Role of Echinocandins in Fungal Biofilm–Related Disease: Vascular Catheter–Related Infections, Immunomodulation, and Mucosal Surfaces

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Biofilm-related infections have become an increasingly important clinical problem. Many of these infections occur in patients with multiple comorbidities or with impaired immunity. Echinocandins (caspofungin, micafungin, and anidulafungin) exert their fungicidal activity by inhibition of the synthesis of the \((1\rightarrow3)\)-\(\beta\)-d-glucan. They are active among in vitro and in vivo model systems against a number of Candida species and filamentous fungi in their planktonic and biofilm phenotype. Their superior activity against biofilms poses them in an advantageous position among the antifungal armamentarium. However, additional studies are warranted to expand our knowledge on the role of echinocandins against biofilm-related infections.

Keywords. biofilm; caspofungin; micafungin; echinocandin; anidulafungin.

Fungal biofilms are complex, 3-dimensional communities attached to abiotic or biotic surfaces and embedded in an extracellular matrix. Biofilms can form on indwelling medical devices or mucosal surfaces, causing a spectrum of clinical entities ranging from catheter-related fungemia to endocarditis, oropharyngeal infections, and vaginal infections, which are mostly prevalent among patients with weakened, suppressed, or underdeveloped immune systems [1]. Biofilms are critical virulence determinates and they are able to influence the outcome of an infection. Their importance lies in that they are recalcitrant to the majority of current treatment modalities and host defenses. Furthermore, the polymicrobial nature of mucosal biofilms and their interaction with host defenses can cause adverse clinical outcomes, as the implication of host defenses facilitate biofilm development and complicate their eradication [2].

Echinocandins (caspofungin, micafungin, and anidulafungin) exert their fungicidal activity by inhibition of the synthesis of the \((1\rightarrow3)\)-\(\beta\)-d-glucan. According to in vitro and in vivo studies, echinocandins retain their antifungal activity against Candida biofilms [3, 4].

In this review, we briefly describe the role of fungal biofilms in human disease with emphasis on vascular catheter–related and mucosal infections and the role of echinocandins in the management of these infections.

BIOFILMS ON MUCOSAL SURFACES OF THE GASTROINTESTINAL TRACT

Esophageal candidiasis and oral mucosal Candida infections are among the most common opportunistic fungal infections in immunocompromised patients, including human immunodeficiency virus (HIV)–infected patients and those who are immunosuppressed as a result of underlying diseases or medications [1, 5]. Evidence that mucosal candidiasis is associated with persistent or recurrent infection in the absence of cultivable organisms in tissue exudates, combined with its recalcitrance to antifungal treatment, led to the notion that mucosal candidiasis is related to biofilm formation [6–8]. Experimental evidence showed that fungal mucosal biofilms consist of complex structures containing...
yeast, hyphae, commensal bacterial flora, and host cells or cell-derived products such as neutrophils and keratin from desquamating epithelial cells [6]. Microscopic analysis of oral mucosal biofilms revealed structural similarities with abiotic surface biofilm architecture [1]; however, the composition of mucosal biofilms is inherently more complex than abiotic surface biofilms in that they are polymicrobial, with complex interactions occurring between fungal and bacterial species [6, 9, 10]. One notable feature of mucosal fungal biofilms is the abundance of glucans extracted from the extracellular material [6, 11]. Among the mucosal fungal biofilms, denture C. albicans biofilms seem to represent a unique niche of infection as they show resistance to echinocandins (micafungin) [12]. This finding is in contrast with the in vitro and in vivo catheter biofilm studies where echinocandins are effective in the treatment of biofilm-related infections.

The antifungal efficacy of anidulafungin was demonstrated in an experimental immunosuppressed rabbit model of fluconazole-resistant oropharyngeal and esophageal candidiasis. Vascular access was established in each rabbit by the surgical placement of a tunneled silastic central venous catheter, which permitted non-traumatic venous access [13]. Methylprednisolone was used for suppression of mucosal cellular immunity and oral vancomycin to reduce mucosal bacterial colonization competitive with C. albicans. To establish durable infection or colonization, Candida species was administered orally [14]. As shown in Figure 1, among the antifungal agents tested, only anidulafungin induced notable morphological changes to Candida mucosal infection. Anidulafungin demonstrated concentration-dependent fungicidal activity in time-kill assays (Figure 2). These concentration-dependent fungicidal properties of anidulafungin demonstrated in the in vitro time-kill assays were reflected in the dosage-dependent and concentration-dependent fungicidal activities in the tongue, oropharynx, and esophagus (Figure 2B).

