Recent developments in inhaled triazoles against invasive pulmonary aspergillosis

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Abstract

Invasive pulmonary aspergillosis (IPA) presents high incidence and high mortality in immunocompromised patients. To combat or prevent IPA, triazoles such as voriconazole or itraconazole and posaconazole are becoming the first- or second-line therapy, respectively. However, triazoles present issues of oral bioavailability, high liver metabolism and/or drug-drug interactions, increasing the variability of systemic concentrations. To overcome these issues, inhalation is a promising route for delivering triazoles for prophylactic or curative therapy of IPA. Indeed, pulmonary drug delivery increases the drug *in situ* drastically while decreasing the systemic exposure, therefore limiting drug metabolisation, side effects and

drug-drug interactions. Development of triazoles for inhalation has focused on voriconazole and itraconazole, drugs which are both highly permeable but present high solubility differences. In this review, the most complete and promising pharmaceutical developments for voriconazole and itraconazole are described.

Keywords: aerosol, antifungal, aspergillosis, fungal infection, pulmonary delivery, dry powder inhaler, dry powder for inhalation, nebulizer, nebulization, cyclodextrin, nanoparticle, solid dispersion, controlled release drug delivery.

Abbreviations: **AI90H**, amorphous itraconazole with Phospholipon® 90H; **AI** ,amorphous itraconazole; **AIN**, amorphous ITZ nanoparticles-based aggregates; **AIP1**, ITZ nanoparticle-based aggregates with polysorbate 20; **AIP2**, ITZ nanoparticle-based aggregates with polysorbate 80 and Poloxamer 407; **AmphB-deox**, amphotericin B deoxycholate; **AUC**, aera under curve; $\mathbf{C_{max}}$, maximal concentration peak; **CI**, crystalline itraconazole; **CIN**, crystalline ITZ nanoparticles; **CIP**, crystalline ITZ nanoparticle-based aggregates; $\mathbf{d_{ae}}$, aerodynamic diameter; **DPI**, dry powder inhaler; **FPF**, fine particle fraction; **HPβCD**, hydroxyl-β-cyclodextrin; **ICD**, amorphous itraconazole-based inclusion complex with HPβCD; **IPA**, invasive pulmonary aspergillosis; **IPEC**, international pharmaceutical excipients council; **ITZ**, itraconazole; **MIC**, minimum inhibitory concentration; **MMAD**, median mass aerodynamic diameter; **PEG**, polyethylene glycol; **PLGA**, poly-lactide-co-glycolide; **Seq**, saturation solubility at the equilibrium; **SeβCD**, sulfobutyl ether-β-cyclodextrin; $\mathbf{t_{1/2}}$, life half-time; **VCZ**, voriconazole

Introduction

Among the different pulmonary fungal infections, aspergillosis, and in particular invasive pulmonary aspergillosis (IPA), are becoming the most worrying diseases in immunocompromised patients. This is due to their high incidence and mortality. Indeed, IPA represents 50-60% of all invasive pulmonary diseases, with a mortality of 50-90% in severely immunocompromised patients [1]. The triazoles act by inhibiting the 14-α demethylase, a fungal cytochrome P450 enzyme implicated in the synthesis of ergosterol, a fungal cell wall's essential constituent. Moreover, they interact with the same cytochrome present in large quantities in the human liver inducing possible drug-drug interactions in IPA patients [2,3]. Consequently, interactions resulting from inhibitors, inductors or substrates of cytochromes can modify the plasmatic concentrations of triazoles or others drugs administrated concomitantly. Moreover, the genetic polymorphism can also induce unpredicted plasmatic pharmacokinetic profile. To overcome these important issues, pulmonary delivery of triazoles could be an interesting alternative to conventional routes.

Pulmonary aspergillosis

The term aspergillosis includes various diseases caused by ubiquitous saprophyte fungi of the type *Aspergillus*. In view of the large number of species of *Aspergillus* (about 900 species), it is important to note that the majority of diseases are caused by four species in particular: *A. fumigatus* (65-75% of aspergillosis), *A. flavus* (5-10%), *A. niger* (1.5-3%) and *A. terreus* (2-3%) [2,4]. *Aspergillus* contamination was made in 90% of cases by the entry of spores into the body through the respiratory system [2]. Indeed, *Aspergillus* spores present a respirable size (i.e., 1.9-6 microns) that can be inhaled and deposited deep in the lungs [5]. Despite inhaling a few hundred *Aspergillus* spores every day, healthy people usually develop no pulmonary aspergillosis [4-7]. Indeed, an immunocompetent person is able to eliminate these intrusions by the mucociliary escalator, the alveolar macrophages and/or the immune system, for example via neutrophils [7-9].

In immunocompromised patients or in specific bronchial diseases, *Aspergillus* spores deposited in the lungs can be only partially or not eliminated and can germinate into hyphae inducing pulmonary aspergillosis such as aspergilloma, allergic bronchopulmonary

aspergillosis, chronic necrotizing aspergillosis or IPA [2]. In recent decades, the incidence of IPA (and opportunistic aspergillosis in general) has dramatically increased due to the increased number of immunocompromised patients [1]. Indeed severe neutropenia (< 500 cells/blood microliter) due to long corticosteroid therapy (> 3 weeks), organ transplantation, leukemia, cytotoxic chemotherapy and the presence of HIV is important risk factor for IPA [4,10-12]. According to the immunodeficiency of the host, hyphae will grow, and by infiltration of the lung tissue, spread to other organs such as the liver, kidneys, brain and heart [2,9]. Once disseminated, the survival rate of treated patients is less than 10% [3]. Hyphae are responsible for the majority of symptoms and produce toxins inhibiting mucociliary and macrophage activities, reducing clearance systems present in the lungs [9,13]. However, in low neutropenia (1 500 to 500 cells/blood microliter), neutrophils could combat or limit the fungal infection but still generate a strong inflammatory response and pulmonary lesions, which could lead to the death of the patients. The diagnosis of this pathology is carried out by radiography and by isolating *Aspergillus* by endoscopy with a positive polymerase chain reaction analysis. However, for severely neutropenic patients or patients at high risk, evocative aspect radiography is sufficient to start treatment due to the high mortality rate associated with IPA [10,12,14].

Conventional treatments of severe pulmonary aspergillosis

Among the five existing antifungal classes, the genus *Aspergillus* is sensitive to only three classes, including polyenes (e.g., amphotericin B), azoles (e.g., fluconazole, itraconazole, voriconazole, posaconazole) and echinocandins (e.g., caspofungin, micafungin, anudilafungin). The recommendation of the Infectious Diseases Society of America (IDSA) for severe pulmonary aspergillosis such as chronic necrotizing aspergillosis and IPA is primary therapy with voriconazole (VCZ) [15,16]. Indeed VCZ shows better efficacy compared with amphotericin B in the treatment of IPA [17]. For these indications, alternatives are amphotericin B as a liposomal or lipid complex for patients taking medications that can interact with triazoles, such as drugs metabolized by CYP450, or in the context of triazole-resistant *Aspergillus* species. In the context of a disease caused by *A. terreus*, VCZ is preferred because this species is resistant to amphotericin B [16]. Itraconazole (ITZ),

posaconazole, caspofungin and micafungin are also used as alternatives when primary treatment is incompatible or fails, or are used in combination to avoid the development of resistance. Nevertheless, antifungal combinations are not recommended routinely by the IDSA [14,16]. All these molecules are formulated for oral (e.g., VCZ, ITZ, posaconazole) and/or parenteral administration (e.g., VCZ, ITZ, amphotericin B, caspofungin, micafungin), which may explain the higher risk of treatment failure. Such failure is caused by the use of high doses to obtain sufficient concentration at the pulmonary site of infection, low oral bioavailability, high liver metabolism and hepatic first-pass effect, low blood flow to the target area due to angio-invasive hyphae of the fungus, etc. Consequently, the pulmonary route is a promising alternative in the prophylaxis and treatment of pulmonary aspergillosis [8,18-19].

