle with high pressurized inactive gas at a temperature higher than the vaporization point of the solvent. The fine droplets emitted are quickly dried, and then the generated particles are collected in a cyclone chamber or an electrostatic precipitator. The dry powders formulated by the spray-drying method contain an accurate amount of API and excipients [30, 34]. It is noteworthy that the spray-drying method is available to formulate proteinaceous APIs into particles. Exubera, known as the first commercial proteinaceous inhalable dry microparticle product but now withdrawn from the market, which consisted of recombinant human insulin, as an API, and mannitol, glycine, and sodium citrate, as the excipients, was produced by the spray-drying method [35]. The spray-drying method is more advantageous than the emulsion/solvent evaporation one for the preparation of a large amount of stable particles at the industrial level.

A number of inhalable particles containing anti-TB drugs have been prepared by spray-drying. RFP was formulated in a form of inhalable dry particles with a carrier material of PLGA by the spray-drying method [36–39], and it was found that the release period of RFP from the particles increased with an increase in the molecular weight and ratio of lactic acid to glycolic acid in the PLGA. Dry powders containing PA-824, an anti-TB drug under development or multiple drugs such as hydrophilic INH and hydrophobic RFP could also be prepared by the spray-drying method [40, 41]. For the treatment of MDR/XDR-TB, capreomycin was spray-dried into an inhalable formulation; and a phase I clinical trial of the formulation was completed [42, 43]. In addition, it is noteworthy that the spray-drying approach allowed for preparing dry powders containing viable vaccine strain Bacillus Calmette–Guérin (BCG) without freezing [44].

Spray-freeze-drying, similar to the spray-drying, consists of the combination of spray-drying and freeze-drying methods; and it is useful for the preparation of dry powders, especially for those to be used with heat-sensitive molecules such as proteins and peptides without causing a decrease in their activities [45, 46]. The spraying is performed into a cryogenic liquid such as liquid nitrogen, and the drying is conducted at ambient temperature. This method is efficient for the preparation of lighter and more porous dry powders than those obtained by spray-drying, with a high yield of almost 100 %.

5.3.3 *Liposomes*

The delivery of drugs directly to the lungs by nebulization has been attempted by the use of aerosolized liposome suspensions. Lipids, such as phospholipids, are major components of mammalian organisms; and hence they are regarded as biocompatible, and the aqueous space inside them is favorable for trapping hydrophilic drugs. Liposomes containing AMK, an aminoglycoside antibiotic, known as Arikace, are being used for inhalation treatment of gram-negative infections [47]. Arikace is currently proceeding under a phase II clinical study.

The specific targeting of liposomes toward nests of *Mtb*, namely, infected alveolar macrophages, was achieved by modifying the surface of the liposomes with maleylated bovine serum albumin (MBSA) and *O*-steroyl amylopectin (*O*-SAP) [48]. These macrophage-specific ligands increased the delivery of anti-TB drugs encapsulated by the liposomes into the macrophages, which delivery was followed by efficient attacking of the *Mtbs* residing in the intracellular space. Liposomal formulations are likely to progress to routine clinical usage within a short period compared with the polymer particles due to their biocompatible safety, as was shown in pediatric use such as treatment of neonatal respiratory distress syndrome [49]. However, several problems, such as a low encapsulation rate of certain polar drugs, high burst release of drugs, and a short shelf-life, need to be overcome for their practical application [50].

5.4 Effect of Inhaled Particles on Macrophage Functions

As mentioned above, macrophages infected with *Mtbs* should be an effective target for the delivery of anti-TB drugs [21]. Particle formulation is considered to be important for the import of anti-TB drugs into macrophages and is based on the high endocytic activity of macrophages. Endocytic uptake of bacteria and exogenous particles is regarded to be mainly dependent on their sizes and vesicle formation upon ingestion [51–54]. Phagocytosis, one of the ingestion routes, covers the uptake of particles having a diameter of more than 0.5 μm; macropinocytosis, those with one from 0.1 through 5 μm; and pinocytosis, those less than 120 nm in diameter [55–57]. The optimum size for attaining efficient uptake of anti-TB drugs by alveolar macrophages was reported to be around 3 μm [24].

