

Inhalational Drug Delivery in Pulmonary Aspergillosis

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ABSTRACT: Pulmonary infections have long represented one of the major threats to humans. These vary from acute to chronic conditions, depending upon the underlying disease of the airways. Pulmonary aspergillosis (PMA) has raised vital concerns in the immunocompromised patients. The fungal infection is difficult to diagnose in the early stages, often making the disease more complicated. Currently, three classes of antifungal agents are available on the market for the treatment of pulmonary infections. These agents are available in oral and intravenous forms only, which limits the availability of therapeutic concentrations of drug in the lungs for longer durations. Consequently, this leads to therapeutic failure and/or resistance of the organism(s) towards the antifungal agents because the optimum amount of drug does not reach the infection site. To combat the issues associated with the conventional regimens, inhalation of antifungal agents is gaining importance because administration to the lungs offers huge advantages of localized and targeted delivery. A wide range of inhalational devices such as nebulizers, dry powder inhalers, and metered dose inhalers are available on the market to deliver drug molecules to the lungs effectively. However, their clinical utility is limited to conditions such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis only. For a few decades, inhalation therapy has also been gaining importance to treat infectious diseases such as tuberculosis and aspergillosis, though more research efforts are required to make the transition from bench to bedside. The current review provides an explicit account of the potential role of inhalation drug delivery in PMA.

KEY WORDS: invasive aspergillosis, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis, aerosol therapy, nebulization, dry powder inhalers, metered dose inhalers, antifungal agents

I. INTRODUCTION

Respiratory tract infections are one of the most common infective disorders encountered during inpatient and outpatient clinical care. Each year, respiratory diseases account for

one-quarter of all deaths worldwide.¹ These vary from acute infections such as pneumonia, aspergillosis, and bronchitis to chronic conditions such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF).² The infections are primarily caused by a variety of microorganisms such as bacteria, fungi, viruses, etc., residing deep inside the airways. The treatment regimen includes the usage of various antimicrobials depending upon the cause and severity of the underlying infection.

Over the past few decades, one type of respiratory infection that has raised major concern in public healthcare is pulmonary aspergillosis (PMA), a fungal lung infection. The epidemic of PMA has markedly increased because of the increased number of patients with impaired immune function associated with HIV, prolonged neutropenia, intensive chemotherapy, solid organ transplantations, autoimmune diseases, asthma, COPD, etc.^{3,4} Established infection in this patient population becomes difficult to eradicate despite the noteworthy advancements in antifungal therapy and diagnostic techniques.⁵ The mortality rate associated with aspergillus infection ranges from 50% to 85% in populations at high risk and is dependent on factors such as type and site of infection, immune status of the host, and the therapy applied.⁶ Newer strategies with discernible potential are therefore urgently needed for the prevention and management of PMA.⁷

PMA is caused by a ubiquitous fungus, *Aspergillus*, known for causing both invasive and non-invasive infections. Although several studies have reported the effects of *Aspergillus* infection on different organs, the most affected organs are the lungs.⁸ In 1729, Micheli named and described the genus *Aspergillus* for the first time. This mold is found in the soil, water, air, and decaying organic matter. There are approximately 200 species of *Aspergillus*, but four major ones are responsible for causing most of the infections: *Aspergillus fumigatus* (66%) is the most prominent one, followed by *Aspergillus flavus* (14%), *Aspergillus niger* (7%), *Aspergillus terreus* (4%), and others (9%).⁵

The clinical manifestations associated with PMA can vary depending upon the interaction of fungus with the host immune system.⁵ The spectrum of PMA can range from non-invasive conditions such as aspergilloma (AG) and allergic bronchopulmonary aspergillosis (ABPA) to invasive conditions such as chronic necrotizing aspergillosis (CNA) and, the most threatening, invasive aspergillosis (IA).⁹ AG is characterized by a fungus ball growth due to colonization of fungal hyphae amalgamated with mucus, inflammatory cells, and cellular debris within the lungs. It usually develops within the pre-existing lung cavities primarily associated with tuberculosis. The fungus ball may move inside the cavity without invading the surrounding lung tissues or blood vessels. ABPA is a hypersensitive response generated due to inhalation of *Aspergillus* antigens. Generally, patients with conditions such as asthma, CF, and atopy are at higher risk of ABPA. Immunoglobulin E (IgE)- and IgG-mediated hypersensitivity reactions are most frequently observed in patients with ABPA. Patients usually present with symptoms of wheezing, chest pain, sputum with brown plugs, and fever.¹⁰ CNA is a semi-invasive aspergillosis in which a pre-existing cavity may or may not be present, but is generally formed in time. At times, it becomes difficult to distinguish between CNA and AG because both result in cavities. Some researchers put them in common group of chronic cavitary PMA. However, in patients with CNA, local parenchyma destruction is also

observed.^{11,12} IA is the most devastating form of aspergillosis, in which hyphae disseminates into systemic circulation, affecting other organs too. Incidence of IPA is reported to exceed 50% in patients with neutropenia¹³ and to around 90% in hematopoietic stem-cell transplantation (HSCT) patients.¹⁴ The spectrum of IA is expanding, not only in immunosuppressed patients, but also in critically ill patients without risk factors of neutropenia, leukemia, and HSCT. It is one of the major causes of morbidity and mortality among PMAP.¹⁵ Table 1 demonstrates the risk factors and treatment regimens throughout the spectrum of *Aspergillus* infection.

Early diagnosis and proper treatment regime is critical in PMAP infection, which can otherwise result in life-threatening conditions. The introduction of newer techniques for the early detection of radiological and pathological findings, along with the usage of better-tolerated antifungal drugs, can reduce the mortality rates associated with the disease.^{4,21}

The current treatment options available for PMAP are limited to antifungal drugs administered by oral and intravenous routes only. The therapy lasts from weeks to years, depending upon the type of PMAP,²² and is often associated with off-target side effects such as nephrotoxicity, hepatotoxicity, and visual disturbances. Moreover, fungal spores affecting deep airways are difficult to treat because the optimum concentration of antifungal is not available at the site of infection because of conditions such as poor solubility, permeability, and rapid clearance of drug due to high blood flow turnover in the tissue. As a consequence, high doses of drugs are required by conventional routes of administration to maintain drug levels above their minimum inhibitory concentrations (MICs) at the infection sites, which could cause systemic toxicity problems.²³ Therefore, the immediate need is to develop highly effective therapy that will provide localized action, minimize dosing frequency, and prevent off-target side effects. This can be achieved by localizing the drug in the lungs through inhalation route. Many reports from the literature have evidenced the usage of inhalation in effective prevention and management of PMAP.^{23,24} The research data demonstrate achievement of high drug concentrations at the infection site through aerosolized drug delivery.

TABLE 1: Types of pulmonary aspergillosis

Disorder	Treatment	Risk-Prone Factors	Reference(s)
AG	Surgical resection	Old cavitary diseases such as tuberculosis, bronchiectasis, sarcoidosis, CF, carcinoma, etc.	16
ABPA	Corticosteroids alone or in combination with antifungal drugs	Asthma, COPD, CF	17
CNA	Antifungal drugs	CF, COPD, pulmonary infarction, pulmonary resection, radiation-induced pulmonary fibrosis.	18
IA	Antifungal drugs	HIV, prolonged neutropenia, organ transplantations	19,20

Use of nanotechnology-enabled systems has been gaining significant importance in delivering antifungals through inhalation route because these offer distinct advantages of improved solubility, permeability, and dissolution rate profile. Furthermore, these nanocarriers have the ability to minimize premature mucociliary clearance and improve internalization into the cells.²⁵ A diverse range of drug delivery systems has been explored for administering drug to the lungs. The choice of delivery system depends upon the physicochemical properties of the drug and the type of inhalation device used.