Pulmonary Drug delivery

Pulmonary administration presents several advantages for local delivery. Indeed, locally, the drug dose is readily available *in situ*, without being diluted in the general circulation. This *in situ* availability increases its efficiency and speed of action. Moreover, systemic exposure is reduced, decreasing the potential systemic side effects or drug-drug interactions. Therefore, inhalation has become the preferential route of administration to treat respiratory disorders such as asthma, chronic obstructive pulmonary disease and cystic fibrosis, and is in development for other pulmonary disorders [18, 20-24]. The effectiveness of treatment by the pulmonary route depends mainly on the deposition of drug-based particles and therefore on their aerodynamic diameters (d_{ae}). The d_{ae} depends on the geometric diameter, shape and density of the particle. It is considered that particles with a d_{ae} between 0.5 µm and 5 µm will be deposited in the lower airways (i.e., from the trachea up to alveoli) during inhalation [25-28]. Once deposited, particles can be cleared according to various mechanisms, including the mucociliary escalator, alveolar macrophage phagocytosis, absorption through the lung epithelium and metabolism. Mucociliary clearance occurs in the conducting zone through ciliated and goblet cells. Both carry the bronchial secretions to the pharynx, where they will be swallowed or expectorated [29]. The mucus protects the epithelium against dehydratation, heats and humidifies the air and forms a barrier against the penetration of foreign particles

such as undissolved drug particles [29-30]. In the respiratory zone, the undissolved drug particles tend to be phagocytised by alveolar macrophages [31-32]. However, for particles having sizes greater than 6-10 μm or below 0.2 μm, and/or with a hydrophilic particle surface (e.g., PEG molecules), phagocytosis seems to be prevented or reduced [33-35]. Consequently, according to their dissolution velocity, deposited drug particles can be progressively eliminated by these mechanisms or dissolved to act on their pharmacological target and/or be metabolised or absorbed through lung epithelium [36-37]. Indeed, lipophilic drugs pass easily through the cell membranes of the lung epithelium by passive diffusion (i.e., transcellular transport). Hydrophilic drugs, for their part, are absorbed more slowly through extracellular channels of tight junctions by passive diffusion (i.e., paracellular transport). Some drugs could also be absorbed by active transport processes, by endo- or exocytosis [28, 37-39].

To obtain an optimal deposition of drug in the lungs, different formulation strategies can be applied according to the drug's physico-chemical properties [40-43]. In most cases, the formulation strategies require the use of various solvents and/or excipients during the production step. These excipients have to be approved or recognized as "generally recognized as safe" (GRAS) substances for pulmonary drug delivery [40, 44-45]. Moreover, the residual solvents have to be below some acceptable limits [46]. Hence, various guidances for clinical toxicities and pharmacokinetics studies of these excipients are proposed by the Food and Drug Administration (FDA) and the International Pharmaceutical Excipients Council (IPEC) [47-49]. Each drug formulation is designed in relation to its corresponding inhalation device in order to be administered by oral inhalation efficiently. Indeed, the function of inhalation devices is to deliver drug formulations reproducibly as solid or liquid particles suspended in the air (i.e., aerosol of fine particles) that present a d_{ae} between 1 and 5 μm [34,50-51]. There are three types of inhalation devices: nebulizers, metered pressurized dose inhalers and dry powder inhalers (DPIs) [52].

Pharmaceutical development of triazoles delivered by inhalation

To date, only VCZ and ITZ, have been developed for inhalation against fungal infections such as pulmonary aspergillosis. The pharmaceutical developments for each triazole have been described in terms of formulations for inhalation in **Table 1** and each formulation was

described in terms of solubility/dissolution profile, *in vivo* pharmacokinetic and *in vivo* efficacy on immuncocompromised murine model of IPA in **Table 2**. To date in the literature, the formulations designed for nebulizers or DPIs have been tested only on preclinical models of IPA and no clinical trials have been yet performed in humans.

Voriconazole

VCZ is considered as Class II by the biopharmaceutical classification system (BCS) due to its low solubility and its high intestinal permeability [53]. However, its aqueous solubility of ~0.7 $mg.mL^{-1}$ is just at the limit for considering VCZ as Class I. VCZ presents a minimal inhibitory concentration (MIC) against *A. fumigatus* of 1 $\mu g.g^{-1}$ lung weight [3]. VCZ is commercialized as Vfend® in the form of oral tablets, powder for oral suspension and powder for perfusion solution. The latter is formulated by means of sulfobutyl ether-β-cyclodextrins (SeβCD) to enhance VCZ solubility by formation of an inclusion complex. VCZ presents a high oral bioavailability (i.e., 96%), which can be drastically reduced by a concomitant meal to reach an oral bioavailability of 20% [54]. VCZ is highly metabolised (only 2% of VCZ was found unchanged in the urine) by the enzymes of hepatic cytochrome P450 2C19 and, to a lesser extent, 2C9 and 3A4. Its metabolites, with the main N-oxide, have no antifungal actions. Moreover, VCZ has a nonlinear pharmacokinetic due to the saturation of its metabolisation mechanism and its rate of binding to plasma proteins (approximately 58%). In plasma, VCZ concentration should be kept between 1 $\mu g.mL^{-1}$ and 5 $\mu g.mL^{-1}$ to obtain an optimal therapeutic action without severe side effects [55]. VCZ is generally well-tolerated by patients, but side effects may occur such as visual disturbances (photopsia in 20.7% of patients) and an increase in hepatic transaminases (observed in 12.4 % of cases) [56].

Pharmaceutical development for inhalation

The first study was performed by Tolman *et al*., which have nebulised a diluted Vfend® solution designed for the intravenous route to deliver VCZ into mouse lungs [57]. They adjusted the concentration of VCZ and SeβCD to obtain adequate pH, tonicity, and aerodynamic performances by nebulisation (**Table 1**). During the pharmacokinetic study, lung VCZ concentration was undetectable after 6-8h after nebulization due to the rapid clearance of this highly permeable drug by absorption into plasma (**Table 2**). Indeed, SeβCD increase

the aqueous solubility of VCZ, increasing its elimination by absorption. Consequently, high VCZ concentrations after inhalation were detected not only in the lungs but also in the plasma following a single or multiple dose administration, as revealed by the low lung to blood ratio (**Table 2**). Tolman *et al.* evaluated also the prophylactic efficacy of nebulised Vfend® solution in an immunocompromised murine model of IPA caused by *A. fumigatus* using nebulized SeβCD solution as a control. Both were delivered from 2 days prior to spore inoculation up to 7 days post-inoculation, and compared to intravenous amphotericin B deoxycholate solution (AmphB-deox) in a treated group delivered from 1 day after inoculation up to 7 days post-inoculation [58]. The prophylactic treatment showed a significant survival difference between the VCZ treated group and the control and AmphB-deox groups at 7 days post-inoculation as well as 12 days after the end of the therapy (**Table 2**). Nevertheless, this significant survival benefit was not linked to the fungal burden. Indeed, the fungal burden in mice 8 days post-inoculation was similar for each group. However, the control and AmphB-deox groups presented more severe invasive disease and abnormalities than the VCZ group, as revealed by lung histopathology. These studies revealed the prophylactic impact that inhaled VCZ could provide but also the high systemic exposure when delivering VCZ as a highly soluble inclusion complex solution by the pulmonary route.

