Mechanisms of echinocandin antifungal drug resistance

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Abstract

Fungal infections due to Candida and Aspergillus species cause extensive morbidity and mortality, especially among immunosuppressed patients, and antifungal therapy is critical to patient management. Yet only a few drug classes are available to treat invasive fungal diseases, and this problem is compounded by the emergence of antifungal resistance. Echinocandin drugs are the preferred choice to treat candidiasis. They are the first cell wall–active agents and target the fungal-specific enzyme glucan synthase, which catalyzes the biosynthesis of β-1,3-glucan, a key cell wall polymer. Therapeutic failures occur rarely among common Candida species, with the exception of Candida glabrata, which are frequently multidrug resistant. Echinocandin resistance in susceptible species is always acquired during therapy. The mechanism of resistance involves amino acid changes in hot-spot regions of Fks subunits of glucan synthase, which decrease the sensitivity of the enzyme to drug. Cellular stress response pathways lead to drug adaptation, which promote the formation of resistant fks strains. Clinical factors promoting echinocandin resistance include empiric therapy, prophylaxis, gastrointestinal reservoirs, and intra-abdominal infections. A better understanding of the echinocandin resistance mechanism, along with cellular and clinical factors promoting resistance, will promote more effective strategies to overcome and prevent echinocandin resistance.

Keywords

echinocandin; caspofungin; micafungin; FKS; glucan synthase; chitin synthase

Introduction

Fungal infections are a major global health problem, with more than 300 million people afflicted, resulting in nearly 1.35 million deaths annually. Invasive fungal infections are a consequence of underlying diseases and conditions such as AIDS, cancer, organ transplantation, and corticosteroid therapies, with most deaths resulting from infection with Cryptococcus, Candida, and Aspergillus species. In all cases, the successful management of patients with invasive fungal disease requires antifungal therapy. Yet treatment options are restricted, as current antifungal drugs comprise only limited chemical classes represented by polyenes, azoles, flucytosine, and echinocandins. Azole drugs (e.g., fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole) inhibit the biosynthesis of the
plasma membrane sterol ergosterol; polyene drugs (e.g., amphotericin B) are pore-forming molecules that bind to ergosterol in the plasma membrane; flucytosine (5-FC) blocks pyrimidine metabolism and DNA synthesis; and the echinocandin drugs (e.g., caspofungin, anidulafungin, and micafungin) are cell wall–active antifungal agents that inhibit the biosynthesis of critical glucan polymers. The echinocandins drugs are now preferred first-line therapy for patients with invasive candidiasis, and it has been reported that more than 60% of candidemia patients receive an echinocandin during therapy. Given the clinical importance of echinocandins, this review focuses on emerging resistance to echinocandin class drugs and underlying mechanisms.

**Echinocandin class drugs**

Echinocandin drugs are lipopeptide molecules that non-competitively inhibit β-1,3-D-glucan synthase, which is responsible for the biosynthesis of β-1,3-D-glucan, a principal structural component of fungal cell walls. The echinocandin drugs were approved by the U.S. Food and Drug Administration (FDA) for the treatment of esophageal and invasive candidiasis, candidemia, and as empirical therapy in febrile neutropenic patients and prophylaxis in patients undergoing hematopoietic stem cell transplantation (HSCT). The Infectious Diseases Society of America (IDSA) recommends an echinocandin for most neutropenic patients with candidemia. In the non-neutropenic patient population, echinocandins are recommended in place of an azole, and they are preferred in patients with moderately severe to severe illness, as well as in patients with recent azole exposure. Echinocandin drugs are broadly active against most prominent *Candida* species, where they display *in vitro* fungicidal activity. In contrast, they are fungistatic against susceptible molds like the *Aspergillus* species, where they lyse the apical tips of expanding hyphae, alter hyphal morphology, and modify cell wall composition and organization. The echinocandins have a limited spectrum and they are inactive against *Mucormycetes*, *Cryptococcus* spp., or *Fusarium* spp. Echinocandins are highly active against azole-resistant yeasts such as *C. glabrata* and *C. krusei*, as well as to some *Candida* biofilms, since their mechanism of action is unrelated to azoles and they are not substrates for multidrug efflux systems found in highly azole-resistant strains.