Lipid-based colloidal drug delivery systems, liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), provide an additional benefit due to the presence of physiological components such as phospholipids that play major role in regular breathing. Liposomes are the most widely studied systems for inhalation of the drugs because they exhibit chemical similarity with the lung surfactant (80% phospholipids, primarily dipalmitoylphosphatidylcholine [DPPC]) that allows rapid uptake by epithelial cells.²⁶ The first liposomal product, Alveofact[®] (a purified bovine surfactant), was introduced for pulmonary instillation in 1990s for the management of acute respiratory distress syndrome in infants.²⁷ Later, amphotericin B-loaded liposomes were introduced for parenteral administration (Ambisome[®]), though it is often given as off-label nebulization to manage non-CF bronchiectasis.²⁸ SLNs are suspensions of physiological lipids, mainly phospholipids and triglycerides, dispersed in an aqueous surfactant solution. These offer unique advantages of small size, large surface area, and low toxicity due to presence of physiological lipids.²⁷ NLCs are composed of liquid lipid in addition to solid lipid generally in the ratio of 30:70. Improved drug loading and reduced drug exclusion are notable advantages of NLCs over SLNs.²⁹ Conversely, to improve the release rate of drug in the respiratory tract, biodegradable polymeric nanoparticles have been gaining immense importance for decades. Polymers are macromolecular systems that are composed of repetitive units of monomers. Mechanistically polymers act by diffusion and swelling, followed by diffusion and degradation. To modify the release of drugs following pulmonary administration, various polymers, both natural and synthetic, can be employed. Among natural polymers, the most widely studied for pulmonary application are chitosan, albumin, and hyaluronic acid; synthetic polymers explored for pulmonary delivery include poly (lactic-co-glycolic acid), poly (lactic acid), and poly (ethylene glycol).^{30,31} We briefly discuss the literature on these later in this review. The main scope of the present review is to highlight the role of inhalational drug delivery in PMAP and to discuss the major key findings in industrial and academic research across the world.

II. PATHOGENESIS

The earliest mechanistic events that occur during the invasion of *A. fumigatus* and the role of diagnostic markers in the identification of fungus are not clearly stated in the literature. However, to gain deeper insights into it, various efforts have been undertaken in recent years to sequence the genomes of *Aspergillus* species and to reveal the immune mechanisms involved therein.³²

Aspergillus produce small, hydrophobic conidia that get easily dispersed into the air.³³ The conidia of *A. fumigatus* account for less than 1% of all air-borne conidio spores. Albeit less common, its unique virulent characteristics have made the species far more devastating. The primary port through which fungus enters the host is through inhalation of contaminated airborne conidia. The size of conidiospores (3–5 μm) is the major trait that drives entry deep inside the airways because the cut-off range for particles to reach alveoli is 5 μm . Moreover, because of the ability of *A. fumigatus* to bind with extracellular proteins such as laminin and fibrinogen, this probably allows better adhesion in the airways and ultimately its colonization and dissemination in the host.³⁴

Both conducting and respiratory airways play a significant role in the eradication of airborne conidia from the host. In normal physiological conditions, mucociliary clearance is the first line of defense that removes the conidia from entering the lungs. The conidia that are not removed by this mechanism are encountered by the alveolar macrophages (AM), which play a key role in phagocytosis and killing of the fungus. If conidia germinate within the AM and result in hyphae growth, these become the target of pro-inflammatory mediators that ultimately destroy them. Moreover, the presence of pathogen recognition receptors such as toll-like receptors, dectin-1, and mannose-binding lectins recognize specific fungal wall components and produce cytokines that further stimulate neutrophils recruitment, the major defense against *Aspergillus* hyphae, thus preventing the plausible fungal infection.³⁵

In the impaired immune response, the conidia may get localized within the lungs or may disseminate into other organs, evading the normal defense mechanisms of the host, as shown in Fig. 1. The primary events occur in the alveolus, where the conidia germinate into hyphae that invade the respiratory epithelium and may get disseminated into the systemic circulation, leading eventually to invasive fungal infection. The innate pulmonary immunity compromises the inhabitant and recruited phagocytes, soluble immune factors, and antimicrobial proteins. Proteases, adhesins, siderophores, pigments, and mycotoxins are some of the virulent factors of *Aspergillus* associated with depressed immunity and form the basis of infection.^{35,36}

Generally, *Aspergillus* is introduced to the lower respiratory tract, but it may also affect other organs such as the brain, which can lead to seizures, cerebral infarctions, hemorrhage, meningitis, and epidural abscesses. Other less commonly involved organs are the skin, kidneys, heart, esophagus, and liver.^{12,37}

III. CONVENTIONAL THERAPY

Antifungals are the mainstay treatment for eradication of the fungus from the airways. The duration of therapy depends upon the condition of host. The drugs approved by United States Food and Drug Administration (FDA) for the management of PMAP include deoxycholate amphotericin B, liposomal amphotericin B, voriconazole (VRC), itraconazole (ITZ), posaconazole, and caspofungin.²⁰

Initially, amphotericin B (AmB) was considered as the gold standard drug, but the development of newer triazoles such as VRC and ITZ limited its usage because the for-

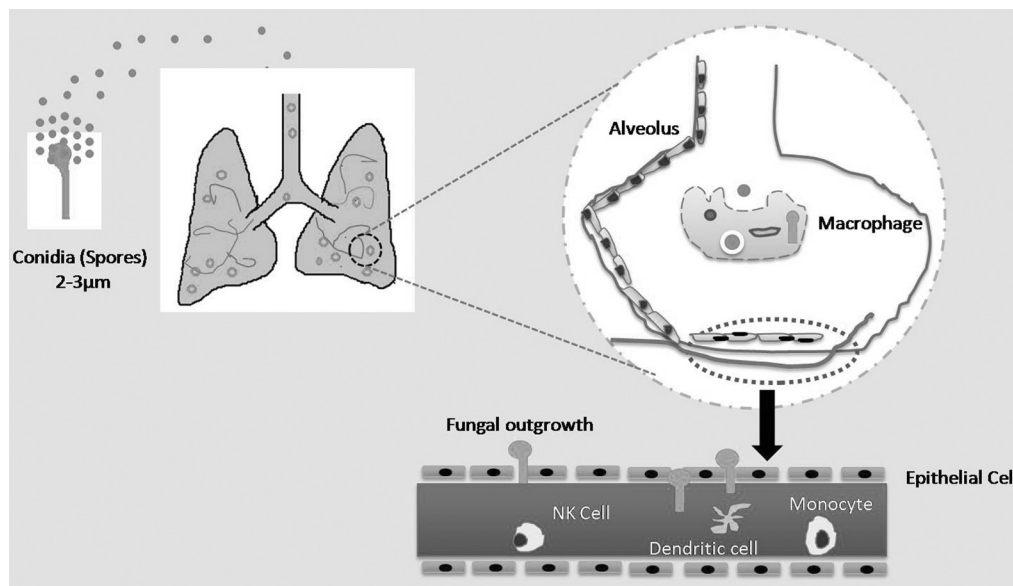


FIG. 1: Mechanistic pathways involved in PMAP. Inhalation of contaminated airborne conidia results into conidial entry into the lungs. Within the lungs, different defense pathways come into play, including mucociliary clearance, phagocytosis, and the release of inflammatory mediators such as neutrophils. Suppression of these will result in the formation of hyphae and ultimate dissemination of disease.

mer is associated with a high risk of nephrotoxicity. To combat the issues of nephrotoxicity, the researchers have formulated lipidic formulations of the same. Few trials have also shown that liposomal AmB can be considered as equivalent to VRC in eradication of infection. However, in a majority of trials, VRC is considered as the standard therapy for the treatment of IA.³⁸

Posaconazole, a new member of azole class, possesses fewer side effects than other triazoles and is the only drug approved for the prophylaxis as well as for treatment.³⁹ Another novel class with different mechanisms and lesser side effects are the echinocandins caspofungin, micafungin, and anidulafungin. Nonetheless, micafungin and anidulafungin are not licensed in the United States for the treatment of IA. This newer class has the potential to be used in combination with the available antifungal regimes.^{19,40} Caspofungin and VRC combinations have shown improved results in preclinical models because they target different cellular sites such as the fungal cell wall and the membrane. To establish the usefulness of these substances in the management of aspergillosis, further clinical trials are needed.⁴¹ A detailed description of antifungal drugs, along with their mechanism of action, is presented in Table 2.