Although fluconazole remains the preferred treatment for esophageal candidiasis, the increasing emergence of azole-resistant strains makes echinocandins an appealing alternative. Two multicenter, double-blind randomized clinical trials with identical study protocols with regard to disease diagnosis, treatment duration, and study endpoints demonstrated that micafungin is efficacious and safe in the treatment of esophageal candidiasis, exhibiting dose-dependent responses and achieving rates of clinical and endoscopic cure similar to that of fluconazole [15–17]. Although further clinical investigation is warranted to identify the optimum micafungin dosing in the treatment of esophageal candidiasis, initial pharmacokinetic/pharmacodynamic analysis indicates that better outcomes occur with higher maximum micafungin concentrations in serum and large, infrequent doses [18].

**VAGINAL CANDIDIASIS**

Vulvovaginal candidiasis is one of the most frequent infections affecting young women. Almost half of women will experience recurrent infection [19]. Candidal mucosal infection is a complex process involving multiple interactions between Candida and the host [20, 21]. Despite the limited understanding in the pathogenesis of this common fungal infection, currently, vaginal candidiasis has been characterized as a biofilm-related infection [1, 22]. Using in vivo and ex vivo approaches, C. albicans vaginitis has the typical biofilm architecture resembling that of in vitro biofilms, consisting of a dense network of yeast and hyphae surrounded by extracellular matrix [23, 24]. Further evaluation of C. albicans vaginal biofilms showed that

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**Figure 1.** Antifungal effects in rabbit glossal tissue infected with fluconazole-resistant Candida albicans. Morphological changes from hyphae and pseudohyphae in untreated controls (A) to an appearance of yeast-like structures in anidulafungin-treated rabbits (B). Amphotericin B- and fluconazole-treated rabbits had no significant changes (C and D) in cell wall morphology. The hyphae of C. albicans in this rabbit model are demonstrated in Gomori methenamine silver stain as dark elongated structures forming in the surface of the glossal stratified squamous epithelium (original magnification ×630). Adapted from Petraitis et al [14].
2 transcriptional regulators of morphogenesis (Efg1) and biofilm formation (Bcr1), required for the production of vigorous biofilms on abiotic surfaces [25, 26], are similarly required for the formation of vaginal biofilms. Both efg1/bcr1 mutants were defective in morphogenesis and biofilm formation, and vaginal biofilms were lacking typical biofilm architecture and extracellular matrix. However, mutant strains were able to extensively colonize the vaginal mucosa, indicating that different pathogenetic mechanisms are implicated in colonization and biofilm formation [1, 22].

The treatment of vaginal candidiasis holds inherent difficulties as, in addition to an effective antifungal agent, the formulation must adhere to the mucosa to bring the active agent in contact with vaginal mucosa. From this perspective, bioadhesive (mucoadhesive) systems represent a promising treatment alternative. Mucoadhesive formulations are held on the mucosal surface and the antifungal agent is released close to the absorptive surface, leading to enhancement of the antifungal agent bioavailability [27]. A cyclodextrin-based, emulsified wax cream containing itraconazole has been developed and found to be an effective delivery system. A phase 3 trial evaluated the efficacy of a 1% itraconazole vaginal cream in patients suffering from vaginal candidiasis. The formulation was well tolerated, not systematically absorbed, and effective in treating vaginal candidiasis [28].

In addition, the widespread occurrence of Candida infections and the development of resistance against the commonly used antifungal agents [29, 30] escalate the need for the development of newer antifungal agents and formulations. One crucial point on the investigation of mucosal biofilms and the assessment of the available treatment options is the model system used. Among the in vivo vaginal candidiasis models, rat and rabbit models respond satisfactorily to standard and investigational agents [31].

