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Drug Resistance Updates 10 (2007) 121–130
DRUG RESISTANCE Updates
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Resistance to echinocandin-class antifungal drugs

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Received 15 January 2007; received in revised form 22 February 2007; accepted 10 April 2007

Abstract

Invasive fungal infections cause morbidity and mortality in severely ill patients, and limited drug classes restrict treatment choices. The echinocandin drugs are the first new class of antifungal compounds that target the fungal cell wall by blocking β-1,3-D-glucan synthase. Elevated MIC values with occasional treatment failure have been reported for strains of *Candida*. Yet, an uncertain correlation exists between clinical failure and elevated MIC values for the echinocandin drugs. Fungi display several adaptive physiological mechanisms that result in elevated MIC values. However, resistance to echinocandin drugs among clinical isolates is associated with amino acid substitutions in two "hot-spot" regions of Fks1, the major subunit of glucan synthase. The mutations, yielding highly elevated MIC values, are genetically dominant and confer cross-resistance to all echinocandin drugs. Prominent Fks1 mutations decrease the sensitivity of glucan synthase for drug by 1000-fold or more, and strains harboring such mutations may require a concomitant increase in drug to reduce fungal organ burdens in animal infection models. The Fks1-mediated resistance mechanism is conserved in a wide variety of *Candida* spp. and can account for intrinsic reduced susceptibility of certain species. Fks1 mutations confer resistance in both yeasts and moulds suggesting that this mechanism is pervasive in the fungal kingdom.

Keywords: Echinocandin; Glucan synthase; Fks1; Caspofungin; Anidulafungin; Micafungin

1. Introduction

The treatment options for invasive fungal infections are limited since there are relatively few chemical classes and targets represented by existing antifungal drugs. Current drugs target cell wall and membrane components. The most broadly used agents are aimed at ergosterol, a predominant sterol within fungal cell membranes, and are either fungicidal but toxic to the host (polyenes) or fungistatic and more vulnerable to resistance (azoles and triazoles). The newest class of antifungal drugs are the echinocandins, which target biosynthesis of β-1,3-D-glucan, a key fungal cell wall component. These drugs are active against clinically relevant yeasts and moulds (Denning, 2003). Caspofungin, the first class member, received FDA approval in 2002 followed by micafungin in 2005 and anidulafungin in 2006. Clinical resistance appears low with sporadic breakthrough cases reported (Morris and Villmann, 2006; Laverdiere et al., 2006; Miller et al., 2006; Hakki et al., 2006). However, as patient exposure to echinocandin drugs broadens, the number of infecting strains with reduced susceptibility is expected to rise. Unfortunately, the relationship between reduced *in vitro* susceptibility to echinocandin drugs and clinical failure is ambiguous (Kartsonis et al., 2005; Pfaller et al., 2005b). This review will address this critical issue for echinocandin drugs by discussing Fks1 modification as a principal resistance mechanism and will define laboratory-based parameters for resistance that can contribute to clinical failure.

2. Echinocandin antifungal drugs

The echinocandins were the first members of the lipopeptide group to be discovered that inhibit β-1,3-D-glucan synthase, which is responsible for biosynthesis of the major cell wall biopolymer (Kurtz and Douglas, 1997). They are cyclic hexapeptides N-linked to a fatty acyl side chain. Only two chemical classes, the lipopeptides and papulacandins are known to inhibit glucan synthase; although, the latter

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doi:10.1016/j.drup.2007.04.002

have not developed as antifungal agents. The echinocandin drugs, caspofungin, micafungin, and anidulafungin are the first of a new class of antifungal compounds that target the fungal cell wall (Denning, 2003; Kartsonis et al., 2003; Wiederhold and Lewis, 2003). These drugs have broad-spectrum antifungal activity against *Candida* and *Aspergillus* spp. without cross-resistance to existing antifungal agents, and therefore are effective against azole-resistant yeasts and moulds (Denning, 2003; Morrison, 2006). The echinocandins are fungicidal against yeasts but fungistatic against moulds, where they block the growing tips of hyphae (Bowman et al., 2002; Douglas, 2006). Echinocandin drugs are highly effective on biofilms (Bachmann et al., 2002b) but are less active against *Zygomycetes, Cryptococcus neoformans* or *Fusarium* spp. (Denning, 2003). They are now widely used for antifungal therapy against yeasts and moulds (Morris and Villmann, 2006; Betts et al., 2006; Bennett, 2006; Joseph et al., 2007). Caspofungin and anidulafungin are FDA approved in the US and other countries for the treatment of serious fungal infections including esophageal candidiasis, candidemia, and other *Candida* infections. Caspofungin is also approved for empirical therapy for presumed fungal infections in febrile neutropenic patients, as well as for treatment of patients refractory to standard treatments for *Aspergillus* infections. Micafungin is approved for the treatment of patients with esophageal candidiasis and for prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation during the period of neutropenia. Overall, these drugs have excellent safety and tolerability profiles with few drug-related adverse events (Ullmann, 2003; Boucher et al., 2004; Higashiyama and Kohno, 2004; Wagner et al., 2006).