IV. CURRENT CHALLENGES

Rising occurrence of aspergillosis is unlikely to diminish primarily due to the increasing number of immunocompromised and fungus-resistant cases. The currently available

TABLE 2: An overview of various drugs available for treatment and management of PMAP

Class	Mechanism(s)	Drug	Standard Dosage	Route(s) of Administration	Side Effect(s)	Reference(s)
Polyenes	Binding to ergosterol, the main sterol of cell membrane; ion channels are formed that lead to fungal cell death	Amphotericin B	L-AMB: (3–5 mg/kg/d IV); ABLC: (5 mg/kg/d IV)	Intravenous	Nephrotoxicity	42,43
Azoles	Inhibiting fungal cytochrome P450-dependent enzyme lanosterol 14- α -demethylase, altering cell membrane functions or cell death	VRC	6 mg/kg IV b.i.d. for 1 d, followed by 4 mg/kg IV b.i.d.; oral dosage is 200 mg b.i.d.	Oral and intravenous	Hepatotoxicity, visual disturbances, skin rashes	44,45
		ITZ	200 mg IV b.i.d. for 2 d, followed by 200 mg IV q.d. or 200 mg PO b.i.d.	Oral and intravenous	Gastrointestinal intolerance	46,47
		Posaconazole	Initially, 200 mg q.i.d., then 400 mg PO b.i.d.	Oral and intravenous	Diarrhea, hypokalemia, fever, and nausea	48,49
Echinocandins	Inhibiting the synthesis of 1,3- β -glucan, a polysaccharide in the cell wall of many pathogenic fungi	Capsofungin	70 mg IV on day one and thereafter 50 mg/d IV	Intravenous	Severe allergic reactions, dizziness, irregular heartbeat, fever, chills	50,51
		Micafungin	100–150 mg/d IV	Intravenous	Nausea, vomiting, diarrhea, flushing	52,53

*ABLC: Amphotericin B lipid complex

weapons to battle the war against PMAP limit its use because of efficacy and safety concerns. The available therapy is administered by oral and intravenous route, thus making it difficult to maintain optimum concentration of antifungal at the infection site. To circumnavigate these issues, the currently combination therapy and/or high doses of drugs are administered to maintain optimum levels of drugs in the lungs. Consequently, this often results to off-target side effects such as nephrotoxicity and hepatotoxicity that invariably cause poor patient compliance and therapeutic failure of the treatment regimen. It sometimes becomes difficult to distinguish between the mortality associated with aspergillosis and that due to the presence of comorbidities.⁵⁴

Furthermore, only three classes of drugs are available on the market. The choice is quite limited compared with treatments for bacterial infections. In the 1980s, two of the classes, polyenes and azoles, entered the market.⁵⁵ The third class, echinocandins, was discovered in the 1970s, but took approximately 30 years to enter the market. The small number of antifungals would not be a problem if the patient's needs were met. However, even with the introduction of the newer class of antifungals, the clinical outcomes are far from ideal. The development pace of newer antifungals is not keeping up with emerging cases of aspergillosis infection.²²

In addition to limited treatment options, resistance cases are also emerging against the current antifungal agents. The factor that may contribute to the development of resistance includes frequency of dosage, fungistatic nature of azoles, drug levels below MIC, and non-compliance to the treatment regimen. In addition, the immune status of the host and other disease conditions may also contribute to the development of resistance against fungus.⁵⁶ Even the diagnosis of the disease is often difficult in the early stages because of the lack of sensitivity and specificity in the existing analytical techniques.⁵⁷

The current need is to develop new molecules that will effectively and specifically target and eradicate the fungus from the host. However, discovery of new molecules is a formidable task because of scientific, economic, and regulatory challenges, including the high cost, time, and money involved.⁵⁸ Therefore, another alternative that is gaining importance in the current era for the treatment of respiratory infections is the use of aerosol drug delivery, which targets the lungs directly and may help in the eradication of the microorganism(s) in a more effective manner than conventional routes of administration. Many of the antibiotics have been approved by the FDA for the treatment of microbial lung infections, including tobramycin (1997) and aztreonam (2010) for management of CF with *Pseudomonas* infection. Moreover, injectable forms of antibiotics such as gentamicin, amikacin, ceftazidime, and AmB are currently administered as “off-label” nebulizers for the management of non-CF bronchiectasis, ventilator-associated pneumonia, and other post-transplantation respiratory infections.^{28,59} The route has been explored for other antifungals apart from AmB, but the literature includes case reports and case studies only. Currently, no antifungal has been approved for aerosol administration through the inhalation route due to a lack of clinical evidence on pulmonary efficacy and safety. More efforts and better strategies are needed to explore the unhidden potential of aerosol drug delivery in the prevention and management of PMAP.

V. AEROSOL DRUG DELIVERY

Drug delivery through aerosols is one of the oldest methods in healthcare, dating back to approximately 4000 years ago.⁶⁰ It has been historically practiced by primeval people to suppress cough by smoking the leaves of *Atropabelladonna*. Medications have also been incorporated into boiling water to allow inhalation for the cure of common infections. Also, artifacts displaced in the museums of countries such as Egypt, India, Greece, and China evidence the practice of inhalation by the ancient civilizations.⁶¹

In the 20th century, aerosols became the first-line therapy for the management of respiratory diseases such as asthma, COPD, and CF.⁶² Delivery of therapeutic agents through aerosols offers numerous advantages such as rapid onset of action because of large surface area, reduced dosage amount, local action, avoidance of first-pass effect, and gastrointestinal problems such as poor solubility, gut irritability, and unwanted side effects due to poor targetability.^{63,64} Over the last few decades, aerosols have been used for systemic disorders such as diabetes.⁶⁵ One example is Afrezza (ManKind Corp./Sanofi-Aventis US), an insulin inhalation powder approved by the FDA in 2014 for the management of type 1 and type 2 diabetes.⁶⁶

The number of aerosol drug-delivery devices has increased significantly over the last decades. Three platforms are available to deliver aerosols, nebulizers, pressurized metered-dose inhalers (pMDIs), and dry powder inhalers (DPIs).⁶⁷ These devices differ in terms of dispersion mechanism (active or passive), type of formulation (solution or dry powder), and the units of dose these contain (single dose or multiple dose). An aerosol is known to deliver the product in the form of spray or mist, which may be made up of small, medium, or large droplets or particles. Depending upon the droplet or particle size, the aerosols get deposited in different regions of the lungs. The mechanism of deposition includes gravitational sedimentation (deposition of particles due the effect of gravity), inertial impaction (deposition of large aerosol particles on the walls of the airways), and diffusion (movement of particles from higher concentration to a region of low concentration due to Brownian motion).⁶⁸ Nevertheless, the deposition of drug in different regions of the lungs is mainly dependent on the aerodynamic particle size, as outlined in Fig. 2.

The terms mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and fine particle fraction (FPF) are used frequently in regard to aerosols. MMAD defines the particle population on the basis of mass, the average diameter at which 50% of the particles by mass are larger and 50% are smaller. GSD measures the spreadability of the aerodynamic particle size distribution and FPF is the fraction of emitted particles that are less than 5 μm .⁶⁹

For successful pulmonary delivery, apart from the formulation characteristics, a harmonic coordination is required between the patient and the inhaler device. Lack of any of these will result in poor therapeutic response and ultimately poor patient compliance. As already stated, three types of inhalers are available on the marketplace to deliver aerosols, nebulizers, pMDIs, and DPIs. Millions of people depend upon these masterpieces to live a normal life. Each device has its own unique combination of design and formulation. Devices are designed to deliver the precise dose with particles in size range of less than 5 μm .^{61,70} Figure 3 shows some of the commercially available inhalers.⁶⁷

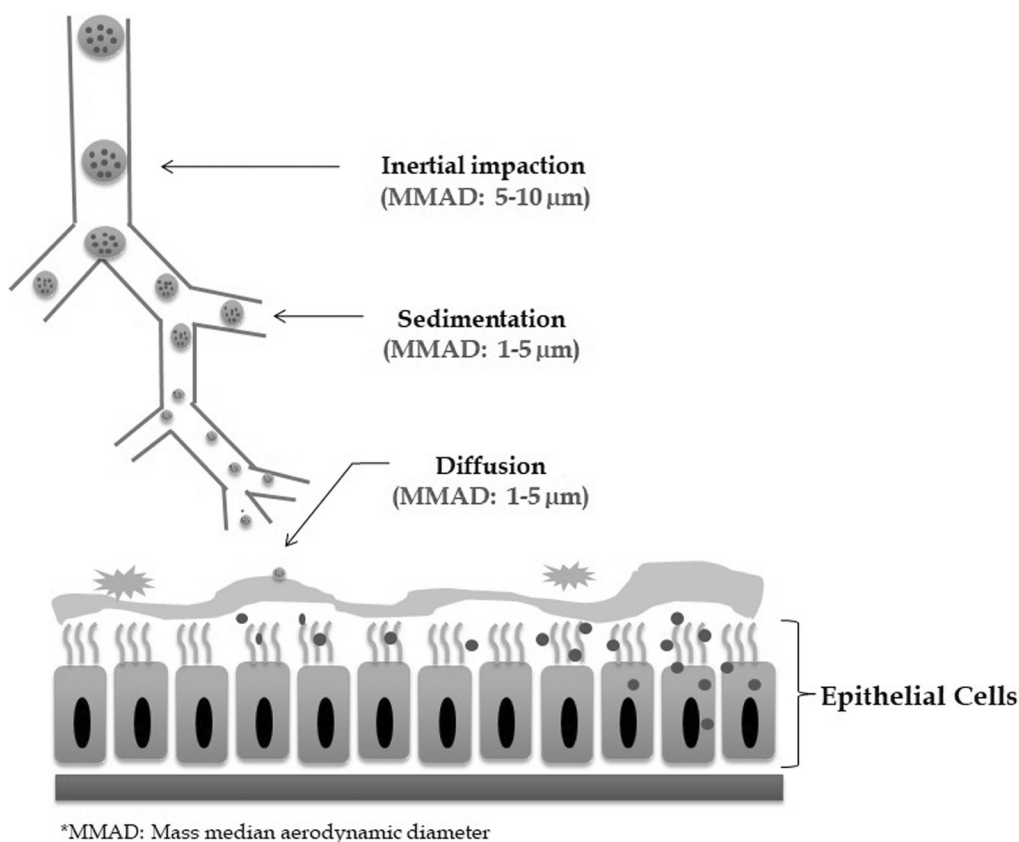


FIG. 2: Mechanism of deposition of particulate systems in lungs.