The echinocandins have an outstanding therapeutic index with a low potential for renal or hepatic toxicity or serious drug–drug interactions. Their low toxicity may reflect the fact that β-1,3-D-glucan synthase, the echinocandin target, is a fungal-specific enzyme not found in humans. All echinocandins have low oral bioavailability and are administered intravenously. Echinocandin drugs are highly serum protein bound, which affects *in vitro* potency. They have a prominent Cmax but are largely AUC/MIC (area under the curve/minimum inhibitory concentration) drugs. They distribute well into tissues, but poorly into the CNS and eye. The β-1,3-D-glucan synthase target comprises a GTP-binding protein, Rho, which helps regulate the biosynthetic capacity of glucan synthase and a catalytic subunit, Fks, encoded by three related genes, *FKS1*, *FKS2*, and *FKS3*. *FKS1* is essential in *C. albicans* and most other *Candida* spp., while *FKS1* and *FKS2* are functionally redundant in *C. glabrata*. *FKS3* is expressed at a very low level relative to the other genes, and it does not appear to be a major contributor to overall biosynthetic capacity.
Breakpoints and the epidemiology of resistance

The predominant *Candida* species causing invasive infections are highly susceptible to echinocandin drugs. The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established standardized microbroth dilution susceptibility tests for *Candida* and echinocandins (36–38), which show uniformly potent activity against most *Candida* species. From 2003 to 2007, 8271 isolates of *Candida* spp. were obtained from over 100 centers worldwide and tested with the CLSI M27-A3 broth microdilution method to define wild-type populations and epidemiological cutoff values (ECV). MIC values of ≤0.06 mg/ml and ECV values ≤ 0.25 mg/ml were obtained for all major *Candida* species against the three echinocandin drugs. Higher MIC values are observed for *C. parapsilosis*, 0.25–2 μg/ml; and *C. guilliermondii*, 0.5–2 μg/ml. Both CLSI and EUCAST have established species and drug-specific clinical breakpoints (CBP) for echinocandin drugs, and epidemiological cutoff values have been defined for anidulafungin and micafungin against common *Candida* species. EUCAST has not established caspofungin breakpoints and does not recommend caspofungin MIC testing for clinical assessment, owing to interlaboratory testing variability, and the CLSI has raised caution when using caspofungin testing, especially with *C. glabrata*.

*Candida* spp. isolates resistant to echinocandin drugs are increasingly reported. However, the overall prevalence remains low at approximately 2–3% with *C. albicans* and most other *Candida* spp. *Candida glabrata* is the major exception for which some centers report high levels of (8–13%) resistance. The emergence of high resistance in *C. glabrata* follows from epidemiologic shifts at some centers in which this *Candida* species is the predominant bloodstream organism recovered from patients, due largely to the rising use of echinocandins and azoles for prophylaxis. Echinocandin resistance typically emerges after prolonged therapy, although it has been reported shortly after initiation of therapy. Echinocandin resistance of 8.0–9.3% was reported in a recent SENTRY program among 1669 bloodstream isolates (BSI) of *C. glabrata*. Similarly, over a 10-year period, echinocandin resistance in *C. glabrata* rose from 2–3% to > 13%. Alarmingly, the rise in echinocandin resistance among *C. glabrata* was accompanied by a parallel increase inazole resistance, resulting in multidrug-resistant strains that in some cases are untreatable (Fig. 1). Overall, resistance rates in *C. glabrata* vary from 3% to 10%, depending on geography and host population. The rapid acquisition of mechanism-specific echinocandin resistance by *C. glabrata* during therapy in an azole-resistant background leading to multidrug resistance with an unfavorable outcome is concerning.