It is noteworthy that inflammatory responses are triggered by the uptake of particles from macrophages. The signals generated during such a response could represent cautious signals to notify the surrounding immune cells of an attack by invaders. These signals are initiated by pattern recognition receptors (PRRs), which identify pathogen-associated molecular patterns (PAMPs) [58]. Hence, contamination by PRR ligands including components of bacterial cell walls, such as lipopolysaccharide and lipoarabinomannan, should be avoided when determining the exact mechanism of interaction between inhaled particles and macrophages.

Nanoparticles associated with polyethylene glycol (PEG) are known as a stealth formulation and are favorable to allow these particles to avoid interacting with immune cells. This formulation is practically utilized for the liposomal formulation of doxorubicin DOXIL (doxorubicin HCl liposome injection) used for the treatment of ovarian cancer and Kaposi's sarcoma [59]. However, the utilization of PEG to obtain these stealth particles caused unintentional production of anti-PEG IgM antibody from splenic B cells, and the subsequent clearance of the particles was consequently accelerated [60–62]. The silent stealth nature and concealment, as in the case of a "Ninja," is a key consideration for the development of formulations for inhalation delivery [63, 64].

5.5 Animal Studies

It is very difficult to deliver particles to the lungs homogenously by inhalation. Delivery is relatively well achieved for humans and large animals having a lung size similar to the human one. However, the delivery to small animals, such as mice, rats, and guinea pigs, is much more difficult, because the lung size of these animals is obviously very small. In the case of mice, the air space of the lungs has a volume of approx. 1 ml; and the internal diameter of the upper trachea is about 1 mm. In order to evaluate efficacies of inhalation formulations in these small animals, whole-body, head-only, and nose-only exposure inhalation chambers have been exploited [65–67]. However, it is difficult to determine quantitatively the amount of drugs delivered into the lung tissue by use of exposure inhalation chambers due to the deposition of the drugs in the unintended tissues, such as the nasal cavity and skin. In addition, the use of such chambers requires a massive amount of dry powders, which is disadvantageous in terms of the handling and cost of dry powders. Consequently, intratracheal administration, such as instillation, nebulization, and insufflation, has been employed to deliver drugs to the lungs of small rodent animals. Characteristics of various methods for drug delivery to the lungs of laboratory animals are summarized in Table 5.2. In the next section, we will review noninvasive methods for drug delivery to the lungs.

5.5.1 Inhalation Administration of Drugs into the Lungs

5.5.1.1 Intratracheal Administration

Accurate delivery of drugs to the lungs is considered to be achievable by intratracheal administration. For this goal, an endotracheal cannula of a suitable size should be prepared for intubation; and a careful examination of the pharynx and larynx is necessary. The internal diameter of the trachea of laboratory rodents is approx. 1–3 mm. Prior to intubation, the animal is anesthetized to a sufficient depth to abolish

Table 5.2 Methods for drug delivery to the lungs

	Nose-only exposure chamber	Intratracheal administration		
		Insufflation	Nebulization	Instillation
Animals	Mice, rats, guinea pigs			
Required dose	Gram order	Milligram order	Microgram to milligram order	Microgram to milligram order
Formulations	Liquid, suspension, dry powder	Dry powder	Liquid, suspension	Liquid, suspension
Synchronization with breath	Yes	No	Yes/no	No
Distribution in the lungs	Homogeneous	Heterogeneous	Heterogeneous	Heterogeneous
Quantitative dosing	No	Yes	Yes	Yes
Repeated dosing	Yes	Yes/no	Yes/no	Yes/no
Delivery to unintended region	Nasal cavity, throat, skin	None	None	None
Anesthesia	Dispensable	Necessary	Necessary	Necessary
Batch size	A large number of animals	A single animal	A single animal	A single animal
Cost	Very high	Low	Low-high	Very low

the cough and swallowing reflexes. The use of anesthetic agents that cause no or only weak irritation of the respiratory tract is important for intubation. The inhalation anesthetics halothane and isoflurane, and the intravenous anesthetic propofol are likely to be favorable to avoid the over-secretion of saliva and airway mucus. In addition, anticholinergic agents are useful for reducing the mucus secretion and preventing tube blockage. Visualization of the entrance of the trachea is also important to perform the intubation properly and may be achieved by using a laryngoscope or an otoscope [68, 69]. Alternatively, illumination of the neck with a powerful light source helps visualization of the oropharynx [70].