Then, Beinborn *et al.* generated dry powders for inhalation containing either amorphous or crystalline VCZ by thin film freezing with or without a stabilizing agent (**Table 1**), respectively, followed by lyophilisation [59]. Dry powder for inhalation containing amorphous VCZ showed a significantly higher *in vitro* dissolution velocity (1.3 times) but also a significantly lower lung residence than the dry powder containing crystalline VCZ in mice (**Table 2**). Indeed, the amorphous state is thermodynamically less stable than its crystalline counterpart due to a lower ordered system, which increases its aqueous solubility and therefore its dissolution velocity. An increase of aqueous solubility promotes the absorption of this highly permeable drug through the lung epithelium, which decreases its lung residence. The crystalline-based dry powder for inhalation seems to be more suitable for pulmonary delivery, with better aerodynamic performances, lung residence and systemic exposure (**Table 1 and Table 2**).

Indeed VCZ presents an aqueous solubility that is at the limit of Class I and passes the lung epithelium easily. Therefore, formulation strategies able to control its release rather than its solubility could be more appropriate for pulmonary VCZ delivery **(Figure 1)**. Consequently, Sinha *et al.* developed dry powders for inhalation that present controlled-release properties (**Table 1**). VCZ was formulated as nonporous and porous nanoparticles with a poly-lactide-co-glycolide (PLGA), produced by multiple-emulsification processes followed by lyophilisation [60]. A drug release study revealed an initial burst effect, delivering 20% of VCZ within the first 2 hours, followed by a sustained release of drug during 15 days, without significant differences between porous and nonporous nanoparticles. The powder aerosol from a powder mixture of either porous or nonporous nanoparticles and micronized lactose were efficiently generated by a custom-made DPI (**Table 1**). Nonporous and porous nanoparticles were delivered in mouse lungs and VCZ was detectable at least until 5 days post-administration. The use of nanoparticles, which limited macrophage phagocytosis, and a sustained-release polymer showed interesting results in terms of increased lung residence of VCZ. However, PLGA is not yet approved for pulmonary drug delivery [61].

Itraconazole

ITZ is a weak base that is practically insoluble in water. Indeed, at neutral pH, its solubility is less than 10 $ng.mL^{-1}$ and at pH 1 it is 4 $\mu g.mL^{-1}$. Moreover, ITZ is highly lipophilic, as revealed by its octanol:water (pH 8.1) coefficient of 5.66 [62]. Consequently, ITZ presents low solubility and high permeability through the intestinal membranes, which classifies it as Class II in the BCS [55]. ITZ presents an MIC against *A. fumigatus* of 2 $\mu g.g^{-1}$ lung weight [3]. ITZ is commercialized as Sporanox® in the forms of capsule, oral or IV solution. In the solutions, ITZ is solubilized by means of hydroxyl-β-cyclodextrin (HPβCD) [63]. Due to its acid dependent solubility, ITZ bioavaibility is erratic. Indeed, in capsule form, the bioavailability of ITZ varies between 15 and 55% [3,64]. In oral solution, the ITZ-HPβCD inclusion complex increases solubility and therefore bioavailability to 20-30% [65]. However, the HPβCD-based formulation induced more gastrointestinal disorders. ITZ is mainly metabolised by the cytochrome P450 3A4 in the liver, and its main metabolite, hydroxy-ITZ, has antifungal properties equivalent to ITZ. Once absorbed, this molecule and its metabolite

are highly bound to plasma proteins, corresponding to 99.8% of the administered dose *per os*. Moreover, ITZ is an important CYP 450 3A4 inhibitor, inducing a strong possibility of interactions with other drugs metabolized by this enzyme. The majority of adverse effects associated with ITZ are due to its inhibition. Besides these interactions, nausea and vomiting (24% of cases), hepatotoxicity (8.5% of cases) and skin rash (5-19% of cases) are the most frequently observed side effects [3]. The minimal plasmatic concentration of ITZ should be 0.5-1 $mg.mL^{-1}$ for either prophylaxis or a therapeutic setting [3,54].

Pharmaceutical development for inhalation

ITZ presents a drastically lower aqueous solubility than VCZ, limiting its pharmacological action and lung residence. Indeed, undissolved ITZ particles are rapidly eliminated by the mucociliary escalator and macrophage phagocytosis (**Figure 1).** Therefore the formulation strategies are focused mainly on increasing its dissolution rate. Formulation strategies used to increase the dissolution rate of poorly water soluble drugs [41] such as ITZ are (i) inclusion complex into cyclodextrin, (ii) particle size reduction to the nanometer scale, (iii) amorphization of the drug, (iv) optimal wettability or a combination of the three latter strategies as (v) a solid dispersion, or in lipid-based carriers such as (vi) a nanostructured lipid carrier or (vii) polymeric micelles, as reported in **Table 1**. In this review, we focus only on the most complete and promising pharmaceutical development of ITZ for inhalation, in this case solid dispersions. However, other formulations are also described in **Table 1** and **Table 2**.

The first pharmaceutical development was performed by the McConville's research group, which generated inhalable amorphous ITZ nanoparticle-based aggregates by means of spray freezing into liquid (AIPI and AIP2) and compared them to inhalable crystalline ITZ nanoparticle-based aggregates by evaporative precipitation into aqueous solution (CIP) [66].

In vitro dissolution analysis revealed that the use of both surfactants improved the ITZ dissolution profile drastically, despite its crystalline (CIP) or amorphous (AIP2) state, in comparison with the use of polysorbate 20 (AIP1) or bulk ITZ (**Table 1**). Similar results were observed during the *in vivo* pharmacokinetic study on mice. Indeed, lung $AUC_{0\text{-}24h}$ (i.e., area under the curve between T=0 and T=24h, which reflects the lung residence) and lung $t_{1/2}$ (i.e., life half-time, which reflects the ITZ elimination rate) were similar for CIP and AIP2 whereas AIP1 showed a higher ITZ elimination, certainly due to a lower dissolution rate and higher

