

Echinocandin pharmacodynamics: review and clinical implications

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Echinocandins have made a significant impact in the treatment of select invasive fungal infections, most notably invasive candidiasis and aspergillosis. However, treatment outcomes for such infections are still less than optimal, prompting an examination of dosing and administration techniques in an attempt to exploit known pharmacodynamic properties and improve outcomes. Echinocandins generally exhibit concentration-dependent, fungicidal activity against *Candida* spp. and fungistatic activity against *Aspergillus* spp. However, increasing drug concentrations of echinocandins above the organism's MIC may result in a paradoxical increase in fungal growth as demonstrated in some *in vitro* and *in vivo* models (known most commonly as the 'Eagle effect'). Therefore, the potential impact of dose escalations on improving the clinical efficacy of echinocandins based on *in vitro* and animal models are uncertain and are still being evaluated. In addition, such strategies have to consider the potential for increased treatment-related toxicities and costs. To date, published clinical studies (both superiority and non-inferiority) demonstrating the potential for dose-related improvements in treatment outcomes have been limited to mucocutaneous and oesophageal candidiasis. Further research is needed to determine if a role exists for optimizing echinocandin pharmacodynamics in various clinical settings.

Keywords: caspofungin, micafungin, anidulafungin

Introduction

Since the approval of caspofungin (MK-0991, Cancidas[®]; Merck & Co., Inc., Whitehouse Station, NJ, USA) by the US FDA in 2001, the echinocandin class of antifungals [which now also includes micafungin (FK-463, Mycamine[®]; Astellas Pharma US, Inc., Deerfield, IL, USA) and anidulafungin (VER-002, LY303366, Eraxis[™]; Pfizer, Inc., New York, NY, USA)] has made a significant impact on the prevention and management of select invasive fungal infections (IFIs).^{1,2} Another glucan synthesis inhibitor, aminocandin, is still undergoing investigation. However, despite numerous desirable characteristics (including fungicidal activity against select *Candida* spp., favourable *in vitro* activity against a variety of non-*albicans* *Candida* and *Aspergillus* spp., once-daily dosing, favourable safety profile and limited drug interactions), treatment outcomes in patients with an IFI are less than optimal.^{1–5} For example, survival rates for patients with invasive aspergillosis treated with echinocandins range from 50% to 67.5%.^{6–8} For invasive candidiasis, survival rates in such patients range from 66% to 89.6%.^{9–12} Therefore, the need to optimize antifungal dosing and administration based on pharmacodynamic properties is imperative. Although characteristics such as concentration-dependent activity have been described in a variety of *in vitro* and animal models, successful exploitation of such properties for improvement of treatment outcomes for invasive infections in humans have been limited to date.

The objectives of this review are to evaluate the existing published data on the pharmacodynamic properties of the

echinocandin class of antifungals and discuss the implications of such data on their clinical application. A summary of the pharmacodynamic parameters of the echinocandin class is provided in Table 1.

In vitro and *in vivo* models

Candida spp.

A variety of *in vitro* studies have been conducted to characterize the pharmacodynamic activity of the echinocandins against a variety of *Candida* spp.^{13–19} In general, echinocandins demonstrate increasing antifungal activity as drug concentrations exceed the MIC for the organism, resulting in a concentration-dependent killing over a broad concentration range. In addition, echinocandin activity can also persist in a drug-free environment following drug exposure [known as the post-antifungal effect (PAFE)].^{14,16–21}

Echinocandins have fungicidal activity against most *Candida* spp. and are considered to be susceptible by the CLSI if MIC breakpoints are ≤ 2 mg/L.^{22,23} Such breakpoints are justified based on pharmacokinetic characteristics of these drugs using standard doses (i.e. producing serum concentrations >1 mg/L). Additionally, favourable clinical response data were observed in Phase II/III trials for echinocandins in infections caused by isolates with MIC breakpoints of up to 2 mg/L.^{9,11,22,24,25} An invasive candidiasis murine infection model (using a single isolate of *Candida albicans*) demonstrated that the target area under the