3. Glucan synthase complex

The β-1,3-glucan synthase is a multi-subunit enzyme complex responsible for fungal cell wall construction and division septum deposition. The enzyme catalyses the transfer of sugar moieties from activated donor molecules to specific acceptor molecules forming glycosidic bonds in the reaction UDP-glucose + {(1,3)-β-D-glucosyl}(N) → UDP + {(1,3)-β-D-glucosyl}(N + 1). Most of our understanding of the genetics of glucan synthase has come from studies in yeast (Inoue et al., 1996; Qadota et al., 1996). The UDP glycosyltransferases are one group of enzymes that carry out this reaction and over 100 members of this protein family are known (Ross et al., 2001). The enzyme complex has a minimum of two subunits, Fks1 and Rho. Fks1 appears to be the catalytic subunit, which is supported by cross-linking studies with a photoaffinity analog of the substrate UDP-glucose (Schimoler-O'Rourke et al., 2003). Rho, a GTP-binding protein in the Rho/Rac subfamily of Ras-like GTPases, helps regulate the activity of the glucan synthase (Mazur and Baginsky, 1996). Biochemical studies have implicated other membrane-associated components including Pma1 which appears in close association with the glucan synthase complex (Schimoler-O'Rourke et al., 2003). Pma1's role in maintaining transmembrane electrochemical proton gradients (Monk and Perlin, 1994) may be important to glucan synthase by providing the driving force for translocating product and/or in maintaining an acidic cell wall environment close to the membrane, as glucan polymers are laid down. Additional information about potential interacting proteins has come from labeling studies with the photoactivatable cross-linking echinocandin LY303366. The photoaffinity probe identified two proteins of 40 and 18 kDa in membrane preparations (Radding et al., 1998). It was suggested that the 40 kDa protein could be a homolog of Pil1 and Lsp1, sphingolipid-dependent regulators of cell wall integrity signaling (Edlind and Katiyar, 2004). These gene products may interact with the glucan synthase complex, although genetic/biochemical confirmation is lacking. The mechanistic nature of the interaction between echinocandins and glucan synthase remains ambiguous.

4. Elevated MIC and clinical outcome

Large-scale surveillance studies have documented the outstanding potency of echinocandin drugs against clinical isolates of *Candida* species in routine susceptibility assays (Espinel-Ingroff, 2003; Pfaller et al., 2003b, 2005a, 2006). These studies also reveal the presence of occasional strains from highly susceptible species that display uncharacteristically high MIC values. They further highlight the presence of less-susceptible non-*albicans Candida* spp., such as *C. parapsilosis* and *C. guilliermondii*, which have routine MIC values 4–100-fold greater than those observed for *C. albicans*. The clinical significance of this reduced susceptibility is unclear as infections with these organisms generally respond to current echinocandin therapy (Mora-Duarte et al., 2002; Bennett, 2006). In general, an uncertain correlation exists between clinical failure and elevated *in vitro* MIC values for echinocandin drugs. In two well-documented candidiasis studies, elevated MIC was not a reliable predictor of treatment outcome (Kartsonis et al., 2005; Pfaller et al., 2005b). This discordance may reflect the fact that the majority of elevated MIC values reported in these studies were relatively modest (MIC $\leq$2 μg/ml). Yet, sporadic treatment failures consistent with clinical resistance have been documented with high MIC isolates (Hakki et al., 2006; Laverdiere et al., 2006; Miller et al., 2006). In this context, it is important to distinguish between adaptive mechanisms by fungi that can cause elevated MIC values *in vitro* but do not influence clinical outcome and those that result in treatment failure. As patient exposure to echinocandin drugs broadens, it is anticipated that the number of clinical isolates with elevated MIC values will rise and an increasing number of patients may fail therapy due to resistance. Thus, it is vital to understand the nature of developing resistance mechanisms to this class of drugs.