A. Nebulizers

Nebulizers are one of the oldest inhalation devices. These convert liquid formulations into droplets of 1–5 μm . One of the advantages associated with the use of nebulizer is that it does not require patient coordination between inhalation and actuation, thus making it relatively easier for pediatric, geriatric, and ventilated patients. Moreover, these can deliver larger doses of drug compared with other inhalation devices. Nebulizers are further subdivided into three categories, jet nebulizers, ultrasonic nebulizers, and mesh nebulizers.⁷¹ Jet nebulizers are also called “pneumatic nebulizers” and are based on the Bernoulli principle for the generation of aerosols. The major structural components of jet nebulizers are compressors, nebulizer chambers, mouth pieces, and facemasks. These can nebulize all types of liquids, solutions, suspensions, oils, etc. Conversely, ultrasonic nebulizers use high-frequency (1–3 MHz) vibrations of piezoelectric crystals for the generation of aerosol. These are not used to nebulize high-viscosity liquids. Mesh nebulizers are the most advanced form of nebulizers and use micropump technology for the generation of aerosol. The liquid is passed through multiple apertures of a mesh for the production of aerosol droplets. It can be used to deliver suspensions, liposomes, etc.⁷²

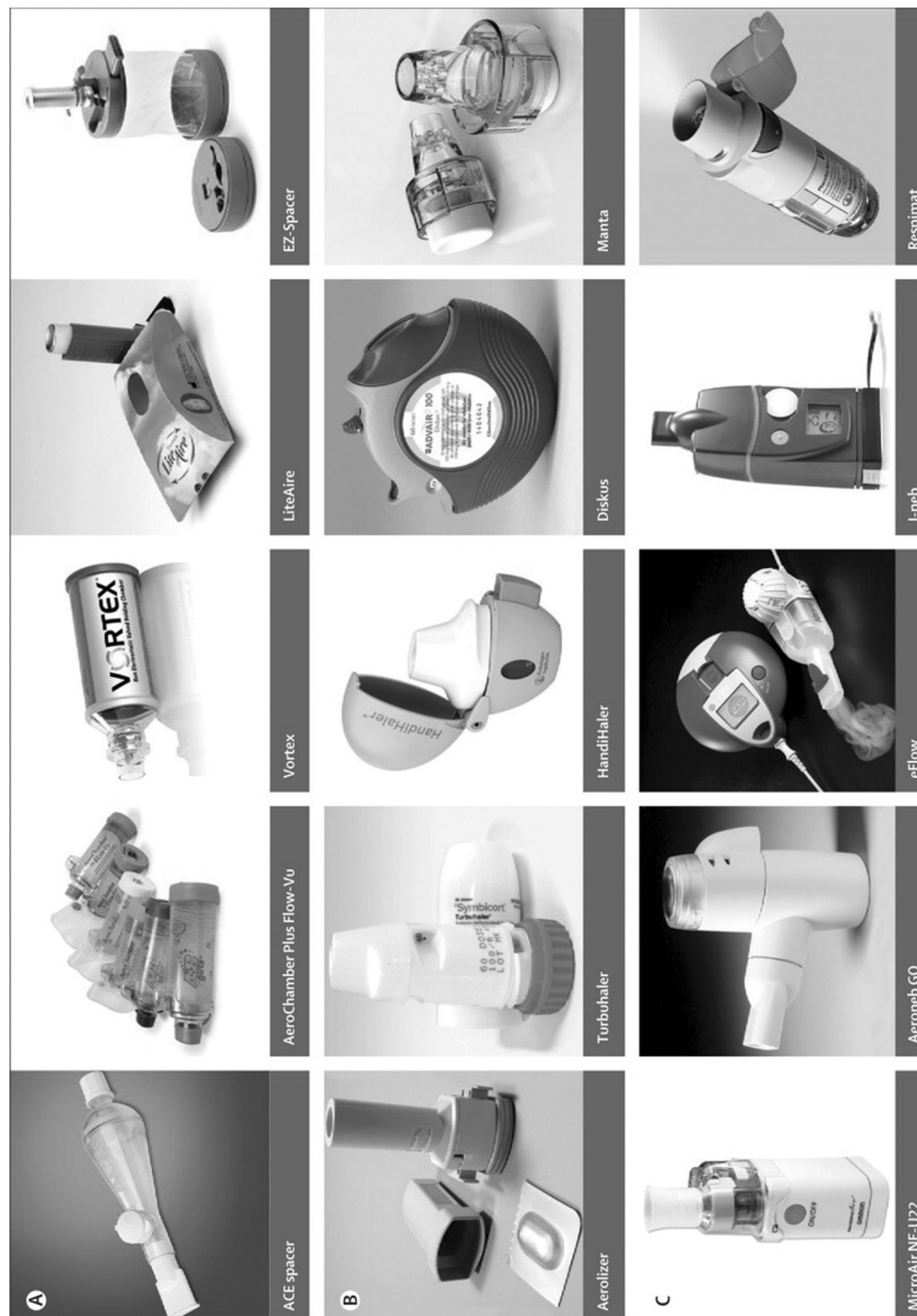


FIG. 3: Examples of commercially available inhalers. (Reprinted with permission from Dolovich and Dhand, 2011.⁶⁷)

B. pMDIs

pMDIs were first marketed in 1956 for the delivery of epinephrine (Medihaler-Epi™) and isoproterenol (Medihaler-Iso™).⁶⁸ The pMDIs deliver medical aerosols using propellants, the principal components of pMDI. Ideally, the propellants should be nontoxic, non-flammable, and compatible with the formulation components and provide constant vapor pressure during the entire life of the product.⁷³ Initially, chlorofluorocarbons were used as the propellants in pMDIs, but due to their harmful effects on the environment such as ozone layer depletion, they were banned by the Montreal Protocol in 1987.⁷⁴ Hydrofluoroalkane replaced chlorofluorocarbons in all pMDIs, but the change was not easy. These pMDIs consist of either a solution or suspension in a single or mixture of propellants. Other excipients such as surfactants and organic solvents are also used to stabilize these formulations. A canister, metering valve, actuator, and a mouth piece are the major structural components of a pMDI. These can be classified into breath-actuated and breath-coordinated devices. Breath-actuated inhalers address the problems associated with patient actuation and inhalation because they sense the patient's inhalation pattern with the help of actuator and deliver the dose automatically.⁷³ Conversely, breath-coordinated inhalers synchronize the discharge of dose with patient's inspiration. During inhalation, an actuator coordinates with the inhalation flow rate, thus helping the patient to have enough time to actuate the pMDI.^{75,76}

C. DPIs

DPIs, as the name indicates, are a device that delivers medication in a form of dry powder to the deep lungs. The DPIs are considered as alternatives and the major competitors of pMDIs. The first commercial DPI, Spinhaler™, was introduced by Aventis in 1970 for the delivery of a mast cell stabilizer, sodium cromoglycate.⁷⁷ Later, a large number of DPIs were introduced onto the market and are classified depending upon: (1) the number of doses a device could deliver, such as single unit to multi-unit devices; (2) patient contribution to aerosolizing the powder, such as breath-activated devices; and/or (3) by the mechanism of powder dispersion, passive or active.