Table 1. Summary of pharmacodynamic parameters for echinocandins

Echinocandin	Predictor of outcome by model			Reference(s)
	<i>in vitro</i>	animal model	human studies	
Caspofungin				
<i>Candida</i> spp.	concentration dependent, fungicidal	AUC:MIC ^{a,b}	unknown	14, 16, 26
<i>Aspergillus</i> spp.	concentration dependent, fungistatic	C _{max} :MEC ^c range of 10–20	unknown	79, 93
Micafungin				
<i>Candida</i> spp.	concentration dependent, fungicidal	AUC:MIC near 20	unknown	17, 20, 21, 42–46
<i>Aspergillus</i> spp.	concentration dependent, fungistatic	C _{max} :MEC ^b	unknown	45, 90, 94, 95
Anidulafungin				
<i>Candida</i> spp.	concentration dependent, fungicidal	C _{max} :MIC ^{d,b} and AUC:MIC ^b	unknown	18, 47, 48
<i>Aspergillus</i> spp.	concentration dependent, fungistatic	none noted	unknown	48, 81
Aminocandin				
<i>Candida</i> spp.	concentration dependent, fungicidal	C _{max} :MIC near 10	unknown	19
<i>Aspergillus</i> spp.	ND ^e	ND	ND	

^aAUC:MIC=area under the concentration–time curve:minimum inhibitory concentration.

^bActual ratio not stated in references utilized.

^cC_{max}:MEC=peak concentration:minimum effective concentration.

^dC_{max}:MIC=peak concentration:minimum inhibitory concentration.

^eND=no data available at this time.

concentration–time curve to MIC (AUC:MIC) ratio was the best predictor of efficacy for caspofungin.²⁶ A limitation of clinical studies to date has been the low number of *Candida* isolates with MICs of >2 mg/L. In addition, the limited number of clinical failures in these studies made breakpoint determination problematic. Acquired resistance to echinocandins resulting in clinical failure has been infrequently reported in the literature. This resistance is thought to be secondary to the FKS1 mutation, in which the glucan synthesis enzyme complex is less sensitive to echinocandin therapy.^{27–36} However, data to establish a MIC that would be predictive of failure in patients are lacking.^{9,22,24} Data on the correlations between clinical failures and the MIC seem to be lacking and failures may be due to host factors rather than lack of drug efficacy.²⁴ Furthermore, a recent *in vitro* comparison of 5346 *Candida* isolates collected between 2001 and 2006 from 91 medical centres in patients with invasive disease demonstrated that most had caspofungin, micafungin and anidulafungin MICs of ≤2 mg/L with no significant changes in susceptibilities over the 6 year time course.³⁷

In vitro studies have been conducted to describe the activity of caspofungin against *C. albicans*, *Candida glabrata* and *Candida tropicalis* using time–kill analysis at concentrations ranging from 0.125 to 16 times the MIC,¹⁴ and against *Candida guilliermondii*, *Candida kefyr* and *Candida lusitanae* at concentrations ranging from 0.125 to 8 times the MIC.¹⁶ With the exception of one isolate of *C. tropicalis* and one isolate of *C. albicans*, fungicidal activity (defined as a 99.9% reduction from the initial fungal load within 48 h) was reported for all isolates.^{14,16} Similar findings have been reported for both micafungin and anidulafungin.^{17,18} Micafungin's fungicidal activity was reported at concentrations of 1–16 times the MIC for three out of four isolates of *C. albicans*, at concentrations of

4–16 times the MIC for two isolates of *C. glabrata* and at concentrations of 0.25–16 times the MIC for two isolates of *Candida krusei*. Caspofungin was not fungicidal for either *C. tropicalis* isolate tested in this study.¹⁷ Anidulafungin also appeared to display concentration-dependent, fungicidal activity against *C. albicans*, *C. glabrata*, *C. krusei* (MICs ranging from 0.008 to 0.1256 mg/L) and *Candida parapsilosis* (MICs 1.0–2.0 mg/L).¹⁸ For aminocandin against *Candida* spp. *in vitro*, peak:MIC ratios of 3 (mean ± SD 3.72 ± 1.84) produced a net static effect, with maximal fungal kill occurring at ratios near 10.¹⁹