The secretion of hydrolytic proteins such as secretory aspartate proteinases has been shown to play a major role in the pathogenesis of vaginitis [32, 33]. From this perspective, cream compounds containing small-molecule protease inhibitors may aid Candida clearance from the vaginal mucosa. In vivo studies conducted in rats and rabbits testing the efficacy, pharmacokinetics, and tolerability of the intravaginal cream formulation of 2 protease inhibitors (APG12 and APG19) concluded that these compounds significantly accelerate Candida elimination.
early in the course of the infection, making these compounds acceptable candidates for clinical trials [31].

In view of the clinical concerns about increasing fluconazole resistance and the intrinsic resistance of some Candida strains such as C. glabrata or C. krusei to triazoles, clinical development of compounds containing echinocandins as a cream formulation for the treatment of refractory or recurrent candidemia may provide another therapeutic alternative that warrants further investigation. Nevertheless, although echinocandins such as micafungin are under discussion in various ethical guidelines, they are not approved for this indication and are not supported by clinical evidence for their safety and efficacy [34].

**Fungal Endocarditis**

Fungi account for 1%–2% of all cases of endocarditis and as much as 4% of those involving prosthetic valves [35–37]. The description of fungal endocarditis pertains to native and prosthetic valve endocarditis. While recognizing the differences in the likely pathogenesis of prosthetic vs native valve fungal endocarditis, the available literature usually combines both types of infection in the same report. Fungi (Candida species and Aspergillus species) are the third or fourth leading cause among infective endocarditis cases. The formation of biofilms on native or bioprosthetic heart valves (medically termed as vegetations) is a well-documented biofilm process [2, 38, 39]. As fungi produce bulky and friable vegetations, they are more prone to detachment and to the creation of clinically overt embolic events with alarming mortality rates [2, 35, 37]. Fungal endocarditis crude mortality rates have improved over the years but they remain excessively high, reaching 70%. For Candida species, endocarditis mortality is around 50%, and >90% for Aspergillus species endocarditis cases [35, 37]. Improved survival rates have been noted among patients with fungal endocarditis who received combined surgical–antifungal treatment, were infected with Candida species, and had univalvular involvement [35].

Guidelines from the Infectious Diseases Society of America and the European Society of Clinical Microbiology and Infectious Diseases recommend a combined medical (amphotericin B with or without flucytosine) and surgical approach for the treatment of fungal endocarditis [40, 41]. However, current recommendations are based on experimental studies, expert opinions, and small case series, and there are no randomized clinical data to define the most appropriate therapy for fungal endocarditis [35, 42, 43]. The inherent biofilm resistance to conventional antifungal therapies makes surgical resection and valve replacement a prudent approach [44]. Historically, surgery has been the fundamental approach for fungal endocarditis treatment. By the advent of newer antifungal agents such as echinocandins, however, surgical treatment as the ultimate therapeutic approach appears outdated [45]. The rationale behind the use of echinocandins for Candida endocarditis lies in the rapid fungicidal activity against most Candida species, their superior activity against Candida species biofilms, and their better tolerability compared to polyenes [46].

There is increasing clinical experience showing the efficacy of echinocandins in the treatment of Candida endocarditis [45, 47–53]. In vitro studies and recent cohorts suggest the use of an echinocandin for Candida endocarditis [37, 45, 54, 55]. A recent summary of case reports and small case series reports that 53% of the Candida endocarditis cases were cured without surgery, being treated only with an echinocandin [45]. In a meta-analysis, which is widely referenced in the treatment of Candida endocarditis guidelines, the better survival rates among patients who received combined surgical–antifungal treatment is underscored [35]. There were some concerns about the higher echinocandin minimum inhibitory concentrations (MICs) in Candida parapsilosis; however, they have not been verified in the clinical setting [40, 41, 56]. Furthermore, in a large epidemiological study of candidemia caused by the genetically heterogeneous taxon of C. parapsilosis, all isolates were susceptible to caspofungin [57].

In a systematic review of published literature reported between 1966 and 2002, the clinical outcomes were similar between the patients who received combination antifungal treatment without surgery and the patients who underwent surgical intervention. These observations underline the role of combination antifungal therapy, especially for patients who cannot undergo surgery [58]. Especially for Aspergillus endocarditis cases, where surgical intervention is endorsed as the attempts to manage cases with antifungal agents alone are rarely successful [59], combination antifungal therapy with voriconazole or liposomal amphotericin B and echinocandins appears to be an attractive therapeutic option [37].