elimination by the mucociliary escalator (**Table 2**). The prophylactic efficacy of CIP and AIP2 was tested by nebulization in an immunocompromised murine model of IPA infected by *A. flavus* in comparison with the Sporanox® solution delivered by the oral route [67]. All formulations were delivered from one day prior to spore inoculation, for 12 days. AIP2 showed the highest median survival in comparison with CIP; both were much higher than the median survival of the Sporanox®-treated and control groups (**Table 2**). This superiority of AIP2 seems to be because of a higher lung exposure and lower lung elimination observed in comparison with those from CIP and Sporanox®, respectively. Then, Vaughn *et al.* studied the systemic exposure and side effects of pulmonary delivery of AIP2-based dispersion [68-69]. Higher ITZ concentrations in lung tissue and lower plasmatic concentrations were observed after repeated pulmonary delivery of AIP2 by nebulization in comparison with repeated oral delivery of Sporanox® solution (i.e., inclusion complex with HPβCD), resulting in a drastically higher lung-to-serum ratio [70]. AIP2 administrated by oral route showed no difference in comparison with the Sporanox® solution in terms of lung concentrations or serum concentrations but showed lower side effects. Indeed, oral administration of the Sporanox® solution was associated with several side effects such as diarrhoea and dehydration due to the presence of HPβCD [68]. Furthermore, Vaughn *et al.* examined the inflammatory response in mouse lungs and showed no inflammatory response from the pulmonary nebulization of AIP2 or from their excipients (i.e., poloxamer 407 or polysorbate 80) [69]. Finally, AIP2 was tested by nebulization in an immunocompromised murine model of IPA infected with *A. fumigatus* [70]. Pulmonary delivery of AIP2 at 30 $mg.kg^{-1}$ twice a day by nebulization or oral delivery of the Sporanox® solution at the same dosage three times a day were delivered two days before spore inoculation, for 14 days. The median survival and percentage of survival at day 12 of AIP2 were significantly higher in comparison with the Sporanox®-treated or the control group (**Table 2**). Moreover, the AIP2-treated mice showed fewer necrotic foci and vascular lesions but a similar fungal burden compared with the control group. Consequently, reducing particle size, using the amorphous state and using an adequate surfactant system for optimal ITZ wettability are interesting tools for increasing the dissolution rate of ITZ and therefore reaching much higher lung exposure and residence to obtain an optimal prophylactic activity against IPA.

Yang *et al.* applied another processing system, the ultra-rapid freezing technique, to generate amorphous ITZ nanoparticle-based aggregates (AIN) [71]. This technique differs from spray freezing into liquid process in that it uses rapid freezing of a drug/excipient solution onto a cryogenic substrate of desired thermal conductivity to obtain a solid dispersion/solution. The excipients used were mannitol and lecithin. Mannitol acted as a hydrophilic excipient, aiding water to penetrate and dispersing ITZ nanoparticles from the aggregates. Lecithin presents surface-active properties that improved ITZ wettability and therefore the ITZ surface area in contact with the dissolution medium. During a pharmacokinetic study in mice similar to those conducted by Vaughn *et al.* 2006, they observed a higher lung C_{max}, shorter t_{max} and a 10-times higher serum C_{max} [68,71]. These results revealed a drastic increase in the absorption constant of ITZ from AIN in comparison with AIP2. This absorption increase is attributed to the presence of lecithin. Lecithin is composed mainly of phosphatidylcholines, comprising 70-80% of those occurring in the lung surfactant. Exogenous phospholipids are known to be able to increase absorption by decreasing the bilayer stability [72]. They estimated that appropriate size reduction, supersaturated ITZ concentration and the presence of absorption enhancers such as lecithin are responsible for the drastic increase in the ITZ absorption rate. In the aim to evaluate the impact of supersaturated ITZ concentration, Yang *et al.* compared the dissolution improvement in supersaturation conditions of AIN with the dissolution of crystalline ITZ nanoparticles produced by wet-milling (CIN) that had a similar surface area [73]. AIN was able to supersaturate the simulated lung fluid 27 times more than CIN after 15 and 30 min, the latter being at its saturation solubility at the equilibrium (Seq). This supersaturation concentration then decreased progressively to 7 times after 2 h and 5 times after 24h. An *in vivo* pharmacokinetic study on rats then revealed a similar lung deposition after nebulization of both formulations, with a similar elimination after 24 h. However, the plasma C_{max} and $AUC_{0\text{-}24h}$ of ITZ were nearly 4 times higher for AIN than CIN. These results revealed that there was a higher ITZ dissolved part in the lung, leading to a higher absorption into the blood, while the ITZ from CIN was mainly undissolved and eliminated by the non-absorptive clearance systems such as the mucociliary escalator. Then, Yang *et al.* evaluated the impact of lecithin in the ITZ absorption by comparing the lung and serum pharmacokinetic from a nebulized solution of amorphous ITZ-based inclusion complex with

HPβCD (ICD) with that from AIN [74]. Suitable aerodynamic performances were obtained for ICD and AIN (**Table 1**). Lung pharmacokinetic data revealed a similar C_{max} and lower elimination, leading to a slightly higher lung $AUC_{0\text{-}24h}$ for ICD in comparison with AIN (**Table 2**). In terms of serum pharmacokinetic data, AIN showed higher systemic exposure than ICD, with a lower $t_{1/2}$ into the blood from the lung, leading to a higher $AUC_{0\text{-}24h}$ (**Table 2**). Indeed, despite the dissolution step of AIN not being encountered with ICD, higher ITZ concentrations were detected over time in serum than from ICD, revealing an increase in the absorption rate related to an increase in permeability. The latter was certainly due to the permeation enhancing properties of lecithin. From these studies, Yang *et al.* demonstrated the advantage of the dissolution rate of the amorphous state of ITZ in the dissolved part of ITZ in the lung but resulting also in a higher elimination by absorption. Moreover, they showed the permeability enhancer properties of lecithin.

Recently, Duret *et al.* produced solid-dispersion-based dry powders for inhalation. They did so by means of spray-drying with the aim of increasing the ITZ dissolution rate by reducing the size to the nanometer scale, using the amorphous state and generating optimal wettability/hydrophilic environment by using surfactant and/or hydrophilic matrix. The spray-drying process is an easily scaled-up one-step process, contrary to the spray freezing into liquid method. Spray-drying converts atomized liquid droplets to respirable dried microparticles of 1-5 μm. Moreover, compared to the use of liquid formulations aerosolized by nebulisers, the use of dry powder medicines for inhalation could reduce administration time and environmental drug propagation, increase long-term drug stability and lung-deposited dose and be produced with disposable devices, limiting the maintenance procedure. Solid dispersions were produced from hydro-alcoholic solutions containing amorphous ITZ and mannitol with or without surfactant (i.e., tocopherol polyethylene 1000 succinate or Phospholipon® 90H) [75-76]. Moreover, the resulting dried microparticles presented good aerodynamic performances and improved ITZ dissolution properties (**Table 1 and Table 2**). Indeed, the presence of amorphous ITZ in the mannitol matrix increased the dissolution profile significantly in comparison with crystalline or amorphous ITZ without hydrophilic matrix. Moreover, the presence of 10% of Phospholipon® 90H related to ITZ weight had a significant positive impact on the dissolution profile in comparison with solid dispersions