A PAFE has been demonstrated for echinocandins against *Candida* spp. *in vitro*.^{13,18,19,21,38} For caspofungin against *C. albicans*, a PAFE was >12 h when concentrations exceeded the MIC.³⁸ Others reported the lack of a PAFE when caspofungin was tested against other *Candida* organisms (with the exception of one isolate of *C. lusitanae*), including *C. guilliermondii*, *C. kefyr* or *C. lusitanae*.¹³ Similar to that observed with caspofungin, a PAFE has also been observed for micafungin against *Candida* spp., and ranged from 0.9 to ≥20.1 h depending upon the concentration tested.²¹ In this study, concentrations that were four times the MIC produced the longest PAFE. For anidulafungin a PAFE >12 h was observed against *Candida* spp.¹⁸ The PAFE reported with aminocandin against *Candida* spp. *in vitro* ranged from 8 to 80 h, with the higher dose of 4 mg/kg producing a longer PAFE.¹⁹ The PAFE is important so that fungal killing can still occur even when plasma concentrations are below the MIC or the MEC (minimum effective concentration) of the organism; this may not be as important with high tissue concentrations as both caspofungin and anidulafungin are reported to have 'extensive tissue distribution'.^{39,40} Additionally, current dosing of the echinocandins is once daily; while the half-lives range from 13 to 27 h for the various echinocandins, a PAFE may not be an added advantage given the

current dosing recommendations.^{39–41} However, given the PAFE, extended dosing intervals could be an option, although further study is needed.

Animal models have also been used to describe the concentration-dependent pharmacodynamic properties of various echinocandins against *Candida* spp. In a murine model, the AUC:MIC appeared to be the best predictor of caspofungin efficacy against *C. albicans*.²⁶ Various animal models have also consistently shown concentration-dependent killing of *Candida* spp. with micafungin.^{42–46} In a disseminated *C. glabrata* neutropenic murine model, mice were administered a single dose of micafungin intraperitoneally at doses ranging from 0 to 100 mg/kg (doses were determined by multiplying daily doses by 7 for a 7 day cumulative dose).⁴² Maximal fungicidal activity occurred in mice that received ≥ 50 mg/kg. Another disseminated candidiasis neutropenic murine model with 4 strains of *C. albicans* and 10 strains of *C. glabrata* (with MICs ranging from 0.008 to 0.25 mg/L) reported that free drug 24 h AUC:MIC ratios for a static effect and killing were 7.5 ± 6.2 and 14.3 ± 13.1 , respectively.^{43,44} The free drug 24 h AUC:MIC demonstrated a strong relationship with reductions in kidney organism burden ($R^2=0.86$ for *C. albicans* and $R^2=0.58$ for *C. glabrata*). In neutropenic rabbits with disseminated *C. albicans* infection, a dose-dependent clearance of yeast in most tissues (liver, spleen, kidney, lung, vena cava and vitreous) was observed, with higher doses of 2 mg/kg/day required for brain tissue clearance.⁴⁵ The concentration-dependent activity of anidulafungin has been studied in a murine candidiasis model (inoculated with *C. albicans*, *C. tropicalis* and *C. glabrata*)⁴⁷ and in a rabbit model of invasive candidiasis caused by *C. albicans*.⁴⁸ In both studies, C_{max} :MIC as well as the AUC:MIC ratio best predicted outcome.^{47,48} A PAFE of 19 to >96 h (depending on the *Candida* isolate) was observed in the murine model.⁴⁷

Increasing drug concentrations of echinocandins above the organism's MIC has resulted in a paradoxical increase in fungal growth *in vitro* against select *Candida* spp. isolates.^{20,21,49–53} Known most commonly as the 'Eagle effect', it has been theorized that changes in fungal cell morphology result in increases in 'enlarged, globose cells'.⁵⁰ Therefore it is this compensatory homeostatic stress response that becomes activated in response to echinocandin-mediated cell wall damage that underlies this phenotype. Others believe changes in the fungal cell wall content result from a reduction in β -1,3-glucan content and increased chitin content,⁵¹ or that elevated drug concentrations may stimulate an unknown resistance mechanism.⁵² Additionally, up-regulation of several different pathways such as the protein kinase C (PKC) cell wall integrity pathway and the calcineurin pathway have been proposed to contribute to the Eagle effect, with several studies demonstrating an increase in the expression of genes for cell wall proteins in response to cell wall damage with higher concentrations of caspofungin.^{54–59} These pathways also seem to be coordinated with increases in chitin content.^{56,58,60} Furthermore, this paradoxical effect with the calcineurin pathway was negated when a calcineurin inhibitor such as cyclosporin A was given with caspofungin.^{54,55,58} The Eagle effect has been observed with caspofungin against various *Candida* spp.^{20,21,50,61,62} In one study, certain isolates of *Candida* spp. [including *C. albicans* (14/101) and *Candida dubliniensis* (112/124)] maintained growth at both low and high drug concentrations, yet displayed no fungal growth at moderate drug

