Drug Resistance Updates 17 (2014) 37–50

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

The role of azoles in the management of azole-resistant aspergillosis: From the bench to the bedside

Seyedmojtaba Seyedmousavi[a,b], Johan W. Mouton[a,b], Willem J.G. Melchers[a], Roger J.M. Brüggemann[c], Paul E. Verweij[a,*]

[a] Department of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands
[b] Department of Medical Microbiology and Infectious Diseases, Erasmus MC, The Netherlands
[c] Department of Pharmacy, Radboudumc, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 21 June 2014
Accepted 29 June 2014

Keywords:
Aspergillus fumigatus
Invasive aspergillosis
Voriconazole
Posaconazole
Azole-resistance
Management

ABSTRACT

Azole resistance is an emerging problem in *Aspergillus fumigatus* and is associated with a high probability of treatment failure. An azole resistance mechanism typically decreases the activity of multiple azole compounds, depending on the mutation. As alternative treatment options are limited and in some isolates the minimum inhibitory concentration (MIC) increases by only a few two-fold dilutions steps, we investigated if voriconazole and posaconazole have a role in treating azole-resistant *Aspergillus* disease. The relation between resistance genotype and phenotype, pharmacokinetic and pharmacodynamic properties, and (pre)clinical treatment efficacy were reviewed. The results were used to estimate the exposure needed to achieve the pharmacodynamic target for each MIC. For posaconazole adequate exposure can be achieved only for wild type isolates as dose escalation does not allow PD target attainment. However, the new intravenous formulation might result in sufficient exposure to treat isolates with a MIC of 0.5 mg/L. For voriconazole our analysis indicated that the exposure needed to treat infection due to isolates with a MIC of 2 mg/L is feasible and maybe isolates with a MIC of 4 mg/L. However, extreme caution and strict monitoring of drug levels would be required, as the probability of toxicity will also increase.

1. Introduction

Azole resistance is an emerging problem in species of the genus *Aspergillus* (Denning and Perlin, 2011; Snelders et al., 2008). The polyphasic approach to the taxonomic classification of aspergilli has resulted in the recognition of new species (Peterson et al., 2008). These new or sibling species are difficult to identify using conventional methods, often requiring molecular techniques (Alcazar-Fuoli et al., 2008; Geiser et al., 2007). Recent epidemiologic research indicates that sibling species of *Aspergillus* may cause invasive aspergillosis in susceptible hosts (Balajee et al., 2005; Gerber et al., 1973; Guarro et al., 2002; Hedayati et al., 2007; Jarv et al., 2004; Latge, 1999). Many of these species show a susceptibility profile that differs from the conventional species, usually with reduced activity of specific antifungal agents (Alcazar-Fuoli et al., 2008).

In addition to intrinsic resistance within the aspergillus family (van der Linden et al., 2011a), there are increasing reports of acquired resistance to azoles (Denning and Perlin, 2011). The majority of reports concern *Aspergillus fumigatus* (Verweij et al., 2009a), although azole resistance has been reported sporadically in other species as well, such as *A. flavus* (Liu et al., 2012) and *A. terreus* (Arendrup et al., 2012b).

In *A. fumigatus* two routes of resistance selection have been reported; Azole resistance has been reported in patients with chronic cavitating aspergillus diseases that receive long-term azole therapy (Howard et al., 2009). In these patients the initial infection is caused by an azole-susceptible isolate, but through therapy azole-resistant isolates may be cultured. A second route of resistance selection is believed to occur through exposure of *A. fumigatus* to azole compounds in the environment (Snelders et al., 2008, 2009, 2012; Verweij et al., 2009b). Azoles are commonly used for crop protection or material preservation. Some of the fungicides were found to have a molecule structure very similar to that of the medical triazoles (Snelders et al., 2012; Verweij et al., 2009b). The fungus is believed to develop mutations that confer resistance to fungicides, but due to the molecule similarity with the medical triazoles, the latter become inactive as well.

A wide range of mutations in *A. fumigatus* have been described conferring azole-resistance commonly involving modifications in the *cyp51A*-gene, the target of antifungal azoles. Cyp51A mutations

* Corresponding author.
E-mail address: Paul.Verweij@Radboudumc.nl (P.E. Verweij).

http://dx.doi.org/10.1016/j.drup.2014.06.001
1368-7646/

in *A. fumigatus* commonly affect the activity of all mold-active antifungal azoles. Specific mutations correspond with various phenotypes characterized by complete loss of activity of a specific azole, and with decreased activity of others (Verweij et al., 2007).

If a role for the azoles remains in the management of azole-resistant aspergillosis (Walsh et al., 2008), optimizing drug exposure appears critical to increase the probability of treatment success. In this context, understanding of the pharmacokinetics (PK) and pharmacodynamics (PD) and more importantly defining the pharmacodynamic target of the azole compounds is crucial to increase the probability of a favorable clinical response (Andes, 2004). Reduced susceptibility of the fungus for azoles has significant impact on the ability to achieve the PD-target, and sometimes targets can only be achieved at the cost of increased probability of toxicity. Many variables, such as the underlying azole resistance mechanism and PK/PD properties of the antifungal agent, are important to determine if treatment with an azole remains feasible (Andes et al., 2009). Furthermore, in the absence of extensive clinical experience with the treatment of azole-resistant aspergillosis, data obtained through *in vitro* susceptibility testing and experimental models of infection are needed to design treatment strategies.

We reviewed our current understanding of azole resistance and the potential role of voriconazole and posaconazole in order to guide clinicians to manage patients with azole-resistant aspergillus disease. The results of *in vitro* and preclinical studies were extrapolated to humans to provide evidence that may support the use of voriconazole and posaconazole in isolates with attenuated azole susceptibility.