The powder blends of DPIs include a mixture of micronized drug with coarse carrier particles such as lactose, mannitol, trehalose, and sucrose. Carrier particles are added to improve aerosolization characteristics such as flowability and dispersibility.⁷⁸ The DPIs offer greater stability than liquid formulations and require minimum patient coordination between breathing and actuation of the device to deliver the powdered dose. These are widely accepted and used by approximately 40% of people suffering from asthma and COPD.^{79,80}

Several recent innovations have emerged in the aerosol industry in the last a few decades. The increased number of these devices on the marketplace also is evidence of their success at potentially curing respiratory disorders. Nevertheless, reinforced efforts are further required to improve clinical outcomes and to achieve the desired effect. Some of the available aerosol drug-delivery devices that occupy the largest share of the market are listed in Table 3.

TABLE 3: Brief account of key inhaler devices available on the market

Device	Type	Company	Reference
Nebulizers			
Omron NE-U17 Nebuliser	Ultrasonic nebulizer	Omron Healthcare, The Netherlands	68
Aeroeclipse® II BAN	Breath-actuated jet nebulizer	Monaghan Medical Corporation, USA	81
Compair™ NE-C801	Jet nebulizer with virtual valve technology	OMRON Healthcare, The Netherlands	82
Akita2 Apixneb	Vibrating mesh nebulizer	Activaero GmbH, Germany	83
Micro Air® Ne-U22	Passive mesh nebulizer	Omron Healthcare, The Netherlands	84
Aeroneb	Active mesh nebulizer	Aerogen, Ireland	72
PARI eFlow®	Active mesh nebulizer	PARI, Germany	85
Pressurized Metered Dose Inhalers			
Easi-Breathe®	Breath-actuated	Norton Healthcare, UK	86
K-Haler	Breath-actuated	Clinical Designs, UK	73
MD-Turbo	Breath-actuated	Respirics, USA	73
SmartMist	Breath-actuated	Aradigm, USA	87
Easidose	Breath-coordinated	Bespak, UK	88
DPIs			
Diskhaler®	Multidose blister pack	GlaxoSmithKline, UK	89
Diskus®	Multidose blister strip	GlaxoSmithKline, UK	79
Aerohaler®	Single-dose capsule	Boehringer-Ingelheim, Germany	90
Nexthaler®	Multidose, disposable passive DPI	Chiesi Farmaceutici, Italy	91
Ellipta	Single- or two-strip, single-step activation, multidose DPI	GlaxoSmithKline, UK	92
Genuair	Multidose, disposable inhalation device	Almirall Sofotec GmbH, Germany	93
Podhaler	Single-dose, capsule-based passive inhaler	Novartis Pharmaceuticals, USA	94
Conix™	Single-dose reusable, single-dose disposable, multidose DPI device	3M, USA	95
Twincaps	Multidose	Hovione, Portugal	96

VI. CLINICAL EVIDENCE OF AEROSOL THERAPY IN PMAP

The number of patients with PMAP is escalating, primarily due to the increasing high-risk population with an impaired immune system.⁹⁶ Nonetheless, these infections show poor response to the available treatment therapy because of their low accumulation in the lungs and systemic adverse effects. Several attempts have been made worldwide to

overcome the problems associated with the conventional route by delivering antifungals through inhalation.⁹⁷ A number of such studies have demonstrated favorable outcomes with aerosolized therapy in preclinical studies and clinical investigations indicating the feasibility and effectiveness of aerosolized therapy in PMAP. Zhou et al. reported the case of a 42-year-old patient with severe influenza virus infection and secondary bacterial and *Aspergillus* co-infection. For the *Aspergillus* infection, therapy was started with intravenous VRC for 3 weeks, followed by oral VRC for 13 weeks, along with inhaled AmB for the first 3 weeks. The final chest CT scan following treatment revealed the disappearance of cavities and nodules in the lungs. The study demonstrated the role of inhalation therapy as an adjunct in the treatment of PMAP, which, if not efficiently managed, will complicate influenza infection leading to high mortality rates.⁹⁸

Xia et al. performed a meta-analysis to evaluate the efficacy of aerosolized AmB in prophylaxis of IPA. A total of 768 high-risk patients with IPA were included in the study. It was observed that the occurrence of IPA in patients who undertook aerosolized AmB therapy were lower than that in those who had taken placebo (4.4% vs. 10.4%, respectively). Therefore, the meta-analysis provides a proof-of-concept that prophylactic therapy of aerosolized AmB can successfully minimize the incidence of IPA among high-risk patients.⁹⁹

Ghong et al. supported the notion that the use of liposomal AmB (L-AmB) inhalation can minimize the risk of IPA considerably in acute myeloid leukemia (AML) patients. In this study, a total of 127 subjects with AML received chemotherapy and prophylactically inhaled L-AmB during their chemotherapy cycles. The incidence rate of IPA was significantly reduced in patients inhaling aerosolized L-AmB compared with the control group (9.5% vs. 23.4%, respectively). The study concluded that administration of inhalable L-AmB as a prophylactic twice a week to AML patients undergoing chemotherapy resulted in considerable reduction in the incidence of IPA. Moreover, the total cost of therapy was improved compared with the diagnostic costs and VRC.¹⁰⁰

Canetti et al. presented a case report of IPA in a kidney transplantation patient who was successfully treated with aerosolized AmB lipid complex. The therapy was started with VRC 200 mg q12h and intravenous AmB lipid complex. High-resolution CT scans were conducted regularly to monitor the fungus cavities in the lungs. After 50 days, the therapy with VRC was discontinued because of increased transaminases and gamma-glutamyl transferase levels and monotherapy with aerosolized AmB lipid complex (50 mg/d) was started. Partial cleansing of fungus was observed after 3 months of therapy in the medium lobe and complete cleaning in the lower left lobe, suggesting higher potential of inhalation of AmB lipid complex in IPA.¹⁰¹

Fujita et al. reported a case study of aerosol administration of AmB in a lung transplantation patient with PMAP. The 33-year-old female was diagnosed with PMAP one year after lung transplantation and was treated with the antifungals micafungin and ITZ. ITZ was continuously administered thereafter. After some time, the infection relapsed and the patient was diagnosed with recurrent PMAP. Initially, the treatment was started with intravenous administration of micafungin, but was then changed to aerosolized AmB. Continuous administration of aerosolized AmB resulted in improving and clearing the fungal infection from lungs and no reoccurrence was found later.¹⁰²

Hilberg et al. presented three cases of IPA treated with inhaled VRC solutions. Initially, therapy was started with oral VRC, but was switched to nebulization because, after a few weeks of oral therapy, an increase in liver enzymes was observed. The study reported the safety and therapeutic effectiveness of inhaled VRC in the subjects compared with oral administration.¹⁰³

Monforte et al. conducted a clinical trial involving 104 patients with lung transplantations having high risk of IPA. The L-AmB was nebulized and given prophylactically. The treatment included 25 mg three times a week continuing from 1 to 60 days, 25 mg once a week from 60 to 180 days, and 25 mg once every 2 weeks thereafter. Out of 104 patients, only 8 (7.7%) developed *Aspergillus* infection. The study concluded higher efficacy and safety of nebulized L-AmB in the dose and frequency used for the prevention of *Aspergillus* infection in lung transplantation patients.¹⁰⁴

Slobbe et al. performed a randomized double-blind placebo-controlled trial on 271 patients (>17 years) with hematologic diseases undertaking intensive chemotherapy. The aim of the trial was to check the efficacy of inhaled L-AmB in the prevention of IPA. Nebulization was performed for 30 min per day for 2 consecutive days with a maximum of 6 weeks. The results obtained from the study suggested that the prophylactic use of L-AmB for short-term nebulization is more efficacious and well-tolerated, with no clinically significant systemic side effects in prevention of IPA.¹⁰⁵

Borro et al. performed a retrospective analysis to study the efficacy and safety of AmB-lipid complex (AmB-LC) in 60 lung transplantation patients. AmB-LC was administered once every 2 days for 2 weeks and then once per week for at least 13 weeks. Fluconazole was also added for the first 21 days. The results from the study demonstrated that 59 patients (98.3%) responded well to the prophylactic therapy of AmB-LC. The study demonstrated the potential of nebulized AmB-LC for the prophylaxis of aspergillosis in lung transplantation patients.¹⁰⁶