Administration of liquid and suspended materials is carried out by intratracheal instillation. The volume for instillation is an important factor to achieve wide spreading of the drug in the lung tissue. Namely, 1–2 ml/kg body weight seems to be a favorable one. It is noteworthy that the amount of test drugs in the liquid dosing is restricted by their solubility in the limited volume of the liquid medium. In general, a slight amount of less than 1 mg may be favorable. Viscous liquid samples can be delivered into the lungs by intratracheal instillation, but the possible occurrence of respiratory failure should be taken into account.

Intratracheal nebulization is also useful to deliver low viscous liquid and suspended drugs into the lungs, and making use of a specialized apparatus, such as the MicroSprayer Aerosolizer Model IA–1C with FMJ-250 High Pressure Syringe (Penn-Century, Inc., Philadelphia, USA), is effective. The droplet size of aerosolized

phosphate-buffered saline obtained with the MicroSprayer was reported to be around 8 μm in terms of mean mass aerodynamic diameter [70]. Other specialized apparatuses, such as the nebulization catheter AeroProbe (Trudell Medical International, London, Canada), allow for pulmonary aerosol administration with a breath-controlled dosing. About 12 % of the aerosol dosed by use of this catheter device was reported to be delivered to the rat lung parenchyma [71]. However, the aerosols delivered into the lungs were mainly trapped at tracheobronchial sites owing to large mass median diameter of the droplets, which was 11 μm [71]. It is important to design nebulizers targeting deep into the lung in such a way that they administer aerosol particles having aerodynamic diameters between 1 and 5 μm, which is the optimal aerodynamic size for delivering drugs deep into the lung tissue for efficient intratracheal delivery [27].

Models of Penn-Century type intratracheal insufflator are commonly employed for pulmonary administration of dry powders. The standard chamber of the device holds dry powders up to 5 mg, and the insufflation of the powders is executed by forcing air through an actuating syringe. Air pressure and volume must be adjusted to accommodate well the respiration of small rodents having a tidal volume of less than 2 ml. In general, the insufflation of a small amount of dry powder, less than 1 mg, is difficult.

5.5.1.2 Exposure Inhalation Chambers

There are numerous reports on exposure inhalation chambers, such as whole-body, head-only, and nose-only types [65, 66]. These chambers are equipped with various types of airflow generation systems. Of these, the flow-past chamber is favorable for exposing all test animals with the same aerosol concentration to attain uniform delivery of drugs and for reducing the amount of drugs expended [72]. In general, a dust generator, a nebulizer, or a metered dose inhaler is equipped as an aerosol generator. Particle sizes and amount of inhaled particles in the test animals can be estimated from the analysis of particles in the chamber atmosphere. However, the exposure inhalation chamber system is much more expensive than intratracheal administration apparatuses. Alternatively, hand-crafted inhalation chambers based on a plastic centrifuge tube have been utilized for dry powder administration to mice and rats [32, 73].

One of the major problems in using an inhalation exposure chamber is that the actual amount of test materials deposited in the lungs cannot be determined accurately. In general, the received dose is estimated by the following equation:

$$D = C \times V_{\mathrm{m}} \times t \times \mathrm{Fr},$$

where D = dose, C = concentration of test sample, V_{m} = volume for the minute ventilation, t = exposure duration, Fr = fraction of test sample deposited or absorbed [74]. Alternatively, the dose deposited in the lungs can be determined by measuring the plasma concentration of the inhaled agent [75].

5.5.2 Pulmonary Deposition of Inhaled Particles

Evaluation of pulmonary deposition of inhaled particles provides important information regarding drug action. The amount of particles in the lungs is determined from that in the bronchoalveolar lavage fluid and in the lung homogenate of the test animals. In general, the amount of drugs deposited in the lungs by inhalation increases more rapidly with time than that administered by intravenous injection. As a result, the total amount in the lungs is greater than that by intravenous administration. However, the drug administered by inhalation disappears similarly as that by injection within 12 h [76]. The drug in the lungs is eliminated mainly by the pulmonary defense system, such as mucociliary clearance toward the upper airways and by transference into the blood. Hence, it is necessary to construct a strategy to minimize such a rapid disappearance of the deposited drug for achievement of the long-lasting effect of inhaled particles in the lung tissue.