without Phospholipon® 90H [76]. A pharmacokinetic study in mice was performed to evaluate solid dispersions made with crystalline ITZ (CI) or amorphous ITZ with (AI90H) or without (AI) 300% Phospholipon® 90H in relation to ITZ weight [77]. In terms of solubility, IC showed no improvement in solubility, while AI and AI90H presented supersaturated solubilities 45 times and 50 times higher than Seq at 30 min and at 5 min, respectively. However, supersaturation levels decreased over time to lower values (44 and 87 $ng.ml^{-1}$ at 24h, respectively). Consequently, the dissolution profiles of AI and AI90H were drastically and significantly improved in comparison with CI. Moreover, the presence of Phospholipon® 90H significantly improved the dissolution profile of AI90H in comparison with AI. Lung and plasma pharmacokinetic studies revealed higher lung $AUC_{0\text{-}24h}$ for AI compared to CI and AI90H (**Table 2**) corresponding on dissolved and undissolved part of ITZ in the lungs. The plasma concentrations reflect the dissolved part of ITZ able to be absorbed. The highest plasma exposures are seen with AI and AI90H in comparison with CI (**Table 2**). AI provided the best balance between sufficient lung residence and dissolution rate of ITZ. Indeed, AI90H showed too fast elimination by absorption due to excessive dissolution rate of ITZ and CI showed rapid elimination by the mucociliary escalator due to insufficient dissolution, as revealed by their respective lung $t_{1/2}$ (**Table 2**). Moreover, the presence of Phospholipon® 90H (i.e., saturated phosphatidycholine including 85% of 1,2-distearoyl-*sn*-glycero-3-phosphocholine and 15% of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) not only increased the ITZ dissolution rate, it could also increase the ITZ permeability through the lung. Finally, Duret *et al.* tested the prophylactic efficacy of AI in an immunocompromised murine model of IPA infected with *A. fumigatus* [78]. AI at 5 $mg.kg^{-1}$ every 48h was insufflated into mouse lungs and was compared with a non-treated group and a group treated orally with 12.5 $mg.kg^{-1}$ of VCZ twice a day. All treatments began two days prior the spore inoculation and lasted for 7 days. Survival curves showed a significant increase in the survival rate in comparison with the non-treated and oral VCZ treated group. Moreover, AI showed similar prophylactic efficacy, with a higher survival percentage than the conventional oral VCZ treatment (**Table 2**).

Conclusion

Inhalation of triazoles, such as ITZ and VCZ, is promising for preventing or treating IPA by controlling dissolution characteristics (improving in the case of ITZ and prolonging release in the case of VCZ) and by escaping clearance systems for insoluble ITZ and for controlled release VCZ delivery systems. Moreover, pulmonary administration allows systemic exposure and therefore side effects or drug-drug interactions to be decreased while increasing therapeutic activity by targeting the infection locus directly *in situ*. However, the residence time of the solubilized drug in the lungs is the cornerstone of the formulation strategies to aim for the balance between pharmacological action and elimination. According to established guidelines, this strategy may be also applied to posaconazole and fluconazole to prevent or treat other kinds of aspergillosis or other pulmonary fungal infections such as candidiasis, hystoplasmosis, mucormycosis, cryptococcosis, blastomycosis and coccidiodomycosis.

Acknowledgment

The authors declare no conflict of interest.

Legends of Tables

Table 1. Description of formulations developed for inhalation with voriconazole (VCZ) or itraconazole (ITZ), their aerosol performances with the respective inhalation device characterized by the mass median aerodynamic diameter (MMAD) and the fine particle fraction (FPF), the excipients used and their approval status for pulmonary drug delivery and the main properties observed in terms of solubility, dissolution and/or pharmacokinetics.

Triazole	Formulation	Inhalation device	MMAD (µm)	FPF (%)	Excipient (approval status)	Properties	Reference
VCZ	SeβCD-based solution	Aeroneb Pro micropump jet nebulizer	2.98	72	SeβCD (NA)	excessive absorption and systemic exposure	[57]
	amorphous VCZ nanostructured aggregate-based dry powder	Handihaler DPI	5.2	32	polyvinylK25 (A)	excessive absorption and systemic exposure	[59]
	crystalline VCZ microstructured-based dry powder		4.2	38	/	more suitable lung residence and systemic exposure	
	PLGA nanoparticle-based dry powder	hand-made DPI	2.29-2.58	NR	PLGA (NA), lactose carrier (A)	*in vitro* and *in vivo* controlled release properties	[60]
ITZ	aqueous suspension of amorphous ITZ nanoparticle-based aggregates= AIP1	Aeroneb Pro micropump jet nebulizer	2.76	71	polysorbate 20 (A)	improved slightly dissolution rate, increased slightly the lung residence and serum exposure	[66]
	aqueous suspension of amorphous ITZ nanoparticle-based aggregates = AIP2		2.82	85	poloxamer 407 (NA), polysorbate 80 (A)	improved dissolution rate, increased lung residence and serum exposure	
						no local inflammation	[69]
	aqueous suspension of crystalline ITZ nanoparticle-based aggregates = CIP		2.70	76	poloxamer 407 (NA), polysorbate 80 (A)	equivalent lung residence to AIP2	[66]
	aqueous suspension of amorphous ITZ nanoparticles = AIN		2.40	54	lecithin (A), mannitol (A)	supersaturation solubility, higher absorption and systemic exposure	[71,73]
	HPβCD-based solution = ICD		3.19	53	HPβCD (NA)	soluble ITZ, high absorption and systemic exposure	[73]

	aqueous suspension of crystalline ITZ nanoparticles = CIN		2.80	47	/	equivalent lung residence to AIN but lower solubility and therefore lower systemic exposure	[72]
	solid dispersion-based dry powder	Axahaler DPI	1.61-4.5	16-47	mannitol (A) with or without tocopherol PEG1000 succinate (NA)	supersaturation solublity, improved dissolution rate with or without surfactant	[75,76]
			NR	43-67	mannitol (A), with or without Phospholipon® 90H (GRAS)	improved dissolution rate due 10% Phospholipon® 90H	[75,76]
			1.85-2.10	NR	mannitol (A), with or without Phospholipon® 90H (GRAS)	lower residence time and higher lung elimination with solid dispersion based on Phospholipon® 90H or crystalline ITZ	[77]
	crystalline ITZ nanoparticle-based dry powder		2.89-3.47	46-63	mannitol (A), tocopherol PEG1000 succinate (NA), taurocholate Na (NA)	supersaturation solublity, improved dissolution rate	[79]
	aqueous suspension of crystalline ITZ nanoparticle-	jet/ultrasonic/vibrating mesh nebulizers	NR	NR	Poloxamer 188 (NA) or Solutol H15 (NA) or polysorbate 80 (A)	no macroscopic or microscopic observation of inflammation, high lung-to-serum ratio due to ITZ dissolved part and lower lung-to-serum ratio due to the undissolved part acting as a reservoir	[80]
	aqueous suspension of nanostructured lipid carrier	PARI Boy junior jet nebulizer and beurer IH50 ultrasonic nebulizer	NR	NR	Precirol ATO 5 (NA), acid oleic (A), polysorbate 20 (A)	long-term stability, no controlled release properties, stable during nebulization	[81]
	aqueous solution of chitosan micelles	Hudson jet nebulizer	NR	38-47	hydrophobically-modified chitosan (NA)	stable during nebulization, controlled release properties	[82]
	chitosan nanoparticles encapsulating HPβCD-ITZ complex-based dry powder	Cyclohaler DPI	NR	16-43	chitosan (NA), HPβCD (NA)	*in vitro* burst effect followed by controlled release properties, ability to be aerosolized after co-spray drying with lactose, mannitol and/or leucine	[83]

A = approved for pulmonary administration, DPI = dry powder inhaler, FPF = fine particle fraction, GRAS = generally recognized as safe, MMAD = mass median aerodynamic diameter, NR = non-reported NA = not approved for pulmonary administration