Acquired resistance mechanism

It is well established that mutations in the *FKS* genes encoding the catalytic subunits of glucan synthase confer echinocandin resistance in otherwise susceptible *Candida* species resulting in therapeutic breakthrough infections. Amino acid substitutions in Fks subunits induce elevated MIC values (10–100 fold) and reduce the sensitivity of glucan synthase (IC50) to drug by as much as 3000-fold. Prominent mutations in *FKS* genes are associated with poor pharmacodynamic responses and diminished clinical outcome.
presence of an FKS mutation is an independent risk factor for echinocandin failure in patients with C. glabrata infections, and the presence of an FKS mutation was superior to MIC in predicting clinical response, especially when caspofungin is used for testing.63

Amino acid substitutions associated with resistance occur in two limited but highly conserved hot-spot regions of Fks36,64,65 encompassing residues Phe641–Pro649 and Arg1361 (or equivalent) in C. albicans and most other Candida spp. Amino acid substitutions at Ser645 and Phe641 cause the most pronounced phenotypes7,29,36,66 and they are the most abundant, accounting for more than 75% of resistance in C. albicans.7 These fks mutant strains are largely insensitive to drug and respond poorly or not at all in pharmacodynamic studies of murine models of infection.67–70 In C. glabrata, resistance occurs in homologous regions of FKS1 and FKS2.29,61 Amino acid substitutions occur at twice the frequency in Fks2 relative to Fks1.7,29,66 and modifications at amino acid positions Ser629 and Ser663 and Fks2 position F659S confer the highest IC50 and MIC values. In some cases, nonsense mutations occur in either FKS1 or FKS2 (C. glabrata), which confer prominent resistance.29,61,71 Resistance conferring amino acid substitutions can alter the catalytic capacity of glucan synthase;36 which is sensed by the cell, resulting in altered gene expression for FKS1 and FKS2, which may impact susceptibility.28,29 FKS2 expression is also downregulated by the immunosuppressant tacrolimus (FK506).72 and it can be used to overcome resistance conferred by FKS2 via suppression of gene expression.28 The FKS echinocandin-resistance mechanism resulting in elevated MIC values and clinical failures is widely observed in Candida species including C. tropicalis, C. krusei and C. kefyr55,73,74 (Fig. 1A). Mapping of the mutational hot spots on a topology map for Fks1 indicates that amino acid substitution occur near the extracellular membrane surface within transmembrane segments 6 and 7 (Fig. 1B). The external location may suggest a potential interaction site for echinocandin drugs that does not require entry into the cell.75

**Hot spot polymorphisms and inherent reduced susceptibility**

Mutations in the FKS hotspot regions cause a range of phenotypes, which correlate with modification of drug–target interactions, resulting in altered kinetic inhibition (IC50).29,36 Some mutations, like those at positions Phe641 and Ser645 in C. albicans and related species, confer the most pronounced phenotypes. However, other mutations in the hot spot region, especially those near the C-terminal end of hot spot 1, cause less pronounced phenotypes. Notable examples are the naturally occurring polymorphisms at Pro649 in the C. parapsilosis complex (C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis) and at Met633 and Ala634 in C. guilliermondii, which display inherently high MIC values relative to other Candida species.24,54,76 Intrinsic reduced susceptibility carries an uncertain clinical significance, as these infecting strains are often successfully treated with echinocandins at established dosages,77–79 but it varies with patient population.80–82 It has been shown for C. parapsilosis that glucan synthase is 10- to 50-fold less sensitive to the echinocandins relative to enzymes from C. albicans,83 which accounts for the higher MIC values. But the enzyme, while less sensitive, is still inhibited at typical therapeutic drug concentrations, which accounts for clinical response. A third region defined by W695 (outside clinical hot spots 1 and 2) of Saccharomyces cerevisiae Fks1 was found,84 but it is not associated with clinical failures.
Fks defects and fitness

In *C. albicans*, amino acid substitutions in Fks1 conferring echinocandin resistance carry a fitness cost, as they compete much less efficiently with isogenic FKS wild-type strains in murine models of infection. This is largely due to the fact that these substitutions decrease the catalytic $V_{\text{max}}$ for the biosynthesis of $\beta$-1,3-D-glucan, which alters the cell wall composition and morphology, making mutant cells somewhat less fit relative to wild type. Collectively, this data is consistent with the observation that horizontal transmission is rarely, if ever, encountered, and resistance involves *de novo* acquired resistance.