Inhaled particles deposited in the lungs can be detected microscopically in lung sections by use of particles labeled with fluorescent probes. Fresh lung tissue is sliced to a thickness of approx. 2 mm without being embedded in paraffin and is then observed under a confocal laser scanning microscope [77]. In addition, it is worth noting that the Kawamoto method enables the preparation of whole-lung sections with a thickness of 10 μm from frozen lungs [78]. The distribution of inhaled particles in the lungs was easily determined by using such whole-lung sections [79]. In contrast, scintigraphic imaging using radioisotopes such as ^{99m}Tc is favorable for real-time monitoring of drug deposition in lung tissue in 2D or 3D without sacrificing the test animals [80]. The radiolabeling of the drug with ^{99m}Tc is conducted by mixing the two in a solvent in which the drug is insoluble but ^{99m}Tc is soluble, such as water, followed by drying of the mixture [81]. Such a labeling procedure is of importance for tracking the pulmonary deposition of drugs time-dependently and accurately.

5.5.3 Anti-TB Activity of Inhaled Particles

The efficacy of inhaled anti-TB drugs is evaluated by measuring the anti-TB activity in terms of a decrease in the number of colony-forming units (CFU) in TB animal models. As summarized in Table 5.3, various anti-TB drugs, along with various dose regimens, have been used to treat animal models of TB [37, 75, 82–85]. Among them, the guinea pig is favorable as a model animal in TB studies; because its pathogenesis in terms of the formation of granulomas and caseous necrosis regions is similar to that seen in human TB [86].

As shown in Table 5.3, a significant decrease in mycobacterial burden in the lungs seemed not to be achieved in most of the cases; even though a large amount of anti-TB drugs was delivered in a form of an inhalation formulation to the lungs [37, 75, 82–84]. The lesser effectiveness of the inhaled anti-TB drugs than expected

Table 5.3 Drugs formulated for inhalation treatment of TB and their experimental results

Drug	Carrier	Dose and repeat times	Delivery method	Animal	TB model	Drug concentration	CFU[a]	Ref
Rifampicin	PLGA	12 mg/kg and 5 mg/kg Each once	Insufflation and nebulization	Guinea pig	*Mtb* H37Rv, subacute	N.D.[b]	0.71	[37]
Rifampicin	PLGA	540 mg/kg Four times	Nebulization	Guinea pig	*Mtb* H37Rv, subacute	N.D.	N.E.[c]	[82]
Rifampicin	PLGA	300 μg/kg 14 times	Insufflation	Rat	*Mtb* Kurono, acute	N.D.	0.23	[83]
Capreomycin	Leucine	1.80 mg/kg[d] 28 times	Insufflation and inhalation	Guinea pig	*Mtb* H37Rv, chronic	C_{max}: 6.70 ± 1.26 μg/mL in plasma (at 19 min)	1.03	[84]
PA-824	Leucine, DPPC	9.7 mg/kg[d] 28 times	Inhalation	Guinea pig	*Mtb* H37Rv, chronic	C_{max}: 0.59 μg/mL in plasma (at 3 h)	0.80	[75]
Clofazimine	Leucine	28.8 mg/kg Eight times	Inhalation	Mouse	*Mtb* H37Rv, chronic	N.D.	2.61	[85]

[a]Decrease in CFU from that with no treatment (log scale)
[b]N.D. not determined
[c]N.E. not effective
[d]Calculated from plasma concentration

might have been because the inhaled anti-TB drugs had not been delivered deep enough into the lungs, where *Mtb* resides. Hence, it is necessary to develop devices that enable the delivery of an effective amount of anti-TB drug to the tuberculosis granulomatous nests, which reside deep in the lungs, as well as to prepare more efficient inhalable formulations of anti-TB drugs. Of the inhalation drugs under development, capreomycin dry powders have successfully proceeded to a phase I clinical study [43].

5.6 Conclusions

Mtbs that have invaded the lungs during respiration are easily taken up by alveolar macrophages. Hence, delivery of anti-TB drugs directly to the alveolar macrophages that have been infected with this organism is expected to be more effective in terms of anti-TB activity than their oral administration. However, such an inhalation therapy for TB has not yet been established and remains in the development stage. In order for inhaled anti-TB drugs to exert potent mycobactericidal activity, the development of inhalable formulations containing anti-TB drugs and that of devices that achieve efficient delivery of a curable amount of these formulations to the macrophages are necessary. Inhalation therapy for TB will surely contribute to radical treatment of TB including MDR/XDR TB.