concentrations.²¹ The MIC₉₀ for 98% of the *C. albicans* was ≤ 0.125 mg/L, with the Eagle effect being noted at concentrations ranging from 2 to 32 mg/L. Additionally, the MIC₉₀ for 90% of the *C. dubliniensis* was ≤ 0.125 mg/L for caspofungin, with the Eagle effect being noted at concentrations ranging from 0.5 to 32 mg/L (83% from 1 to 16 mg/L). Similar results were observed in *Candida* bloodstream isolates from cancer patients, with the paradox observed in isolates such as *C. albicans* (12/20), *C. parapsilosis* (9/10), *C. tropicalis* (4/10) and *C. krusei* (1/10).²⁰ Interestingly, this effect was not seen among 10 clinical isolates of *C. glabrata*. Against *C. parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis*, this paradoxical phenomenon was noted to have occurred in as many as 37% (7/19) of *C. parapsilosis* isolates, although it was not observed with other *Candida* isolates in this study.⁶²

The paradoxical effect of echinocandins on *Candida* spp. has also been evaluated with other agents within this class. Micafungin at concentrations ranging from 0.125 to 64 mg/L against *C. albicans* and *C. dubliniensis* has been analysed using a broth microdilution test.²¹ While the Eagle effect did not occur with any of the *C. albicans* isolates ($n=72$), 80/126 (63%) of *C. dubliniensis* isolates exhibited this effect at micafungin concentrations ranging from 0.5 to 64 mg/L. Growth of the isolates occurred at both low and high micafungin concentrations, but no growth occurred at intermediate levels. An earlier study evaluating other *Candida* spp. [*C. albicans* ($n=20$) and 10 isolates each of *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata*] and micafungin also found that this phenomenon occurred in 70% and 60% of the *C. tropicalis* and *C. krusei* isolates, respectively, but not in the other *Candida* spp.²⁰ Consequently, isolates with lower MICs were more likely to experience this paradoxical growth when compared with those with median MICs ($P<0.0001$). Two reports cite the paradoxical effect for anidulafungin against *Candida* spp.^{20,21} In one report, only *C. albicans* and *C. tropicalis* demonstrated the effect, although five species of *Candida* were tested (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. glabrata*).²⁰ Contrary to these findings, another *in vitro* study evaluating anidulafungin at concentrations of 0.125–64 mg/L did not observe an Eagle effect with either *C. albicans* ($n=103$) or *C. dubliniensis* ($n=127$).²¹

Murine models have also been utilized to examine the Eagle effect.⁴⁹ In mice inoculated with different isolates of *C. albicans*, higher cfu values were observed in the highest caspofungin dosage group (20 mg/kg) relative to the lower dosage group (5 mg/kg).⁴⁹ However, the results were not reproducible when the experiment was repeated.⁴⁹ To date, animal studies with anidulafungin and micafungin and various *Candida* spp. have failed to detect an Eagle effect when doses were escalated.^{42–48,63–66} In contrast, a decrease in fungal burden was demonstrated as doses were increased up to 10–20 mg/kg/day. Higher doses of aminocandins are also associated with more fungal burden reduction and improved survival.^{67–70} A potential dose-limiting toxicity was noted in one study at doses of 100 mg/kg.⁶⁷ In this study, the investigators reported that rapid death occurred in mice within 1 day of treatment with this dose.⁶⁷ Although not specifically evaluated, the Eagle effect was not reported for the available *in vitro* and *in vivo* studies for aminocandins.^{67–71}

In contrast to their activity in the planktonic state, the effect of select antibiotics (including antifungals) may be different in the biofilms secreted by microorganisms. Caspofungin,

micafungin and anidulafungin have shown favourable activity against biofilms in *C. albicans*, *C. dubliniensis*, *C. glabrata* and *C. krusei*.^{72–78} In a study using time–kill analysis to evaluate caspofungin against *C. albicans* biofilms, >99% of the attached fungal cells were killed at concentrations ranging from 0.125 to 1 mg/L.⁶¹ The highest concentration in this study (8 mg/L) was not as effective as lower concentrations. In a similar study of biofilms, this paradoxical effect was again demonstrated in several *Candida* spp. isolates, including *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*.⁵⁰