Majority of the clinical studies available in the literature are limited to AmB only, stating its role in the prevention and management of PMAF.²⁴ Prophylactic therapy with nebulized AmB has proved efficacious with minimum systemic exposure in the management of IPA. Furthermore, the Infectious Disease Society of America (IDSA), in their recent guidelines, has recommended the nebulized therapy of AmB for IPA prophylaxis in patients with lung transplantations and/or prolonged neutropenia.¹⁰⁷ Conversely, the guidelines of Society of Infectious Diseases Pharmacists (SIDP) contradict the routine use of nebulized AmB from the results based on randomized clinical trials. Instead, the SIDP recommends the use of triazoles in the treatment and management of IPA.¹⁰⁸

Although the aerosolized formulations are an attractive means to prevent IPA, the paucity of data on pulmonary safety, efficacy, and pharmacokinetics is the major concern. In addition, the superiority of aerosolized triazoles over AmB is still considered debatable. More clinical investigations are urgently needed to compare the effectiveness of these aerosol agents in the prevention and management of PMAF. At present, the promising potential benefit of aerosol antifungal therapy remain restricted to literature reports and case studies only.²⁴

VII. RECENT ADVANCES IN AEROSOL THERAPY FOR PMAP

Inhalational systems have been generating enormous interest in the field of microbial lung infections such as PMAP and tuberculosis. An account of published reports on inhaled antifungals in PMAP between 1990 and 2017, pictorially depicted in Fig. 4, testifies to an escalating trend in this regard. Moreover, as evidenced from the clinical investigations, inhalation of antifungals plays a pivotal role in the prevention and management of PMAP. A number of clinical trials have been performed and many more are in development. Initially, the available intravenous forms of the drug were administered as aerosols, but this was not ideal because safety and efficacy was compromised due to the presence of added preservatives such as phenols, which are generally responsible for causing bronchospasm.¹⁰⁹ In 1997, major advancements took place in the field of inhalation antimicrobial therapy when tobramycin inhalation solution was approved by the FDA for the management of CF.¹¹⁰ Since then, other antibiotics, such as aztreonam and colistin,²⁸ have also been approved for directly targeting the lungs and many more are in clinical phase of development, such as ciprofloxacin,¹¹¹ vancomycin,¹¹² etc.

Lately, advancements in the field of nanotechnology have also made an impact in the inhalation delivery of therapeutics. Nano-based systems offer the advantages of increased drug solubility, permeability, sustained drug release, uniform distribution, and improved internalization of drug in the cells.¹¹³ This will not only reduce the drug dose, but also minimize dosage frequency, which consequently improves patient compliance. Depending upon the type of inhalation device, the nanosystems either form droplets or aggregates of micrometer size during the process of inhalation, but their behavior in the lungs after deposition depends upon their nano-dimensions.¹¹⁴ The formulation type also affects the size of the particles in the respiratory tract because volatile aerosols

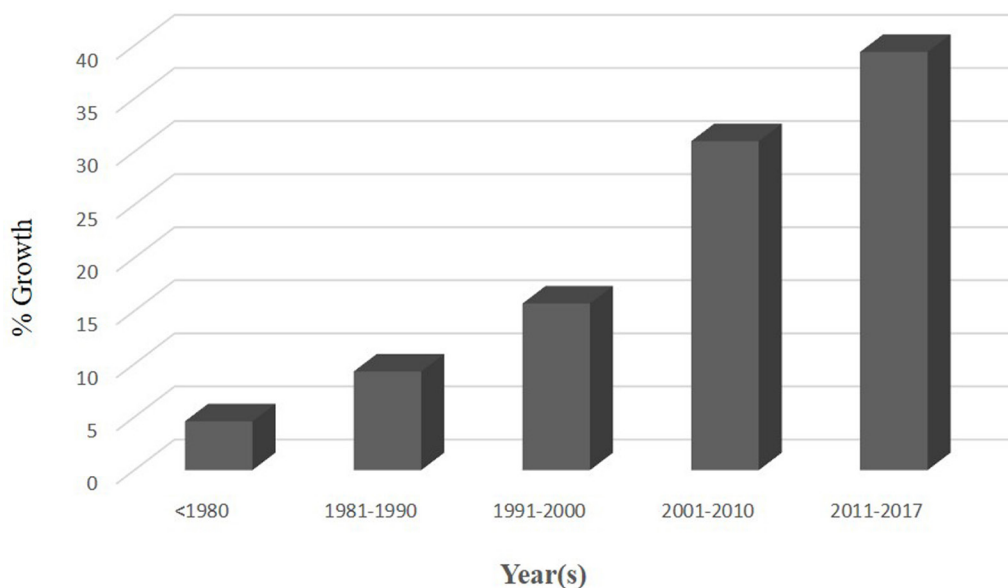


FIG. 4: Published reports on inhalation drug delivery in PMAP over the last five decades.

show reduced size due to evaporation, hygroscopic aerosols show bigger size because of moisture effects, and particulate aerosols show aggregation.¹¹⁵

In the context of PMAP, a diverse range of micro-/nano-drug-delivery systems have been studied by researchers worldwide. The commonly studied delivery systems include polymeric,¹¹⁶ liposomes,¹¹⁷ cyclodextrin complexes,¹¹⁸ nanomicelles,¹¹⁹ NLCs,¹²⁰ nano-emulsion,¹²¹ large porous particles,¹²² etc. (Table 4). These delivery systems improve the transport of the therapeutic moiety to the affected site and also prolong the residence time of the drug in the lungs compared with their conventional counterparts. The choice of excipients is also of critical concern in the case of pulmonary drug delivery because it could augment the disease further. Excipients such as polymers, lipids, sugars, amino acids, and surfactants have been employed to deliver drugs by the pulmonary route. Chronic administration of these excipients may sometimes result in depletion of lung surfactant and may result in immunogenicity, local irritation, and toxicity.¹²³ The use of lung endogenous lipids such as phosphatidylcholine, DPPC, cholesterol, etc., may result in successful outcomes because of their non-immunogenicity and ability to overcome clearance mechanisms. Moreover, the presence of residual solvent is also of major concern because it may lead to toxicity issues. The safety and compatibility studies with lung tissue should be of prime importance in the development of aerosol formulation. Therefore, the processing techniques and formulation components should be thoroughly screened before formulating any delivery system.^{124,125}

Arora et al. developed highly respirable dry powders of VRC by using spray drying technology. The powders were characterized based on MMAD, GSD, FPF, surface morphology, and moisture content. The optimized dry powder formulation was also studied for cellular uptake and viability on an air-interface Calu-3 model. Furthermore, lung bioavailability and tissue distribution studies were conducted in mice. From the data, it was observed that the optimized formulation showed MMAD of 3.79 μm , FPF of 60.0%, GSD of 1.70, irregular morphology, and low moisture sensitivity. The cellular viability assay showed the safety of the optimized dry powder on Calu-3 cells. Moreover, the delivered dry powder maintained VRC concentrations in the lungs above MIC of *Aspergillus* species for more than 8 hours. The study demonstrated the potential of aerosolized VRC dry power in the management of PMAP.¹²⁶

In another study, Pardeike et al. formulated inhalable NLCs of poorly soluble ITZ for the management of *Aspergillus* infection in captive falcons. The optimized formulation of NLCs showed particles in nanometer range with an entrapment efficiency of 99.98%. Moreover, MTS and LDH assay revealed no cytotoxicity effect on A549 cell lines. The developed formulation was also tested for the lung deposition studies using gamma scintigraphy. The results obtained showed the effective deposition of NLCs in the lungs and air sacs of the falcons. The study provides evidence of inhalation drug delivery in the management of PMAP.¹²⁷

Although nano-based drug-delivery systems have shown great potential in pulmonary drug delivery, yet the issues related to the pulmonary safety and efficacy are still in debate.^{128,129} More extensive and religious efforts are required at the preclinical level to prove their potential.