References

1. Gler MT, Skripconoka V, Sanchez-Garavito E, Xiao H, Cabrera-Rivero JL, Vargas-Vasquez DE, Gao M, Awad M, Park SK, Shim TS, Suh GY, Danilovits M, Ogata H, Kurve A, Chang J, Suzuki K, Tupasi T, Koh WJ, Seaworth B, Geiter LJ, Wells CD (2012) Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med 366:2151–2160
2. Tasneen R, Li SY, Peloquin CA, Taylor D, Williams KN, Andries K, Mdluli KE, Nuermberger EL (2011) Sterilizing activity of novel TMC207- and PA-824-containing regimens in a murine model of tuberculosis. Antimicrob Agents Chemother 55:5485–5492
3. Global Tuberculosis Report (2012) World Health Organization. http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf. Accessed 15 Apr 2013
4. Barnes PF, Cave MD (2003) Molecular epidemiology of tuberculosis. N Engl J Med 349: 1149–1156
5. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C (2003) Tuberculosis. Lancet 362: 887–899
6. HIV/TB Facts (2011) World Health Organization. http://www.who.int/hiv/topics/tb/hiv_tb_factsheet_june_2011.pdf. Accessed 15 Apr 2013
7. Armstrong JA, Hart PD (1971) Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. J Exp Med 134:713–740
8. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG (1994) Lack of acidification in mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science 263:678–681
9. Ferrari G, Langen H, Naito M, Pieters J (1999) A coat protein on phagosomes involved in the intracellular survival of mycobacteria. Cell 97:435–447

10. Manca C, Paul S, Barry CE 3rd, Freedman VH, Kaplan G (1999) Mycobacterium tuberculosis catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. Infect Immun 67:74–79
11. Voskuil MI, Bartek IL, Visconti K, Schoolnik GK (2011) The response of mycobacterium tuberculosis to reactive oxygen and nitrogen species. Front Microbiol 2:105
12. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F (2009) Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol 10:943–948
13. Russell DG, Barry CE 3rd, Flynn JL (2010) Tuberculosis: what we don't know can, and does, hurt us. Science 328:852–856
14. Ramakrishnan L (2012) Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol 12:352–366
15. Kim MJ, Wainwright HC, Locketz M, Bekker LG, Walther GB, Dittrich C, Visser A, Wang W, Hsu FF, Wiehart U, Tsenova L, Kaplan G, Russell DG (2010) Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. EMBO Mol Med 2:258–274
16. Lee W, VanderVen BC, Fahey RJ, Russell DG (2013) Intracellular Mycobacterium tuberculosis exploits host-derived fatty acids to limit metabolic stress. J Biol Chem 288:6788–6800
17. Eum SY, Kong JH, Hong MS, Lee YJ, Kim JH, Hwang SH, Cho SN, Via LE, Barry CE 3rd (2010) Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. Chest 137:122–128
18. Migliori GB, Hopewell PC, Blasi F, Spanevello A, Raviglione MC (2006) Improving the TB case management: the international standards for tuberculosis care. Eur Respir J 28:687–690
19. Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH (2004) Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. Am J Respir Crit Care Med 170: 1131–1134
20. Shargie EB, Lindtjorn B (2005) DOTS improves treatment outcomes and service coverage for tuberculosis in South Ethiopia: a retrospective trend analysis. BMC Public Health 5:62
21. Gordon S, Rabinowitz S (1989) Macrophages as targets for drug delivery. Adv Drug Deliv Rev 4:27–47
22. Sacks LV, Pendle S, Orlovic D, Andre M, Popara M, Moore G, Thonell L, Hurwitz S (2001) Adjunctive salvage therapy with inhaled aminoglycosides for patients with persistent smear-positive pulmonary tuberculosis. Clin Infect Dis 32:44–49
23. Roy CJ, Sivasubramani SK, Dutta NK, Mehra S, Golden NA, Killeen S, Talton JD, Hammoud BE, Didier PJ, Kaushal D (2012) Aerosolized gentamicin reduces the burden of tuberculosis in a murine model. Antimicrob Agents Chemother 56:883–886
24. Hirota K, Hasegawa T, Hinata H, Ito F, Inagawa H, Kochi C, Soma G, Makino K, Terada H (2007) Optimum conditions for efficient phagocytosis of rifampicin-loaded PLGA microspheres by alveolar macrophages. J Control Release 119:69–76
25. Weibel ER (1963) Morphometry of the human lung. Springer, Berlin
26. Hickey AJ (2002) Delivery of drugs by the pulmonary route. In: Banker GS, Rhodes CT (eds) Modern pharmaceutics. Marcel Dekker, New York
27. Gonda I (1981) A semi-empirical model of aerosol deposition in the human respiratory tract for mouth inhalation. J Pharm Pharmacol 33:692–696
28. Byron PR (1986) Some future perspectives for unit dose inhalation aerosols. Drug Dev Ind Pharm 12:993–1015
29. Batycky RP, Hanes J, Langer R, Edwards DA (1997) A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. J Pharm Sci 86: 1464–1477
30. O'Hara P, Hickey AJ (2000) Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. Pharm Res 17:955–961
31. Makino K, Nakajima T, Shikamura M, Ito F, Ando S, Kochi C, Inagawa H, Soma G, Terada H (2004) Efficient intracellular delivery of rifampicin to alveolar macrophages using rifampicin-loaded PLGA microspheres: effects of molecular weight and composition of PLGA on release of rifampicin. Colloids Surf B Biointerfaces 36:35–42