***Aspergillus* spp.**

Similar to properties demonstrated in select *Candida* spp., concentration-dependent activity of echinocandins has been demonstrated against *Aspergillus* spp.^{79–81} However, in contrast to the fungicidal activity seen against *Candida* spp., caspofungin,^{79,80} micafungin and anidulafungin^{81,82} appear to display fungistatic activity against *Aspergillus* spp.

A much shorter PAFE (≤ 0.5 h) for both caspofungin and micafungin relative to that seen with caspofungin against *Candida* spp. was observed with *Aspergillus* spp. (specifically *Aspergillus fumigatus*).⁸³ To date, there are no published studies evaluating the PAFE of anidulafungin or aminocandin with regard to *Aspergillus* spp.

Due to the fungistatic activity of echinocandins against mould infections, it is difficult to determine a clear MIC at which point mould is inhibited; therefore the MEC rather than the MIC is used to determine the activity of echinocandins against moulds.^{84–87} The MEC is defined as the lowest concentration of drug that causes growth of short, rounded compact hyphal forms of the organism.⁸⁸ The CLSI recommends that *Aspergillus* spp. with MECs of ≤ 1 mg/L should be considered susceptible to echinocandins.⁸⁴ Additionally, *in vitro* susceptibility to anidulafungin, caspofungin and micafungin of 526 *Aspergillus* spp. isolates from >60 medical centres from 2001 to 2007 demonstrated that >99% of the isolates were inhibited at MECs of ≤ 0.06 mg/L.⁸⁹ However, currently, there are limited data to correlate MECs of ≤ 1 mg/L and clinical outcomes in invasive *Aspergillus* infections.⁸⁸

Similar to select *Candida* spp., the Eagle effect was also observed for caspofungin against *Aspergillus* spp.⁷⁹ Specifically, 56% (5/9) of the *A. fumigatus* isolates, 13% (1/8) of the *Aspergillus flavus* isolates and 50% (6/12) of the *Aspergillus terreus* isolates demonstrated this paradox.⁷⁹ An *in vitro* model was also used to determine the MEC of various echinocandins (including micafungin) against *A. fumigatus*, *A. terreus* and *A. flavus*.⁹⁰ Results generally ranged from 0.06 to 0.25 mg/L, but were slightly higher for germinated *A. flavus* (0.25–1.0 mg/L). While metabolic activity decreased as concentrations of micafungin increased for most isolates, paradoxical increases in metabolism occurred in both *A. fumigatus* (2/11 isolates) and *A. terreus* (1/8 isolates). There are limited *in vitro* data regarding anidulafungin and the presence of a paradoxical effect with *Aspergillus* spp. Paradoxical increases in metabolism were detected in *A. fumigatus* (5/11) and *A. terreus* (2/8) isolates in one study at higher concentrations of anidulafungin.⁹⁰

In a recent Monte Carlo simulation study,⁹¹ different doses of micafungin were evaluated in 48 plasma samples donated from 10 patients to determine the optimal effective concentration

target against *Aspergillus* spp. In this study, the target plasma concentration was >0.05 mg/L. To reach this target, the probability of target attainment (PTA) had to be $>95\%$. Micafungin doses ranged from 25 to 300 mg per day for the once-daily regimen and from 12.5 to 150 mg twice a day for the twice-daily regimen. The results of this study demonstrated that the effective concentration target was only achieved with the 250 and 300 mg once-daily regimens and the 100, 125 and 150 mg twice-daily regimens. Therefore, based on the results of this simulation study, micafungin doses of at least 100 mg twice daily or 250 mg once daily may be required for favourable outcomes.⁹¹ While the target concentration in this study was focused on the plasma, this may not be the best marker of activity since plasma concentrations may not correlate with infection site concentrations, which are especially important with *Aspergillus* infections. A recent study⁹² evaluated concentrations of micafungin not only in the plasma, but also in the alveolar macrophages (AMs) and epithelial lining fluid (ELF). This study administered three daily doses of micafungin (150 mg/day) to 15 healthy volunteers. The results of this study demonstrated markedly higher concentrations in the plasma (14.8 ± 1.6 mg/L) compared with the ELF (0.52 ± 0.1 mg/L) and AMs (10.4 ± 5.6 mg/L) at 4 h. However, at 24 h, the AM concentration was the highest (14.6 ± 8.6 mg/L) compared with the plasma (4.8 ± 0.6 mg/L) and ELF (0.43 ± 0.2 mg/L).⁹²