Collectively, current data suggest that surgery may not be universally necessary for all cases of fungal endocarditis treatment. A more individualized approach has evolved. Surgery is indicated in cases of Candida species endocarditis where the risk of embolization is high, and where there is persistent fungemia and myocardial abscess [45]. Undoubtedly, echinocandins lend promise, especially for those patients who cannot undergo surgery. However, the role of newer antifungal agents including echinocandins, as well as surgery, in the management of fungal endocarditis needs further elucidation.

**Vascular Catheter-Related Candida Biofilms**

Among the indwelling medical devices, vascular catheters are the medical device most frequently associated with infections [2]. Catheters provide the surface for a microorganism to colonize and form biofilms [60]. Candida species account for the majority of vascular catheter–associated fungemias [61].
Whereas *Candida* species and strain type seem to affect biofilm formation, collectively, the biofilms of all *Candida* species consist of basal yeast cells with the exception of *C. parapsilosis*, which produces biofilms composed of pseudohyphae and aggregated yeast cells [62–64]. Biofilms, almost cathodically, form on both internal and external surfaces of vascular catheters. The relationship between catheter-related biofilms and vascular infection is conceivable. Sessile cells can serve as a nidus of persistent infection topically or in remote areas throughout the body as microcolonies can be detached, due to increased shear stress or disproportionate increase of biofilm cells [2]. The correlation between biofilm formation and clinical outcome has been shown [65, 66]. *Candida* biofilms adversely affect the health of hospitalized patients, with candidemia being a significant predictor of mortality [66].

Catheter-related biofilm resistance to most of the commonly used antifungal agents has been demonstrated for a number of *Candida* species. Recent studies using a variety of model systems have indicated that *Candida* biofilm resistance to antifungal agents appears to be multifactorial [67]. In biofilms apply both the conventional antifungal resistance mechanisms that occur in planktonic cells as well as those specific for the biofilm phenotype. Among the proposed mechanisms responsible for *Candida* biofilm resistance are the production of extracellular matrix, the slower growth rate, high cell density, quorum sensing, persister cell formation, and the activation of stress-induced pathways, including the calcineurin, HSP90, and mitogen-activated pathways. Most of these mechanisms are overlapping, and each one becomes dominant depending on the stage of biofilm development [67]. For example, efflux pumps account for biofilm resistance during the early phase of biofilm development, whereas as the biofilm matures, biofilm-specific mechanisms such as extracellular matrix play a much more important role [68–70].

In the case of *Candida* biofilm formation in vascular catheters, device removal is indicated as salvage rarely works [71, 72]. However, this is not always effective or feasible [73]. Among the antifungal agents tested, echinocandins have been shown among in vitro and in vivo catheter model systems to have superior activity against *Candida* biofilms [4, 54, 74–76]. In an in vitro model of *C. albicans* biofilm associated with silicone catheters, lock therapy with caspofungin (2 mg/L) and micafungin (5 mg/L) was able to cause a significant and persistent reduction of yeast metabolic activity of intermediate and mature biofilms [77].

Although little distinction is made clinically between the 3 available echinocandins, in vitro studies have shown that there are drug- and species-specific differences against *Candida* species biofilms [78]. While non-*albicans* *Candida* planktonic cells were susceptible to all echinocandins, there are drug- and species-specific differences in susceptibility among biofilms, with *Candida lusitaniae* and *Candida guilliermondii* exhibiting high MICs in all 3 echinocandins. All echinocandins exhibit generally high MICs against *C. parapsilosis* within the biofilm population, with micafungin having the lowest MIC (MICs: micafungin, 4 mg/L; anidulafungin, 32 mg/L; and caspofungin, 64 mg/L) [78]. These high MICs within the biofilm population contrast with the substantially lower MICs observed in the planktonic population.