TABLE 4: Literature on inhalation of antifungals in PMAP

System	Excipient(s)	Inhalation Device	Key Findings	Reference
AmB				
Nanoparticle	Polymethacrylic acid	Nebulizer	Aerosol route was effective in killing > 99% of <i>Aspergillus</i> . Study provides a proof-of-concept in prevention of fungal lung infections.	116
Liposomes	HSPC, DSPG, AT, cholesterol	Nebulizer	Multistage impinger studies indicate the capability of L-AmB aerosols to reach deep lungs. Moreover, cellular uptake studies have shown the concentrations above MIC within alveolar epithelial cells.	117
Nanomicelle	Chitosan, stearic acid	Nebulizer	AmB nanomicelles improved the antifungal activity and could prove a suitable choice for pulmonary delivery of AmB.	119
Nanoemulsion	Intralipid® or Clinoleic®	Nebulizer	Clinoleic® emulsion had higher FPF than the Intralipid®. Lipid nanoemulsions could be successful carriers for delivering AmB to the peripheral airways.	121
Non-ionic surfactant vesicles (NIVs)	Cholesterol, hydroxypropyl- γ -cyclodextrin	Nebulizer	AmB-NIV significantly reduce fungal lung burden in a rat model of IA ($p < 0.05$) compared to treatment with the carrier alone.	130
Hybrid systems	Phosphatidylcholine, chitosan	Nebulizer	Chitosan-coated liposomes could be successful carriers for AmB delivery with higher drug-loading that is likely to effectively deliver the drug to the peripheral airways.	131
Liposomes	PC, cholesterol, OPM, OPP	Nebulizer	<i>In vivo</i> tissue distribution studies on albino rats showed higher drug concentrations in the lungs with ligand-anchored liposomal aerosols. The drug was estimable after 24 h in the lungs.	132

TABLE 4: (continued)

System	Excipient(s)	Inhalation Device	Key Findings	Reference
Nanoparticles	SDS	DPI	The SDS-AmB formulation has shown significantly reduced toxicity compared to AmB alone on <i>in vitro</i> cell lines.	133
ITZ (ITZ)				
Cyclodextrin complex	2-Hydroxypropyl- β -cyclodextrin	Nebulizer	Slower systemic absorption of ITZ across lung epithelium was observed with HPbetaCD-ITZ compared to URF-ITZ.	118
NLC	Precirol ATO, oleic acid, eumulgin SLM 20	Nebulizer	NLC showed high entrapment efficiency with smaller particle size. In addition, the formulations were stable during nebulization using a jet and ultrasonic nebulizer.	120
Nano-structured aggregates	Mannitol, lecithin	Nebulizer	Nebulization of colloidal dispersions of the ITZ nanoparticles exhibited good aerodynamic properties and confirmed significant lung deposition.	134
Fine solid-crystal suspension (FSCS)	Mannitol	DPI	Solubility enhancement of approximately 145-folds was observed with FSCS ITZ as compared with raw ITZ. The formulation showed high fine-particle fraction of $50.59 \pm 0.67\%$, suggesting FSCS a promising technique for DPI formulation of poorly soluble drugs.	135
Solid dispersions	Mannitol	DPI	Optimized formulation resulted in high yield of 60% with good aerodynamic properties ($FPF = 33 \pm 2\%$)	136
Microparticles	Mannitol, phospholipon 90 H	DPI	Amorphous ITZ-based SD formulations showed extended pulmonary retention <i>vis-à-vis</i> crystalline ITZ formulation	137

TABLE 4: (continued)

System	Excipient(s)	Inhalation Device	Key Findings	Reference
Nanoparticles	Chitosan TPP	DPI	10% addition of leucine to Chitosan-TPP nanoparticles made them more appropriate for aerosolization and increased the pulmonary deposition parameters significantly.	138
Microparticles	PL, Mannitol	DPI	The ITZ-DPI presented very high FPF from 46.9% to 67.0% of the nominal dose. The formulation strategy could be an attractive substitute for treating IA.	139
Transferosomes	Mannitol, lecithin, Span® 60	DPI	Nanotransfersomal DPI co-spray dried with mannitol has the potential to deliver ITZ to the site of infection in the lungs in PMAP.	140
Microparticles	SLS, tocopherol polyethylene 1000 succinate, poloxamer 407, mannitol	DPI	SD ITZ NP using mannitol is a very effective method to produce DPI formulations with optimal aerodynamic properties.	141
Microparticles	Phospholipid, mannitol	DPI	Amorphous ITZ-based SD formulations supersaturate the lung lining fluid and showed extended pulmonary retention <i>w.r.t.</i> micronized crystalline ITZ formulation.	142
VRC (VRC)				
Large porous particles	PLA	DPI	Sustained release of drug was obtained for over 7 d. No cytotoxic and inflammatory (IL-8) effect was observed on A549 cells.	143
Microparticles	Leucine modified	DPI	Leucine-modified dry powder VRC formulation produced by SD can effectively deliver high concentrations of VRC to the lungs with clinically relevant plasma concentrations.	126

TABLE 4: (continued)

System	Excipient(s)	Inhalation Device	Key Findings	Reference
Low-density porous aggregate microparticles	PVP K25, 1,4-dioxane	DPI	High concentrations of VRC in the lungs indicate that micro-structured crystalline VRC could prove to be a favourable strategy for administration of VRC in IPA patients.	144
Microparticles	Aqueous solution of VRC solubilized with sulfobutyl ether-beta-cyclodextrin	Nebulizer	Following a single inhaled dose, maximum drug concentrations were found to be $11.0 \pm 1.6 \mu\text{g/g}$ wet lung weight and $7.9 \pm 0.68 \mu\text{g/mL}$ in the lung tissue and plasma respectively.	145
Microparticles	Aqueous solutions of VRC	Nebulizer	The study demonstrated that inhaled VRC was well-tolerated in rat model. The histopathology studies revealed an increase in the number of alveolar macrophages without inflammation or edema.	146
Microparticles	Aqueous solutions of VRC	Nebulizer	Compared with control and AmB groups, the inhaled VRC solutions exhibited improved survival rate and limited the extent of invasive disease, as assessed by histopathology studies.	147

VIII. INHALATION MODELS FOR PRECLINICAL TESTING

Several animal models have been investigated by researchers to study the efficacy of developed formulations in PMAP. Usually, either normal or immune-suppressed animal models have been used in the preclinical research. In most of the investigations, rodents such as mice, rats, and guinea pigs predominate over the other mammalian animal models such as rabbits and monkeys because they are relatively easier to handle, require a smaller amount of drug, and can include a larger number of animals in the study for improving statistical validity.¹⁴⁸ In veterinary research, however, several avian models have also been used to for preclinical testing because they can acquire *Aspergillus* infection naturally.¹⁴⁹

Literature examples of pulmonary delivery in rodents are majorly restricted to *in vitro* characterization and *in vivo* pharmacokinetic and biodistribution studies only. There are very few studies reporting formulation safety and tolerability studies in rodents. In addition, the facilities available to conduct *in vivo* experiments at the preclinical level

are of major concern associated with this route. Majorly, two types of inhalation exposure chambers, whole-body exposure and head-/nose-only exposure, have been used in the preclinical testing of inhalation drug products. In the whole-body exposure system, the small animals are placed in a chamber coupled with the aerosol generator source.¹⁵⁰ The atmosphere of the formulation or drug being tested is generated in the chamber, which is ultimately inhaled by the animal. This method of exposure can be used for large number of animals without restraining them, offering the distinct advantage of normal breathing. These chambers are available in various sizes, from small exposure chambers holding one animal to large exposure chambers holding many animals, depending upon the size of the chamber. The drawbacks associated with the use of whole-body exposure chamber includes the use of large amount of formulation or drug being tested, exposure of other animal parts such as skin or fur to the aerosol, plausibility of interaction of animal excreta with the aerosol, and requirement of good mixing for uniform distribution of air in the chamber.^{150,151} Conversely, in the head-/nose-only chamber, only the head or nose of the animal is exposed to the aerosol as opposed to the whole body. In this type of exposure system, the animals are placed in tubes connected to the aerosol generator. Optimum airflow rate is required to maintain uniform distribution of aerosol droplets to animals. This type of aerosol exposure offers definitive advantages of reduced drug exposure to other parts such as skin or fur, requires lower amounts of formulations or drug being tested, is suitable for repeated exposures, and is easy to clean up. However, this exposure system could stress the animal because of the necessity for restraining in tubes and the animal may try to turn in the tube, which could alter the output.^{150,152} Other direct instillation methods such as intratracheal instillation or dry powder insufflation have also been used by researchers to introduce therapeutic agents to the lungs.¹⁵³ These available rodent inhalers on the market are too costly to meet standard laboratory requirements. To surmount this, some researchers have built in-house inhalers to conduct these experiments in animal models.