32. Sharma R, Saxena D, Dwivedi AK, Misra A (2001) Inhalable microparticles containing drug combinations to target alveolar macrophages for treatment of pulmonary tuberculosis. Pharm Res 18:1405–1410
33. Ito F, Makino K (2004) Preparation and properties of monodispersed rifampicin-loaded poly(lactide-co-glycolide) microspheres. Colloids Surf B Biointerfaces 39:17–21
34. Atkins PJ (2005) Dry powder inhalers: an overview. Respir Care 50:1304–1312
35. Owens DR, Zinman B, Bolli G (2003) Alternative routes of insulin delivery. Diabet Med 20:886–898
36. Suarez S, O'Hara P, Kazantseva M, Newcomer CE, Hopfer R, McMurray DN, Hickey AJ (2001) Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model. Pharm Res 18:1315–1319
37. Suarez S, O'Hara P, Kazantseva M, Newcomer CE, Hopfer R, McMurray DN, Hickey AJ (2001) Airways delivery of rifampicin microparticles for the treatment of tuberculosis. J Antimicrob Chemother 48:431–434
38. Tomoda K, Kojima S, Kajimoto M, Watanabe D, Nakajima T, Makino K (2005) Effects of pulmonary surfactant system on rifampicin release from rifampicin-loaded PLGA microspheres. Colloids Surf B Biointerfaces 45:1–6
39. Muttil P, Kaur J, Kumar K, Yadav AB, Sharma R, Misra A (2007) Inhalable microparticles containing large payload of anti-tuberculosis drugs. Eur J Pharm Sci 32:140–150
40. Sung JC, Garcia-Contreras L, Verberkmoes JL, Peloquin CA, Elbert KJ, Hickey AJ, Edwards DA (2009) Dry powder nitroimidazopyran antibiotic PA-824 aerosol for inhalation. Antimicrob Agents Chemother 53:1338–1343
41. Sharma R, Muttil P, Yadav AB, Rath SK, Bajpai VK, Mani U, Misra A (2007) Uptake of inhalable microparticles affects defence responses of macrophages infected with *Mycobacterium tuberculosis* H37Ra. J Antimicrob Chemother 59:499–506
42. Fiegel J, Garcia-Contreras L, Thomas M, VerBerkmoes J, Elbert K, Hickey A, Edwards D (2008) Preparation and in vivo evaluation of a dry powder for inhalation of capreomycin. Pharm Res 25:805–811
43. Dharmadhikari AS, Kabadi M, Gerety B, Hickey AJ, Fourie PB, Nardell E (2013) Phase I, single-dose, dose-escalating study of inhaled dry powder capreomycin: a new approach to therapy of drug-resistant tuberculosis. Antimicrob Agents Chemother 57:2613–2619
44. Wong YL, Sampson S, Germishuizen WA, Goonesekera S, Caponetti G, Sadoff J, Bloom BR, Edwards D (2007) Drying a tuberculosis vaccine without freezing. Proc Natl Acad Sci U S A 104:2591–2595
45. Rogers TL, Johnston KP, Williams RO (2001) Solution-based particle formation of pharmaceutical powders by supercritical or compressed fluid CO_2 and cryogenic spray-freezing technologies. Drug Dev Ind Pharm 27:1003–1015
46. Yu ZS, Rogers TL, Hu JH, Johnston KP, Williams RO (2002) Preparation and characterization of microparticles containing peptide produced by a novel process: spray freezing into liquid. Eur J Pharm Biopharm 54:221–228
47. Meers P, Neville M, Malinin V, Scotto AW, Sardaryan G, Kurumunda R, Mackinson C, James G, Fisher S, Perkins WR (2008) Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. J Antimicrob Chemother 61:859–868
48. Vyas SP, Kannan ME, Jain S, Mishra V, Singh P (2004) Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. Int J Pharm 269:37–49
49. Fujiwara T, Konishi M, Chida S, Okuyama K, Ogawa Y, Takeuchi Y, Nishida H, Kito H, Fujimura M, Nakamura H, Hashimoto T, Surfactant-TA Study Group (1990) Surfactant replacement therapy with a single postventilatory dose of a reconstituted bovine surfactant in preterm neonates with respiratory distress syndrome: final analysis of a multicenter, double-blind, randomized trial and comparison with similar trials. Pediatrics 86:753–764
50. Pandey R, Khuller GK (2005) Antitubercular inhaled therapy: opportunities, progress and challenges. J Antimicrob Chemother 55:430–435
51. Aderem A, Underhill DM (1999) Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 17:593–623