Published *in vivo* studies to define the pharmacodynamic properties of echinocandins against *Aspergillus* spp. are sparse, but also suggest that an Eagle effect may occur with this organism. Two murine models of invasive pulmonary aspergillosis (IPA) due to *A. fumigatus* described the activity of caspofungin.^{93,94} Concentration-dependent activity was demonstrated in neutropenic mice administered caspofungin.⁹³ While the C_{\max} :MEC ratio was the best predictor of efficacy, an increase in fungal burden in the lungs was noted in the highest dosage group. An IPA animal model in neutropenic mice also noted an increase in fungal growth as caspofungin concentrations increased.⁹⁴

Animal models have also examined the pharmacodynamic properties of micafungin. IPA animal models in both neutropenic rabbits and mice treated with micafungin demonstrated a dose-dependent response in survival, but reported conflicting results regarding fungal burden and dosage escalation.^{45,94,95} In a neutropenic murine model with a single isolate of *A. fumigatus*, micafungin 0.25–10 mg/kg/day resulted in a dose-dependent reduction in fungal burden.⁹⁴ A murine model of disseminated aspergillosis evaluated micafungin 1–10 mg/kg/day against two different isolates of *Aspergillus* (itraconazole-resistant *A. fumigatus* and amphotericin B-resistant *A. terreus*).⁹⁵ Micafungin MICs were <0.015 mg/L for both isolates. For the *A. fumigatus* isolate, survival rates were higher in the 5 and 10 mg/kg/day arms (100% survival) in comparison with the 1 mg/kg arm ($P=0.02$). Similarly, the *A. terreus* isolate had a higher survival rate in the 10 mg/kg arm compared with the 1 mg/kg arm ($P=0.03$). However, there was no dose-dependent reduction in fungal burden or toxicity noted. A trend toward an increase in fungal burden (with persistent galactomannan antigenaemia), an improvement in rate of survival ($P<0.001$) and a decrease in pulmonary infarction ($P<0.05$) were noted with increasing micafungin doses compared with liposomal amphotericin B in a neutropenic rabbit model of IPA (*A. fumigatus*).⁴⁵

It was speculated that increases in damaged hyphal elements/fragmentation (known to be related to the mechanism of action of the echinocandins in IPA infections) might have caused persistent elevations in the serum galactomannan index despite improvement in outcomes.

Anidulafungin has also been investigated using animal models. A neutropenic rabbit model in animals infected with *A. fumigatus* treated with anidulafungin 1–20 mg/kg intravenously failed to detect a significant reduction in fungal burden at any drug concentration when compared with control ($P=0.3502$).⁴⁸ Improvement in survival ($P<0.05$) was observed in the 1 and 10 mg/kg/day groups, but not in the 5 and 20 mg/kg/day groups. Dose-dependent hyphal injury was observed, with the highest level of cell wall damage occurring in the 20 mg/kg/day group. Possible explanations included either an Eagle effect or a dose-related toxicity in the 20 mg/kg/day arm. Anidulafungin also demonstrated a dose-dependent damage of hyphal structures without elimination of aspergillosis from the tissues in leucopenic rabbits with invasive *A. fumigatus* infection.⁹⁶ While a survival benefit was observed in the 5 and 10 mg/kg/day arms, autopsy results revealed that higher doses of anidulafungin were associated with increased necrotic lungs and mottled livers.