Silva et al. developed a rounded-wall inhalation chamber model that enables toxicological and pharmacological testing in rodents. The inhalation chamber consisted of five animal holders and a central part coupled with a nebulizer. A volume of 15 mL of aqueous dispersion of clove oil 0.5% (v/v), propylene glycol 10% (v/v), and ethanol 10% (v/v) was prepared and nebulized into the chamber. Activation of the nebulizer passed the air directly and continuously from central portion of the chamber to the bifurcations holding animals. Dose uniformity was achieved due to the generation of turbulence at the bottom of central portion, which allowed uniform distribution of dose to each bifurcation. Initially for 1 min, the chamber became saturated with the nebulized dispersion. Afterward, homogeneous and continuous operation was observed for 25 min without any accumulation of the volatile material. For evaluation, the vapors within the chamber were collected and analyzed using gas chromatography. The study revealed a promising approach for preclinical testing of inhalable particulates.¹⁵⁴

Sinha et al. successfully delivered both porous and non-porous VRC-PLGA nanoparticles mixed with microfine lactose to mice using customized dry powder inhalation chamber. The developed polypropylene inhalation chamber was rectangular

and could hold up to six animals at one time, exposing only their noses in the chamber. The major parts of the inhaler included a sample holder, dosing chamber, and tube that connected the sample holder with the dosing chamber. The dry powder was placed inside the dosing chamber and fluidized to deliver the dose. The initial drug deposition of porous particles and non-porous particles was 120.4 $\mu\text{g/g}$ and 74.8 $\mu\text{g/g}$ of lung tissue, respectively, which gradually declined with time and was not detectable after 7 and 5 days, respectively, post-dosing.¹⁵⁵

Yi et al. fabricated and validated a whole-body nanoparticle exposure chamber to study the toxicity profile of titanium oxide nanoparticles in small animals. Herein, the inhalation chamber included nanoparticle aerosol generator, exposure chamber, and monitoring system. The aerosols were generated from dry nano- TiO_2 powders and were delivered to the exposure chamber at a flow rate of 90 LPM. Particle size distribution was monitored using an electric low-pressure impactor (ELPI) and scanning mobility particle sizer (SMPS). With ELPI, the median aerodynamic diameter of the particles was observed to be 157 nm and 145 nm SMPS. During the whole process of the inhalation exposure period, the particle size remained relatively stable. The investigators achieved controlled and uniform nanoparticle aerosol test atmosphere within the exposure chamber.¹⁵¹

Kaur et al. developed and validated a nose-only inhalation chamber for mice for delivering microparticles of two front-line antitubercular drugs, isoniazid and rifabutin. The design of the inhaler was very simple and easy to make. It consisted of a 15 mL poly(propylene) centrifuge tube, C-Flex tubing, and a rubber pipette bulb. The inhaler was optimized on the basis of the fraction of microparticles available at the delivery port. When 20 mg of microparticles was fluidized, about 2.5 mg were collected at the delivery port in 30 s of operation. Of the 2.5 mg of microparticles, the mice inhaled approximately 300 μg .¹⁵⁶

Simple, innovative, reliable, and reproducible inhalation exposure systems are needed to meet the standard requirements of preclinical testing in laboratories. More extensive and intensive efforts are needed to explore this area of inhalational drug delivery further.

IX. PATENT MAPPING

Recently, the delivery of drugs, proteins, or peptides through the pulmonary route is gaining importance, not only in industry, but also in academic research. The pulmonary route involves a variety of applications and advantages of local delivery to the diseased lung cells over conventional available treatments. In PMAP, aerosolization can be used as an effective means to deliver drug deep inside the lungs.²³ In recent years, many new advances have been made in the field of inhaler technology in pulmonary medicine. We have listed some of them in Table 5.

X. FUTURE PROSPECTS

The increase in the number of people having surgery, organ transplantation, HIV, cancer, asthma, and COPD has increased the number of patients at risk of PMAP. The

TABLE 5: Updated list of patents on pulmonary drug delivery of antifungal agents

Title	Publication Number	Publication Year	Inventor(s)	Brief Description	Reference
Lipid-based compositions of anti-infectives for treating pulmonary infections and methods of use thereof	US20160317564 A1	2016	Jeff Weers	Method of reducing the loss of anti-infective encapsulated in a lipid-based composition upon nebulization was disclosed.	157
Formulation for pulmonary administration of antifungal agents and associated methods of manufacture and use	US20140363506 A1	2014	Thomas Tarara, Jeffrey Weers, Michael Eldon, Rangachari Narashimhan, Andrew Clark	Antifungal drugs formulations were developed using a lipid matrix wherein the active agent particles (~90%) in the formulation have an aerodynamic diameter < 3 μ m.	158
Dry powder formulation of azole derivative for inhalation	EP2601973 A1	2013	Francis Vanderbist, Thami Sebt, Arthur Deboeck, Christophe Duret, Karim Amighi, Philippe Baudier	SD powders of azoles with polyol or polysaccharide were prepared. The release composition showed 5% within 10 min, 10% within 20 min, and 40% within 60 min.	159
Nanosuspension with antifungal medication to be administered via inhalation with improved impurity profile and safety	US 20110190245 A1	2010	Chris Rundfeldt, Hartwig Steckel, Holger Scherliess	Nanosuspensions of antifungal drug, particularly, ITZ were developed. Aerosolization results in better therapeutic effect with reduced side effects compared with with conventional therapy.	160

TABLE 5: (continued)

Title	Publication Number	Publication Year	Inventor(s)	Brief Description	Reference
Treatment of pulmonary fungal infection with VRC via inhalation	WO2009137611 A2	2009	Robert O. Williams, Rupert O. Zimmerer, Jason T. McConville, Justin A. Tolman, Nathan P. Wiederhold, Jay I. Peters Menos	The patent provides a method of administering VRC and cyclodextrin solution by inhalation for the treatment of PMAP.	161
Pulmonary delivery of polyene antifungal agents	US7473433 B2	2009	Michael Weickert, Marc S. Gordon, Sandeep Kumar, Bing Yang, Razaq Sarwar	SD powders of polyene antifungals were prepared and demonstrated superior aerosol properties.	162
Aerosolizable pharmaceutical formulation for fungal infection therapy	WO 2004060903 A2	2004	Jeffrey G. Weers, Thomas E. Tarara, Michael A. Eldon, Rangachari Narasimhan	Aerosolizable formulations of AmB were developed and characterized. Effective concentrations of drug was observed upon aerosolization with reduced systemic exposure	163
Pharmaceutical formulation with an insoluble active agent for pulmonary administration	WO2004060351 A2	2004	Thomas E. Tarara, Jeffrey G. Weers	A formulation comprises of particulates of an active agent in a lipid-based matrix. The particles comprise 90% of the active agent with a geometric diameter of 3 μ m.	164

TABLE 5: (continued)

Title	Publication Number	Publication Year	Inventor(s)	Brief Description	Reference
Aerosolized azole antifungal	WO 1990011754 A1	1990	Thomas Samuel, Campbell Orr	A suitable aerosolized formulation was developed to prevent the onset of PMAP in immunocompromised patients.	165
Method of preventing and treating pulmonary infection by fungi using aerosolized polyenes	US4950477 A	1990	Heinz J. Schmitt, Donald Armstrong, Edward M. Bernard	Aerosol of a polyene or a pharmaceutically acceptable derivative thereof was developed to prevent fungal lung infection.	166

current therapeutic regimen is not able to meet clinical needs because of concerns of efficacy, safety, and resistance. Efforts have been lately to understand and explore the potential of aerosol drug delivery in PMAP. Some of the case reports and clinical trials have shown the potential of this route in the prophylaxis and treatment of PMAP. Moreover, formulations of AmB are often recommended as “off-label” nebulization²⁴ for the management of disease. Nevertheless, routine use of antifungal aerosolized therapy is not recommended due to the lack of clinical evidence on pulmonary pharmacokinetics and safety.

The other important goal is to retain the drugs for longer hours in the lungs. Particle engineering approaches and the use of mucoadhesive biomaterials has proven the concept to some extent, but more rigorous studies need to be done. The gathered knowledge would enable the development of next-generation smart aerosol systems that are able to maintain minimum effective concentrations in the lungs for longer hours.

Strategies need to be established to monitor the efficacy and safety of a therapeutic drug administered by aerosol. It is clear that, in order to optimize the immense potential of aerosol drug delivery, more clinical trials should be conducted so that the impending growth of this route in PMAP is not merely restricted to case reports only. Last, an important consideration often overlooked is the requirement to obtain approval from regulatory authorities.

XI. CONCLUSION

Growing incidences of PMAP call for efficient therapeutic strategies toward its prevention and eradication. Because conventional drug delivery approaches are unable to target the infectious cells effectively, the development of creative and innovative aerosol technologies is an acute need to address these issues. Concerted and collaborative efforts by the pharmaceutical industry and academia in this regard have a tremendous potential to provide a tangible remedy to this need with extensive evidence-based clinical reports on patient efficacy and safety. Well-designed clinical investigations need to be undertaken to evaluate the therapeutic benefits and/or risks associated with inhalation drug delivery of antifungals.

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