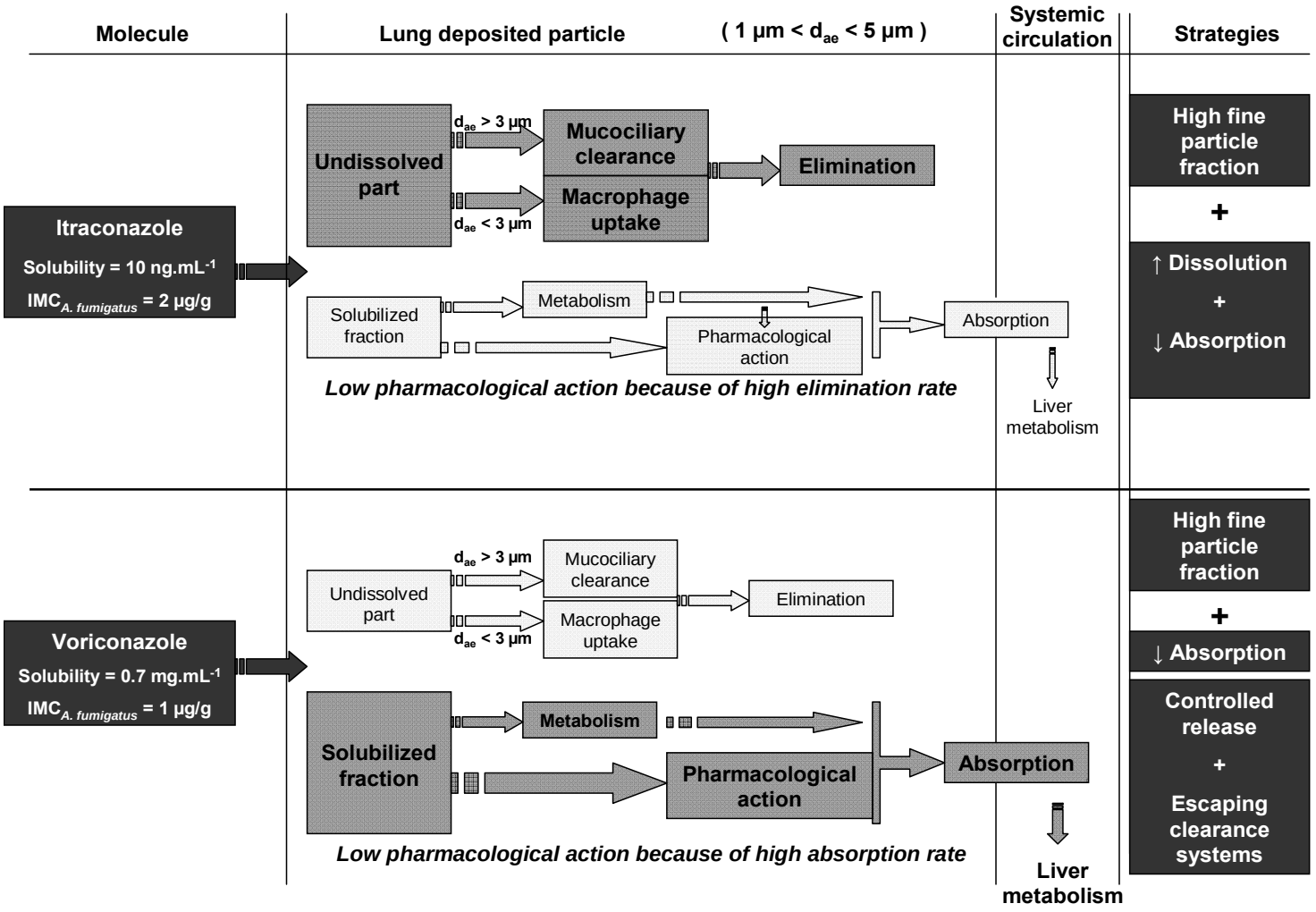
Table 2. Description of formulations developed for inhalation with voriconazole (VCZ) or itraconazole (ITZ), their *in vitro* solubility or dissolution improvements, their lung and systemic pharmacokinetic data revealing the lung exposure (lung $AUC_{0\text{-}24h}$) and lung elimination ($t_{1/2}$) and the systemic exposure (blood $AUC_{0\text{-}24h}$) and the lung-to-blood ratio (lung $AUC_{0\text{-}24h}$/blood $AUC_{0\text{-}24h}$) revealing the difference between the lung and the systemic exposures. However, lung concentration includes dissolved and undissolved drug. The maximal blood concentration reflects the dissolved part of drug which is highly permeable into the lungs.

Triazole	Formulation	*In vitro* solubility	Pharmacokinetic study					Prophylactic efficacity study			Reference
			Lung $AUC_{0\text{-}24h}$ ($\mu g.h.g^{-1}$)	Lung $t_{1/2}$ (h)	Blood $AUC_{0\text{-}24h}$ ($\mu g.h.ml^{-1}$)	Blood C_{max} ($\mu g.ml^{-1}$)	Lung to blood ratio	Preclinical model	Mean survival	% of long-term survival	
VCZ	SeβCD-based solution	soluble inclusion complex	$AUC_{0\text{-}6h}$: 3.4 (HFR) vs 40.1 (LFR)	NR	$AUC_{0\text{-}6h}$: 2.3 (HFR) vs 25.8 (LFR)	1.2 (HFR) vs 7.1 (LFR)	1.5 (HFR) vs 1.6 (LFR)	murine model of IPA by *A. fumigatus*	> 12 days vs 7 days (AmphBdeox) or 7.5 days (CRL)	67% vs 23% (AmphBdeox) or 17% (CRL)	[57-59]
	amorphous VCZ nanostructured aggregate-based dry powder	1.3-time higher velocity (amorphous ITZ vs crystalline ITZ)	232.1		18.6	7.04	12.5	NR			[59]
	crystalline VCZ microstructured-based dry powder		452.6		38.4	6.29	11.8				
ITZ	amorphous ITZ nanoparticle-based aggregates = AIP1	Slightly improved dissolution rate	18.9	2.3	NR	NR	ND				[66]

	amorphous ITZ nanoparticle-based aggregates = AIP2	improved dissolution rate	85.8	5.5	1.69	0.12	50.8	murine model of IPA by *A. fumigatus*	7.5 days vs 6.5 days (CRL) or 5 days (oral Sporanox)	35% vs 10% (CRL)or 0% (oral Sporanox)	[66,68,70]
			28.0	2.9	NR	NR	ND	murine model of IPA by *A. flavus*	>20 days (AIP2) vs 11 days (CIP) vs 4 days (oral sporanox) or 5 days (CRL)	60%(AIP2) vs 40% (CIP) vs 0% (CRL or oral Sporanox)	[67]
	crystalline ITZ nanoparticle-based aggregates = CIP		99.7	6.7	NR	NR	ND				[66,67]
	amorphous ITZ nanoparticles = AIN	27-7 times higher Seq	126.74	7.44	5.53	1.64	22.9	NR			[71]
			27.2	2.91	4.17	0.55	6,5				[73,74]
	HPβCD-based solution =ICD	5.3 mg.ml^{-1}	32.0	4.87	3.26	0.48	9.8				
	crystalline ITZ nanoparticles = CIN	10 ng.ml^{-1} (Seq)	NR		0.662	0.05	ND				[73]
	crystalline ITZ solid dispersion-based dry powder	10 ng.ml^{-1} (Seq)	218.4	6.5	0.182	88	1200				[77]
	amorphous ITZ solid dispersion-based dry powder	45 times higher Seq	332.6	14.7	0.491	249	677	murine model of IPA by *A. fumigatus*	6 days vs 4 days (oral VCZ and CRL)	50% vs 25% (oral VCZ) or 0% (CRL)	[77,78]
	amorphous ITZ solid dispersion with Phospholipon® ^{9}0H-based dry powder	50 times higher Seq	143.8	4.1	0.3768	352	382	NR			[77]
	crystalline ITZ suspension	NR	828.767	25.4	1.761	0.058	471				[80]

AUC = area under curve, C_{max} = maximal concentration peak, CRL = control, HFR = high flow rate , LFR = low flow, ND = non-determined, NR = non-reported, rate, , Seq = equilibrium solubility, $t_{1/2}$ = half life,

Figure 1. Fate and formulation strategies of itraconazole and voriconazole after lung deposition



Disclaimer The findings and conclusions in this report are those of the author(s).

