Chapter 26
Fungal Drug Resistance: Azoles

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1 Introduction. Azole Antifungal Agents: History, Mode of Action, and Clinical Utility

Azole derivatives represent one of the major groups of antifungal drugs used in clinical practice to treat fungal infections in humans, including skin and vaginal infections in the general population, and more serious life-threatening invasive mycoses in severely immunocompromised patients. Although this new class of antifungal agents was developed in the 1960s and 1970s, the first availableazole derivative for the oral treatment of systemic fungal infections, ketoconazole, an imidazole, was released in the early 1980s. A few years later, the introduction of the first-generation triazoles, such as fluconazole and itraconazole, constituted a major advance in the treatment of fungal infections and quickly became the drugs of choice for the treatment of a number of fungal infections, particularly candidiasis (1). The recent introduction of the “new generation” triazoles, including voriconazole, posaconazole, and ravuconazole, at different stages in the development pipeline, represent a welcome addition to the limited arsenal of antifungal agents, mainly due to their increased potency and broader spectrum. Voriconazole and ravuconazole are structurally related to fluconazole, whereas posaconazole bears a close resemblance to itraconazole (1).

The mode of action of azole derivatives is by binding to and inhibiting lanosterol demethylase (Cyp51p or Erg11p), a cytochrome P450 responsible for the 14α-demethylation of lanosterol, thus blocking ergosterol biosynthesis (the major membrane sterol of fungi) and leading to a fungistatic effect in the majority of cases (1, 2). The unhindered nitrogen of the imidazole or triazole ring of azole antifungal agents binds to the heme iron of Erg11p as a sixth ligand, thus inhibiting the enzymatic reaction. The remainder of theazole molecule binds to the apoprotein, in a manner that is dependent upon the individual molecular structure of each azole derivative (2). The exact conformation of the active site differs between fungal species and amongst the many mammalian P450 mono-oxygenases. The precise nature of the interaction between each azole molecule and each kind of P450, therefore, determines the extent of the inhibitory effect of each azole antifungal agent in different fungal species (which means that some fungi could be intrinsically resistant to a given azole derivative). Inhibition of 14α-demethylase by azoles leads to the depletion of ergosterol, which is a major bioregulator of fungal cytoplasm membrane fluidity, and to asymmetry and accumulation of sterol-precursors, including 14α-demethylated sterols, resulting in the formation of a plasma membrane with altered structure and function.

Because of the different characteristics in their activity, pharmacodynamics, pharmacokinetics, and safety profiles, each of these azole agents has found utility in different clinical settings (1, 3). In general, as a class, azole antifungals have a broad spectrum of activity, including activity against Candida species, C. neoformans, dimorphic fungi, and molds. For example, fluconazole has broad clinical efficacy for mucosal candidiasis (vaginal and oropharyngeal), and has also often been considered as a first choice for the prophylaxis and treatment of invasive candidiasis in neutropenic and non-neutropenic patients. It is also active against C. neoformans and some of the causative agents of endemic mycoses. However, fluconazole is not active against Aspergillus and other molds, and some Candida species (namely C. krusei and C. glabrata) are intrinsically resistant to fluconazole. Itraconazole displays potent activity against Candida and Aspergillus spp., dimorphic and dematiaceous fungi, and although it has been used less frequently, the availability of an oral solution and intravenous formulation has recently increased enthusiasm for its application for prevention of mold infections (4). Voriconazole has been shown to be superior to amphotericin B deoxycholate in the primary treatment of invasive aspergillosis, thus representing an important therapeutic advance (5). Posaconazole displays

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potent activity and an expanded spectrum of action, and has the most potential as a treatment strategy for zygomycosis (6).

2 Resistance to Azole Antifungal Agents

2.1 General Considerations and Definitions

Reports on resistance to azole antifungal agents were rare until the late 1980s. However, development of resistance to the current clinically used azole antifungal agents has become an increasing problem. This is particularly true in patients requiring long-term treatment, and in those receiving antifungal prophylaxis (7–9). Thus, azole resistance is frequently described in patients with AIDS and mucosal candidiasis (particularly in the era prior to highly active antiretroviral therapy, HAART), oral candidiasis, and less frequently in invasive infections. Resistance to azole treatment can be stable or transient (10). In addition, there is a growing awareness of the changing epidemiology of fungal infections, with a shift toward species that are intrinsically resistant to the most commonly used antifungal agents (fluconazole) (11, 12). Microbiological resistance is defined as a decrease in antifungal drug susceptibility, which can be measured in vitro by appropriate laboratory methods. This highlights the importance of the development of standardized methods for antifungal drug susceptibility testing in the last decade (13, 14) which are considered milestones in the field of medical mycology. By performing these techniques, a distinction between a susceptible and a resistant fungal isolate can be made according to a threshold drug susceptibility value (i.e. the breakpoint MIC, for Minimal Inhibitory Concentration) which could potentially, and with this drug (8, 9).

2.2 Molecular Mechanisms of Azole Resistance

At the molecular level, different mechanisms contribute to resistance against azole antifungal agents, reviewed in (17, 18). These mechanisms include modification of the antifungal target (in the case of azoles lanosterol demethylase, the product of the ERG11 gene), decreased drug accumulation inside the fungal cells due to the overexpression of multidrug drug efflux pumps, and other alterations in sterol biosynthesis. Deficiency in the uptake of some azole derivatives could also contribute to resistance. Most studies have been performed in C. albicans due to the unique opportunity to analyze series of matched susceptible and resistant isolates recovered sequentially from the same patient (17, 19–24), but recent studies in other pathogenic fungi such as C. glabrata, A. fumigatus, and C. neoformans seem to support these observations (25–32). In most instances, resistance to azoles is a multifactorial process involving several mechanisms. Cross-resistance within the azole class of antifungal agents is common, and is becoming an important issue (33, 34).

2.2.1 Alterations in the Target Enzyme

Alterations in the target enzyme (lanosterol 14-α-demethylase), including point mutations and overexpression, lead to decreased susceptibilities to azole drugs, which may also lead to cross-resistance to other azole derivatives. Pathogenic fungi can overcome the inhibition of azoles by increasing the content of the target enzyme molecules, either by gene amplification or by overexpressing the corresponding gene (ERG11). This results in the need for higher intracellular azole concentration to complex all the enzyme molecules present in the cells. However, this mechanism seems to have a limited impact in resistance to azoles, and does not seem to confer high levels of resistance (17, 18). Point mutations in the gene encoding the target enzyme for azoles (ERG11) result in amino acid substitutions leading to decreased affinity for azole derivatives. In these studies, ERG11 alleles from azole-resistant isolates were sequenced and compared to alleles of matched azole-susceptible isolates. While some ERG11 alleles contain a single mutation responsible for azole resistance, other ERG11 alleles were found to contain several mutations with potential additive effects (21, 35–38). Importantly, some of these mutations have been repeatedly identified by different groups in different geographical locations, and these mutations may represent “hot spots” for the development of azole resistance. Remarkably, most of these substitutions are present in domains that are highly conserved in lanosterol demethylases across fungi, suggesting the importance of these residues for function maintenance through evolution. According to molecular