**ECHINOCANDINS IN FUNGAL BIOFILM-RELATED DISEASE AND IMMUNOMODULATION**

Echinocandins act through inhibition of the biosynthesis of (1→3)-β-D-glucan leading to disruption of the fungal cell wall [79, 80]. The inhibition of (1→3)-β-D-glucan synthesis upsets the necessary equilibrium of the fungal cell wall, which activates compensatory mechanisms, such as upregulation of chitin synthesis [81, 82]. This cell wall remodeling may cause more exposure of (1→3)-β-D-glucan even in the presence of lower bulk levels of (1→3)-β-D-glucan. *Candida albicans* has high levels of the structural molecule (1→3)-β-D-glucan in its cell wall, but the majority of its (1→3)-β-D-glucan is masked by a mannoprotein layer, precluding recognition by the immune cells [83, 84]. Recognition of *C. albicans* (1→3)-β-D-glucan is mediated by mammalian innate immune receptors, such as dectin-1 [85]. Dectin-1 is widely expressed on phagocytes and contributes to the immunological response to (1→3)-β-D-glucan [86, 87]. Dectin-1 signals through the kinase Syk, adaptor CARD9, and Raf1 pathways while it collaborates with Toll-like receptor 2 to trigger proinflammatory responses upon stimulation with *C. albicans* [88]. It was demonstrated that exposure of (1→3)-β-D-glucan by drug treatment alters the way the fungus is recognized by immune cells. Namely, it was found that subinhibitory doses of caspofungin “unmask” the underlying (1→3)-β-D-glucan in the cell wall of *C. albicans*, causing the exposed fungi to elicit a strong immune response [84]. This “unmasking” effect of caspofungin was described in filamentous fungi as well, including *Aspergillus fumigatus*, *Scedosporium apiospermum*, and *Rhizopus oryzae* [87, 89]. In these studies, caspofungin-induced (1→3)-β-D-glucan exposure was associated with enhanced neutrophil-mediated fungal damage damage and increased expression of dectin-1 in neutrophils and tumor necrosis factor (TNF)-α release by macrophages [87, 89].

The “unmasking” effect originally described for caspofungin appears to be class-specific given that all the members of the class, including anidulafungin and micafungin, enhance phagocyte-mediated damage [89]. Unlike the other antifungal agents that directly stimulate immune cells, echinocandins appear to exert their immunomodulating properties in the presence of damaged hyphae [87, 90–93].

The immunomodulatory properties of echinocandins were shown among in vivo studies [94–96]. Mice with disseminated
infections caused by *Rhizopus oryzae*, *Fusarium solani*, or *Scedosporium prolificans*, molds that are intrinsically resistant to echinocandins, showed improved rates of survival when treated with a combination of amphotericin B lipid complex and caspofungin [94–96].

Under in vitro conditions that mimic catheter-related Candida species biofilm infections, anidulafungin demonstrated immunopharmacological effects [97, 98]. Namely, exposure of *C. albicans* biofilms to subinhibitory concentrations of anidulafungin was associated with significant increase in phagocyte-mediated damage. These findings were not evident with other classes of antifungal agents [97]. The patches, like buds or scars, caused by the echinocandin, would be sufficient to activate dectin-1 and trigger potent antifungal inflammatory response to macrophages [83]. It is conceivable that echinocandin-treated biofilms have heightened (1→3)-β-D-glucan exposure, eliciting a larger proinflammatory response from phagocytes [84].

Furthermore, it was found that exposure of *C. albicans* biofilms to different classes of antifungal agents elicited a modified proinflammatory response. Specifically, anidulafungin in subinhibitory concentrations, together with the significant increase in phagocyte-mediated damage to *C. albicans* mature biofilms, elicited a proinflammatory response as indicated by the increased amounts of TNF-α. On the contrary, the elicitation of interleukin 8 (IL-8) was downregulated in anidulafungin-treated biofilms [97]. Subinhibitory concentrations of micafungin modified the activity of human neutrophils against *C. albicans* biofilms. Namely, sub-MIC concentrations of micafungin increased biofilm susceptibility to the antifungal activity of neutrophils against *Candida* biofilms. Whether these findings are of clinical importance in the treatment of biofilm-related infections warrants further investigation [99].

Collectively, in vitro experiments showed that echinocandins exert additive effects with human immune cells against *Candida* biofilms while these interactions result in a differential release of the proinflammatory cytokine TNF-α and the chemokine IL-8. This beneficial type 1 T-helper response observed after treatment of biofilms with echinocandins contributes to the therapeutic effect of these antifungal agents in the treatment of biofilm-related infections.

**Notes**

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