Biofilms

Like bacteria, fungal biofilms are organized in a complex communal structure consisting of cells embedded within an extensive polysaccharide matrix. In *Candida* and *Aspergillus* species, the extracellular biofilm matrix is composed predominantly of $\beta$-glucan, which sequesters drugs and effectively decreases their concentration at the level of the cell membrane. Genetic or chemical modulation of extracellular glucan production enhances cellular susceptibility to antifungal agents. A number of global transcriptional regulators such as Rlm and Smi1 and genes such as FSK regulate glucan formation, leading to resistant biofilms.

Stress adaptation and resistance emergence

The fungal cell wall is a dynamic structure that changes during growth and development, and requires remodeling of the crosslinking of $\beta$-1,3- and $\beta$-1,6-glucans. As the cell wall is critical to fungal cell survival, agents like echinocandin drugs that alter cell wall integrity induce significant cellular stress. In response, fungi possess a repertoire of adaptive response mechanisms that protect against such destabilizing environmental stresses. Echinocandins induce a set of genes from the protein kinase C (PKC) cell integrity–signaling pathway, as well as those required for cell wall maintenance and architecture. Stress signals at the cell surface are transmitted to Rho1 GTPase, which mobilizes a variety of effectors. Activation of cell wall integrity signaling alters the production of various carbohydrate polymers of the cell wall, along with cell wall architecture and remodeling. Inhibition of glucan biosynthesis by the echinocandins induces PKC, Ca$^{2+}$/calcineurin/Crz1, and HOG (high osmolarity glycerol), which mediate the response. Hsp90 is also induced, leading to tolerance to echinocandin drugs through its client proteins calcineurin and Mkc1 and co-chaperone Sgt1. Hsp90 orchestrates cellular stress response circuitry that has a profound impact on both azole and echinocandin resistance, and genetic or chemical modulation of Hsp90 reduces echinocandin tolerance. Echinocandin action also induces compensatory increases in chitin synthesis, which maintains the structural integrity of the cell wall, as chitin replaces $\beta$-1,3-glucan and decreases sensitivity to the drug. For most *Candida* species, activation of Chs2 and Chs8 enables survival in the presence of fungicidal levels of echinocandins. In *C. glabrata*, the terminal MAPK of the PKC signaling pathway, Slt2, controls chitin increase in response to echinocandins. Increases in chitin levels have been linked to paradoxical...
growth observed at very high echinocandin levels exceeding normal therapeutic doses. Finally, membrane sphingolipids can interact with echinocandins and modulate enzyme sensitivity to the drug. Overall, adaptive cellular responses stabilize cells in the presence of the drug, which affords cells time to escape drug action by forming FKS hot-spot mutations (Fig. 3).

Clinical reservoirs and microbial factors driving resistance

The gastrointestinal tract is a normal commensal site for Candida species, and genotyping confirms that colonizing isolates are often the infecting strain for most patients with invasive disease. Candida colonization of the gastrointestinal tract is associated with a mixed bacterial and fungal biofilm. Drug penetration into the glucan matrix of the biofilm is irregular, and there are varying levels of drug exposure resulting in the emergence of drug-tolerant and fks resistant mutants. In the presence of drug, these resistant cells proliferate and are available to cause systemic infections. The biofilm acts as a reservoir that seeds resistant infections. Another important reservoir involves intra-abdominal candidiasis, which occurs in 40% of patients with repeated gastrointestinal surgery, perforation, or necrotizing pancreatitis. These high-burden infections, along with poor drug penetration, are a critical reservoir that promotes resistance.