52. Conner SD, Schmid SL (2003) Regulated portals of entry into the cell. Nature 422:37–44
53. Hansen CG, Nichols BJ (2009) Molecular mechanisms of clathrin-independent endocytosis. J Cell Sci 122:1713–1721
54. Sahay G, Alakhova DY, Kabanov AV (2010) Endocytosis of nanomedicines. J Control Release 145:182–195
55. Swanson JA, Watts C (1995) Macropinocytosis. Trends Cell Biol 5:424–428
56. Tamaru M, Akita H, Fujiwara T, Kajimoto K, Harashima H (2010) Leptin-derived peptide, a targeting ligand for mouse brain-derived endothelial cells via macropinocytosis. Biochem Biophys Res Commun 394:587–592
57. Bhattacharya S, Roxbury D, Gong X, Mukhopadhyay D, Jagota A (2012) DNA conjugated SWCNTs enter endothelial cells via Rac1 mediated macropinocytosis. Nano Lett 12:1826–1830
58. Mukhopadhyay S, Herre J, Brown GD, Gordon S (2004) The potential for toll-like receptors to collaborate with other innate immune receptors. Immunology 112:521–530
59. Prescribing information. Janssen Products, LP, http://www.doxil.com/shared/product/doxil/prescribing-information.pdf. Accessed 6 Sept 2013
60. Dams ET, Laverman P, Oyen WJ, Storm G, Scherphof GL, van Der Meer JW, Corstens FH, Boerman OC (2000) Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. J Pharmacol Exp Ther 292:1071–1079
61. Ishida T, Ichihara M, Wang X, Yamamoto K, Kimura J, Majima E, Kiwada H (2006) Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes. J Control Release 112:15–25
62. Ishida T, Masuda K, Ichikawa T, Ichihara M, Irimura K, Kiwada H (2003) Accelerated clearance of a second injection of PEGylated liposomes in mice. Int J Pharm 255:167–174
63. Hirota K, Hasegawa T, Nakajima T, Inagawa H, Kohchi C, Soma G, Makino K, Terada H (2010) Delivery of rifampicin-PLGA microspheres into alveolar macrophages is promising for treatment of tuberculosis. J Control Release 142:339–346
64. Hirota K, Terada H (2012) Endocytosis of particle formulations by macrophages and its application to clinical treatment. In: Ceresa B (ed) Molecular regulation of endocytosis. InTech, Rijeka
65. Phalen RF (1976) Inhalation exposure of animals. Environ Health Perspect 16:17–24
66. Dorato MA (1990) Overview of inhalation toxicology. Environ Health Perspect 85:163–170
67. Warheit DB, Carakostas MC, Hartsky MA, Hansen JF (1991) Development of a short-term inhalation bioassay to assess pulmonary toxicity of inhaled particles: comparisons of pulmonary responses to carbonyl iron and silica. Toxicol Appl Pharmacol 107:350–368
68. Costa DL, Lehmann JR, Harold WM, Drew RT (1986) Transoral tracheal intubation of rodents using a fiberoptic laryngoscope. Lab Anim Sci 36:256–261
69. Remie R, Bertens APMG, van Dongen JJ, Rensema JW, van Wunnik GHJ (1990) Anaesthesia of the laboratory rat. In: van Dongen JJ, Remie R, Rensema JW, van Wunnik GHJ (eds) Manual of microsurgery on the laboratory rat, part I. Elsevier Science Publishers, Amsterdam
70. Bivas-Benita M, Zwier R, Junginger HE, Borchard G (2005) Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. Eur J Pharm Biopharm 61:214–218
71. Tronde A, Baran G, Eirefelt S, Lennernäs H, Bengtsson UH (2002) Miniaturized nebulization catheters: a new approach for delivery of defined aerosol doses to the rat lung. J Aerosol Med 15:283–296
72. Cannon WC, Blanton EF, McDonald KE (1983) The flow-past chamber: an improved nose-only exposure system for rodents. Am Ind Hyg Assoc J 44:923–928
73. Kaur J, Muttil P, Verma RK, Kumar K, Yadav AB, Sharma R, Misra A (2008) 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 34:56–65
74. Wong BA (2007) Inhalation exposure systems: design, methods and operation. Toxicol Pathol 35:3–14
75. Garcia-Contreras L, Sung JC, Muttil P, Padilla D, Telko M, Verberkmoes JL, Elbert KJ, Hickey AJ, Edwards DA (2010) Dry powder PA-824 aerosols for treatment of tuberculosis in guinea pigs. Antimicrob Agents Chemother 54:1436–1442