Compliance with Ethics Guidelines

Conflict of Interest All authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

[1] Maschmeyer G, Jaas A, Cornely OA. Invasive Aspergillosis – epidemiology, diagnosis and management in immunocompromised patients. Drugs. 2007;67(11):1567-1601

[2] Thompson GR, Patterson T. Pulmonary aspergillosis. Semin Respi Crit Care Med. 2008;29:103-110

[3] Lass-Flörl C, Triazole antifungal agents in invasive fungal infections, a comparative review. Drugs. 2011;71(18):2405-2419

[4] Díaz Sánchez C, López Viña A. Pulmonary Aspergillosis. Arch Bronconeumol. 2004;40(3):114-122

[5] Morris G, Kokki MH, Anderson K, Richardson MD. Sampling of Aspergillus spores in air. J Hosp Infect. 2000;44:81-92

[6] Latgé JP. Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev. 1999;12(2):310-350

[7] Dagenais TRT, Keller NP. Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clin Microbiol Rev. 2009;22:447-465

[8] McConville JT, Wiederhold NP. Invasive pulmonary aspergillosis: therapeutic and prophylactic strategies. In: Williams III RO, Taft DR, McConville JT editors. Advanced Drug Formulation Design to Optimize Therapeutic Outcomes. Informa Healthcare; 2008;172. pp. 53-80.

[9] Hasenberg M et al. Phagocyte responses towards Aspergillus fumigatus. Int J Med Microbiol. 2011;301:436-444

[10] Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest Journal. 2002;121(6):1988-1999

[11] Amchentsev A, Kurugundla N, Saleh AG. Aspergillus-related lung disease. Respir Med CME. 2008;1:205-215

[12] Stevens DA et al. Practice guidelines for diseases caused by Aspergillus. Clin Infect Dis. 2000;30:696-709

[13] Sheppard DC. Molecular mechanism of Aspergillus fumigatus adherence to ghost constituents. Curr Opin in Microbiol. 2011;14:375-379

[14] Segal BH, Walsh TJ. Current approaches to diagnosis and treatment of invasive aspergillosis: State of the art. Am J Respir Crit Care Med. 2006;173:707-717

[15] Traunmüller F et al. Efficacy and safety of current drug therapies for invasive aspergillosis. Pharmacology. 2011;88:213-224

[16] Walsh TJ, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2008;46:327-360

[17] Herbrecht R et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347(6):408-415

[18] Yang W, Wiederhold NP, Williams RO. Drug delivery strategies for improved azole antifungal action. Expert Opin Drug Deliv. 2008;5(11):199-1216

[19] Perfect JR, Dodds Ashley E, Drew R. Design of aerosolized amphotericin B formulation for prophylaxis trials among lung transplant recipients. Clin Infect Dis. 2004;39:207-210

[20] Onoue S, Misaka S, Kawabata Y, Yamada S. New treatments for chronic obstructive pulmonary disease and viable formulation/device options for inhalation therapy. Expert Opin Drug Deliv. 2009;6:793-811

[21] Ibrahim BM, Tsifansky MD, Yang Y, Yeo Y. Challenges and advances in the development of inhalable drug formulations for cystic fibrosis lung disease. Expert Opin Drug Deliv. 2011;8:451-466

[22] Sears MR, Lotvall J. Past, present and future beta-2-adrenoreceptor agonist in asthma management. Respi Med. 2005;99:152-170

[23] Smyth HDC, Saleem I, Donovan M, Verschraegen CF. Pulmonary delivery of anti-cancer agents. In: Williams III RO, Taft DR, McConville JT editors. Advanced Drug Formulation Design to Optimize Therapeutic Outcomes. Informa Healthcare; 2008;172. pp. 81-112.
[24] Depreter F, Pilcer G, Amighi K. Inhaled proteins: challenges and perspectives. Int J Pharm. 2013;447:251-280
[25] Carvalho TC, Peters JI, Williams III RO. Influence of particle size on regional lung deposition – What evidence is there? Int J Pharm. 2011;406:1- 10
[26] Hofmann W. Modelling inhaled particle deposition in the human lung – a review, J Aerosol Sci. 2011;42:693-724
[27] El-Sherbiny IM, Villanueva DG, Herrera D, Smyth HDC. Overcoming lung clearance mechanisms for controlled release drug delivery. In: Smyth HDC, Hickey AJ editors. Controlled Pulmonary Drug Delivery. Springer; 2011. pp. 101-126.
[28] Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. J Clin Pharmacol. 2003;56:588-599.
[29] O'Donnel P, Smyth HDC. Macro- and microstructure of the airways fro drug delivery. In: Smyth HDC, Hickey AJ editors. Controlled Pulmonary Drug Delivery. Springer; 2011. pp. 1-19.
[30] M. Evans C, Koo JS. Airway mucus: the good, the bad, the sticky. Pharmacol Ther. 2009;121:332-348
[31] Harmsen AG,. Muggenburg BA, Snipes MB, Bice DE. The role of macrophages in particle translocation from lungs to lymph nodes. Science. 1985;230:1277-1280
[32] Wang YB, Watts AB, Peters JI, Williams III RO. The impact of pulmonary disease on the fate of inhaled medicines – a review. Int J Pharm. 2014;461:112-128
[33] Chono S, Tanino T, Seki T, Morimoto K, Influence of particle size on drug delivery to rat alveolar macrophages following pulmonary administration of ciprofloxacin incorporated to liposomes. J Drug Target. 2006;14:557-566
[34] Groneberg DA, Witt C, Wagner U, Chung KF, Fischer A. Fundamentals of pulmonary drug delivery. Respir Med. 2003;97:382-387

[35] Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale and clinical applications, existing and potential. Int J Nanomedicine. 2006;1(3):297-315

[36] Olsson B, Bondesson E, Borgström L, et al. Pulmonary drug metabolism, clearance and absorption. In: Smyth HDC, Hickey AJ editors. Controlled Pulmonary Drug Delivery. Springer; 2011. pp. 36-50.