2. Triazole antifungals: mode of action and label indication for invasive aspergillosis

The *antifungal* triazoles are synthetic compounds that have >1 triazole ring attached to an isobutyl core (*e.g.*, voriconazole, ravuconazole, and isavuconazole) or to an asymmetric carbon atom with a lipophilic complex mixed functional aromatic chain (*e.g.*, itraconazole and posaconazole) (Groll et al., 2003). Triazoles inhibit the synthesis of ergosterol from lanosterol in the fungal cell membrane (Groll et al., 2003; Mohr et al., 2008); the target is the cytochrome (CYP)-dependent 14-a-demethylase (*CYP*51 or *Erg*11p), which catalyses this reaction. Thereby, ergosterol is depleted and methyl-sterols accumulate within the cell membrane and lead to either inhibition of fungal cell growth or death, depending on the species and antifungal compound involved. Triazoles are generally fungistatic, although itraconazole, voriconazole, posaconazole and isavuconazole have been shown to be fungicidal against *Aspergillus* spp such as *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *A. versocolor* and *A. sidowii*. (Guinea et al., 2008; Mohr et al., 2008; Pfaller et al., 2002). The various azoles have different affinities for the CYP-dependent 14-a-demethylase, which in return results in various antifungal activities (Warrilow et al., 2010a); and therefore various susceptibilities to *Aspergillus* spp. Four triazole compounds (fluconazole, itraconazole, voriconazole, and posaconazole) have been clinically licensed and are currently in wide use for the prevention and treatment of invasive fungal infections (EMA, 2012a,b). Fluconazole has a lack of efficacy against molds such as *Aspergillus* spp., therefore targeted prophylaxis or treatment against aspergillosis cannot be covered by this agent. Itraconazole is commonly used for the treatment of chronic and allergic conditions (EMA, 2012a,b). Voriconazole has broad *in vitro* activity against *Aspergillus* spp., is recommended first choice treatment of invasive aspergillosis with a label indication in adults and children aged 2 and above (EMA, 2012b). In addition, voriconazole is the drug of choice for treatment of central nervous system aspergillosis (Schwartz et al., 2005). Posaconazole is licensed only for patients aged 18 years or older (EMA, 2012a); for prophylaxis in patients receiving remission–induction chemotherapy for acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) expected to result in prolonged neutropenia and who are at high risk of developing invasive fungal infections; for prophylaxis of invasive fungal infections in hematopoietic stem cell transplant (HSCT) recipients who are undergoing high-dose immunosuppressive therapy for graft *versus* host disease and who are at high risk of developing invasive fungal infections; and for salvage therapy of invasive aspergillosis in patients with disease that is refractory to amphotericin B or itraconazole or in patients who are intolerant of these medicinal products (Cornely et al., 2007; Herbrecht et al., 2002; Ullmann et al., 2007; Walsh et al., 2008).

3. Phenotypic detection of azole resistance and clinical breakpoints for *Aspergillus* spp.

In recent years major advances have been made in the detection of azole resistance in *Aspergillus* spp. Both the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial susceptibility Testing-subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) have developed and standardized phenotypic methods that enable the reliable and reproducible determination of the minimal inhibitory concentration (MIC) for conidia-forming molds such as *Aspergillus* spp. (AFST-EUCAST, 2008; CLSI, 2008). When a collection of fungal strains is tested, typically a Gaussian distribution of MICs is found referred to as the wild type population (Meletiadis et al., 2012). The right side of the distribution, *i.e.* growth of isolates that is inhibited only by a higher concentration of the drug or any isolates/populations to the right side of the wild type distribution might contain isolates that possess a resistance mechanism. These isolates are considered non-wild type (AFST-EUCAST, 2013). Testing of large collections of fungi enables the determination of an epidemiological cut-off, which is the concentration of drug that inhibits 95% of the fungal species. Notably, a clinical breakpoint is needed to obtain a clinically meaningful interpretation of the MIC of individual isolates. A standardized approach is followed, which incorporates standard dosing recommendations and formulations of antifungal agents, the Pk/Pd characteristics, information from experimental models of infection and results from clinical trials. All this information is analyzed and leads to the clinical breakpoint, *i.e.* the classification of the isolate as susceptible to the drug or resistant. There are currently three sets of breakpoints and epidemiological cut-off values (ECVs) available; The breakpoints was published in 2009 by Verweij et al. based on clinical experience and the available knowledge at that time (Verweij et al., 2009a). Since then ECVs have been published by the CLSI (Espinel-Ingroff et al., 2010; Pfaller et al., 2011) and others (Meletiadis et al., 2012) the ECOFF by EUCAST-AFT for azole drugs and *A. fumigatus* (Arendrup et al., 2012a, 2013; Hope et al., 2013; Rodriguez-Tudela et al., 2008). Notably, in all of the above mentioned reports the ECOFF of 1 mg/L is considered as breakpoint for voriconazole against *A. fumigatus*. The breakpoints and ECVs are shown in Table 1. However, our recent analysis on a large collection of *A. fumigatus* isolates (wild-type and *CYP*51A-mutants) collected between 2009–2013, indicated that the ECOFF for voriconazole and *A. fumigatus* should be higher and equal to 2 mg/L (van Ingen et al., 2014).

4. Genotypic detection of azole resistance mechanisms in *Aspergillus* spp.

In addition to the phenotypic methods, significant insight has been obtained regarding the underlying genetic mechanisms that confer an azole resistant phenotype. In *A. fumigatus* two distinct but closely related *cyp*51 genes were found (*cyp*51A and *cyp*51B)