76. Verma RK, Kaur J, Kumar K, Yadav AB, Misra A (2008) Intracellular time course, pharmacokinetics, and biodistribution of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to mice. Antimicrob Agents Chemother 52:3195–3201
77. Lombry C, Bosquillon C, Preat V, Vanbever R (2002) Confocal imaging of rat lungs following intratracheal delivery of dry powders or solutions of fluorescent probes. J Control Release 83:331–341
78. Kawamoto T (2003) Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. Arch Histol Cytol 66:123–143
79. Hirota K, Kawamoto T, Nakajima T, Makino K, Terada H (2013) Distribution and deposition of respirable PLGA microspheres in lung alveoli. Colloids Surf B Biointerfaces 105:92–97
80. Dolovich M, Labiris R (2004) Imaging drug delivery and drug responses in the lung. Proc Am Thorac Soc 1:329–337
81. Pitcairn GR, Newman SP (1998) Radiolabellling of dry powder formulations. In: Dalby RN, Byron PR, Farr SJ (eds) Respiratory drug delivery VI. Interpharm Press, Buffalo Grove
82. Garcia-Contreras L, Sethuraman V, Kazantseva M, Godfrey V, Hickey AJ (2006) Evaluation of dosing regimen of respirable rifampicin biodegradable microspheres in the treatment of tuberculosis in the guinea pig. J Antimicrob Chemother 58:980–986
83. Yoshida A, Matumoto M, Hshizume H, Oba Y, Tomishige T, Inagawa H, Kohchi C, Hino M, Ito F, Tomoda K, Nakajima T, Makino K, Terada H, Hori H, Soma G (2006) 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 8:2484–2491
84. Garcia-Contreras L, Fiegel J, Telko MJ, Elbert K, Hawi A, Thomas M, VerBerkmoes J, Germishuizen WA, Fourie PB, Hickey AJ, Edwards D (2007) Inhaled large porous particles of capreomycin for treatment of tuberculosis in a guinea pig model. Antimicrob Agents Chemother 51:2830–2836
85. Verma RK, Germishuizen WA, Motheo MP, Agrawal AK, Singh AK, Mohan M, Gupta P, Gupta UD, Cholo M, Anderson R, Fourie PB, Misra A (2013) Inhaled microparticles containing clofazimine are efficacious in treatment of experimental tuberculosis in mice. Antimicrob Agents Chemother 57:1050–1052
86. Helke KL, Mankowski JL, Manabe YC (2006) Animal models of cavitation in pulmonary tuberculosis. Tuberculosis (Edinb) 86:337–348