[37] Wiedmann TS, Bhatia R, Wattenberg LW, Drug solubilisation in lung surfactant. J Control Release. 2000;65:43-47

[38] Patton JS. Mechanism of macromolecule absorption by the lungs. Adv Drug Deliv Rev. 1996;19:3-36

[39] Patton JS, Brain JD, Davies LA, et al. The particle has landed – characterizing the fate of inhaled pharmaceuticals. J Aerosol Med Pulm Drug Deliv. 2010;23(2):71-87

[40] Pilcer G. Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm. 2010;392:1-19

- [41] Williams HD et al. Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 2013;65(1):315-499.

Performed an overview of different strategies for poorly water-soluble drug.

[42] Tolman JA, Williams III RO. Advances in the pulmonary delivery of poorly water-soluble drugs: influence of solubilisation on pharmacokinetic properties. Drug Dev Ind Pharm. 2010;36(1):1-30

[43] Ruge CA, Kirch J, Lehr CM. Pulmonary drug delivery: from generating aerosols to overcoming biological barriers – therapeutic possibilities and technological challenges. Lancet Respir Med. 2013;1:402-413

[44] US Food and Drug Administration. 2014. http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=scogsListing&displayAll=true. Accessed June 2014.

[45] Buggins TR, Dickinson PA, Taylor G. The effects of pharmaceutical excipients on drug deposition. Adv Drug Deliv Rev. 2007;59:1482-1503

[46] US Food and Drug Administration. 2014. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073395.pdf. Accessed June 2014

[47] Baldrick P. Pharmaceutical excipient development: the need for preclinical guidance. Regul Toxicol Pharmacol. 2000; 32:210-218

[48] Baldrick P. Pharmaceutical excipient development: a preclinical challenge. In: Katdare A, Chaubal MV. Excipient Development for Pharmaceutical Biotechnology and Drug Delivery System. Informa Healthcare; 2006. pp. 15-36.

[49] Baldrick P. The safety of chitosan as a pharmaceutical excipient. Regul Toxicol Pharmacol. 2010;56:290-299

[50] Islam N, Gladki E. Dry powder inhalers (DPIs) – a review of device reliability and innovation. Int J Pharm. 2008; 360:1-11

[51] Dolovich MB, Ahrens RC, Hess DR, et al. Device selection and outcomes of aerosol therapy: evidence based guidelines. Chest. 2005;127:335-371

[52] Dolovich MB, Dhand R. Aerosol drug delivery: developments in device design and clinical use. Lancet. 2011;377:1032-1045

[53] US Food and Drug Administration. 2014. http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm128219.htm. Accessed June 2014.

[54] Goodwin ML, Drew RH. Antifungal serum concentration monitoring: an update. J Antimicrob Chemother. 2008;61:17-25

[55] Pascual A et al. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis. 2008;46:201-211

[56] Vfend® characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000387/WC500049756.pdf. Accessed June 2014.

[57] Tolman JA et al. Characterization and pharmacokinetic analysis of aerosolized aqueous voriconazole solution. Eur J Pharm Biopharm. 2009;72:199-205

[58] Tolman JA et al. Inhaled voriconazole for prevention of invasive pulmonary aspergillosis. Antimicrob Agents Chemother. 2009;53(6):2613-2615

[59] Beinborn NA, Du J, Wiederhold NP, Smyth HDC, Williams III RO. Dry powder insufflation of crystalline and amorphous voriconazole formulations produced by thin film freezing to mice. Eur J Pharm Biopharm. 2012;81(3):600-608

[60] Sinha B, Mukherjee B, Pattnaik G. Poly-lactide-co-glycolide nanoparticles containing voriconazole for pulmonary delivery: in vitro and in vivo study. Nanomedicine. 2013;9:94-104

[61] US Food and Drug Administration. 2014. http://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm. Accessed June 2014.

[62] O'Neil MJ. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th ed. New Jersey: Merck, 2006.

[63] Sporanox® characteristics. bijsluiters.fagg-afmps.be/DownloadLeafletServlet?id=123908. Accessed June 2014.

[64] Prentice AG, Glasmacher A. Making sense of itraconazole pharmacokinetics. J Antimicrob Chemother. 2005;56:17-22

[65] De Beule K, Van Gestel J. Pharmacology of itraconazole. Drugs. 2001;61:27-37

[66] McConville JT et al.Targeted high lung concentration of itraconazole using nebulized dispersions in a murine model. Pharm Res. 2006,23(5):901-911

[67] Hoeben BJ et al. In vivo efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis. Antimicrob Agents Chemother. 2006;50(4):1552-1554

[68] Vaughn JM et al. Single dose and multiple dose studies of itraconazole nanoparticles. Eur J Pharm Biopharm. 2006;63:95-102

[69] Vaughn JM et al. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int J Pharm. 2007;38:219-224

[70] Alvarez CA et al. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J Infect. 2007;55:68-74

[71] Yang W et al. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. Int J Pharm. 2007;361:177-188

[72] Wauthoz N, Amighi K. Phospholipids in pulmonary drug delivery. Eur J Lipid Sci Technol. 2014; DOI:10.1002/ejlt.201300368

[73] Yang W, Johnston KP, Williams III RO. Comparison of bioavailability of amorphous versus crystalline itraconazole nanoparticles via pulmonary administration in rats. Eur J Pharm Biopharm. 2010;75:33-41

[74] Yang W et al. In vitro characterization and pharmacokinetics in mice following pulmonary delivery of itraconazole as cyclodextrin solubilized solution. Eur J Pharm Sci. 2010;39:336-347

[75] Duret C, Wauthoz N, Sebti T, Vanderbist F, Amighi K, Solid dispersion of itraconazole for inhalation with enhanced dissolution, solubility and dispersion properties. Int J Pharm. 2012;428:103-113

[76] Duret C, Wauthoz N, Sebti T, Vanderbist F, Amighi K. New respirable and fast dissolving itraconazole dry powder composition for the treatment of invasive pulmonary aspergillosis. Pharm Res. 2012;29:2845-2859

- [77] Duret C, et al. Pharmacokinetic evaluation in mice of amorphous itraconazole-based dry powder formulations for inhalation with high bioavailability and extended lung retention. Eur J Pharm Biopharm. 2014;86:46-54

Study demonstrating the various steps of the characterization of a formulation intended to be administered by the pulmonary route from in vitro point of view to pharmacokinetic study on mice.

[78] Duret C, Wauthoz N, Rosière R, Sebti T, Vanderbist F, Amighi K. Prophylactic efficacy of inhaled itraconazole-mannitol dried solid dispersion against invasive pulmonary aspergillosis. Respiratory drug delivery Europe May 2013

[79] Duret C, Wauthoz N, Sebti T, Vanderbist F, Amighi K. New inhalation-optimized itraconazole nanoparticle-based dry powders fro the treatment of invasive pulmonary aspergillosis. Int J Nanomedicine. 2012;7:5475-5489

[80] Rundfeldt C, Steckel H, Scherliess H, Wyska E, Wlaz P. Inhalable highly concentrated itraconazole nanosuspension for the treatment of bronchopulmonary aspergillosis. Eur J Pharm Biopharm. 2013;83:44-53

[81] Pardeike J, et al. Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. Int J Pharm. 2011;419:329-338

[82] Moazeni E, et al. Preparation and evaluation of inhalable itraconazole chitosan based polymeric micelles. Daru. 2012;20:85

[83] Jafarinejad S, et al. Development of chitosan-based nanoparticles for pulmonary delivery of itraconazole as dry powder formulation. Powder Technol. 2012;222:65-70