Like most anti-infectives, a close association exists between drug exposure and the emergence of resistance. It is well-established that FKS-mediated resistance is directly linked to prior, prolonged, and/or repeated drug exposure. As total drug exposure is an important driver of resistance, there is some concern that antifungal prophylaxis, which is intended to prevent infection, may promote the development of breakthrough resistance. The echinocandin drugs have favorable pharmacokinetics and safety profiles that are well suited for prophylaxis. Micafungin is FDA approved for prophylaxis of Candida infections in patients undergoing hematopoietic stem cell transplantation (HSCT) or expected to be neutropenic for at least 10 days, and the European Society of Clinical Microbiology and Infectious Diseases guidelines also recommend micafungin for prophylaxis against Candida infections in allogeneic HSCT adult and pediatric patients, as well as in pediatric patients with acute myeloid and recurrent leukemia. Both micafungin and caspofungin have been used for prophylaxis in adults and children, meta-analyses affirm that prophylaxis reduces the incidence of invasive fungal infections. However, the increasing use of echinocandin drugs for prophylaxis is a concern for increasing resistance. Low-dose prophylaxis has been linked to emergence of echinocandin resistance in C. glabrata, as well as in C. albicans. The expanding use of echinocandin prophylaxis among patients at high risk for invasive fungal infections is likely to fuel an increase in the frequency of isolates that are resistant to multiple classes of antifungal drugs.

Finally, the association between increasing echinocandin and azole resistance in C. glabrata, resulting in multidrug-resistant strains, is a major concern. Selection pressure from prophylaxis and therapy affecting high-burden reservoirs contributes greatly. Microbial factors play a critical role in this process. In contrast to the echinocandins, azole drug resistance resulting in clinical failure may be caused by a variety of genetic changes, most of which affect the expression of fungal drug transporters or the structure and/or expression of...
fungal drug targets. Chromosomal instability is rapidly observed following exposure to azoles or echinocandins, whereby Candida cells can undergo unequal division to produce aneuploid progeny. This intrinsic property of yeasts strongly suggests that cellular stress increases genetic diversity by altering genome integrity. Stress-induced aneuploidy depends on the function of a stress-inducible protein chaperone HSP90, which through its client proteins influences chromosome segregation and cell cycle progression. Aneuploidy can also promote a variety of other genomic rearrangements and mutagenic lesions leading to altered drug phenotypes.

Conclusions

Overall, echinocandin resistance is uncommon, as most infecting Candida species retain drug susceptibility. Yet, acquired drug resistance resulting in therapeutic failures, especially among immunosuppressed patients on long-duration or repeated therapy, is a significant factor in certain clinical high-risk settings. The recent emergence of multidrug resistance to azole and echinocandin class drugs among C. glabrata strains is worrisome. The FKS mechanism conferring stable drug resistance is well defined, but it is important to identify critical genetic factors that promote prominent fks mutant genotypes. Fungi robustly respond to stress through a variety of compensatory mechanisms, as well as through chromosomal modifications, that stabilize cells in response to drug and facilitate escape through the formation of characteristic FKS hot-spot mutations. Finally, total drug exposure and the expanding use of prophylaxis, along with host microbial reservoirs that limit drug access, are important contributors to resistance emergence in critically ill patients. Given these considerations, there is an opportunity to enhance initiation of appropriate therapy by incorporating molecular resistance testing to shorten diagnostic delays and limit resistance emergence by reassessing therapeutic dosing strategies and improved stewardship.

Acknowledgments

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References


Figure 1.
A 10-year profile for antifungal resistance of *Candida glabrata* isolates to azole and echinocandin drugs. Adapted from Ref. 50.
Figure 2.
(A) Spectrum of Fks amino acid changes conferring clinical resistance. Amino acid sequences of Fks hot-spot sequences for major *Candida* species and positions associated with prominent resistance (red), weaker resistance (purple) and naturally occurring reduced susceptibility (blue). (B) Topology model for glucan synthase and predicted positions of amino acid substitutions conferring echinocandin resistance. Adapted from Ref. 75.
Figure 3.
Schema depicting critical stages in evolution of drug resistance following drug exposure.