Other pathogens

Published data are lacking to adequately describe the pharmacodynamic properties of echinocandins against pathogens other than select *Candida* and *Aspergillus* spp. responsible for IFIs. Limited data are available regarding dosing escalation of echinocandins in such organisms as *Rhizopus oryzae* and *Fusarium solani*.^{97,98} In one study, caspofungin was evaluated in mice after they were inoculated with 5×10^2 and 5×10^3 spores of *R. oryzae*.⁹⁷ Caspofungin (at 0.5 mg/kg twice daily) improved the survival of mice with 5×10^2 spores, whereas amphotericin (0.5 mg/kg twice daily) did not ($P<0.05$). Interestingly, higher doses of caspofungin (2.5 and 5 mg/kg both twice daily) did not improve survival. There was also no difference noted in the groups for the 5×10^3 spore inoculum. Neutropenic mice inoculated intravenously with *F. solani* were administered caspofungin (1 or 5 mg/kg/day), liposomal amphotericin B (LAmB) (15 mg/kg/day), LAmB 15 mg/kg/day plus caspofungin 1 mg/kg/day or placebo for 2 days before the infection continuing to 1 day after (continuous therapy).⁹⁸ No survival benefit was observed for any agent in the prophylactic or delayed therapies. For the continuous therapy group, only caspofungin at 1 mg/kg/day (and not 5 mg/kg/day) was associated with improved survival compared with the other groups ($P<0.05$).⁹⁸

Combination therapy

Much of the data regarding combination antifungal therapy are limited by the lack of standardization in the methods used for assessment (i.e. varying combinations tested, different fungal pathogens used) and have been derived from *in vitro* studies or animal models. These models in turn have been difficult to correlate to fungal infections occurring in humans. As a generalization, most antifungal combination studies with echinocandins in the laboratory or in animal models have demonstrated conflicting results, with either no effect or synergy.^{99–102}

Additionally, there are limited data regarding pharmacokinetic interactions and antifungal combinations. Nevertheless, it is postulated that echinocandins may be an attractive option to add to other antifungal therapies due to their efficacy and relative lack of toxicity.^{99,100}

In vitro and animal models of combination therapy (amphotericin B, fluconazole or voriconazole) with echinocandins for candidiasis have been less impressive, with either no effect or slight synergy, and may be difficult to demonstrate due to the fungicidal activity of echinocandins against *Candida* spp. infections at baseline.^{103–108} Further investigation into outcomes in human mycological infections and combination therapy with echinocandins is warranted.

In vitro studies of echinocandins in combination with either amphotericin B or triazoles have demonstrated both synergy and indifference for invasive aspergillosis infections.^{108–117} Animal studies have suggested that the combination of amphotericin B or broad-spectrum triazoles such as voriconazole or posaconazole with an echinocandin may be more effective than using each of these agents individually in invasive aspergillosis infections.^{100,117–120} Clinical outcomes in humans and combination therapies with echinocandins for invasive aspergillosis are limited to case reports, retrospective analyses or small studies. In one open-labelled, non-comparative study in bone marrow transplant patients with refractory aspergillosis, micafungin in combination with amphotericin B had clinical success rates of 39%.¹²¹ However, retrospective studies in humans have demonstrated either a slight benefit or no effect.^{122,123}

Limitations of *in vitro* and *in vivo* models

While much of the pharmacodynamics of echinocandins that are available in the literature are from *in vitro* and animal models, it is difficult to determine if these concepts can be applied to actual patients without extensive research in clinical trials. Although data exist for fluconazole that validate the pharmacodynamics in patients, there are no outcome trials to date evaluating the pharmacodynamic targets (AUC:MIC, C_{max} :MEC or MIC) for the echinocandin class. Additionally, there are inherent limitations to these models.¹²⁴ Some studies may have only used one strain of the organism, limiting the external validity. Also, animal studies generally focus on induced, acute infection rather than chronic conditions. As for the Monte Carlo simulations, common patient variability such as weight, body surface area, genetic polymorphisms and other covariates are hopefully distributed evenly through the random sampling process. However, a small number of patients in a Monte Carlo simulation may skew the data and limit their applicability to the population as a whole. Also, often healthy volunteers are studied, when in fact these subjects may differ significantly from the patient population.¹²⁴ At this point, the clinical data that are currently available for the echinocandin class are primarily focused on dose-ranging studies and are discussed further below.

Alterations of the host immune system

One interesting theory with the echinocandin class involves the changes that occur within the host immune system that aid in

the clearance of the fungal pathogen once the echinocandin has been introduced.¹²⁵ When the echinocandin inhibits the synthesis of β -glucan fungal cell wall, the neutrophils and antibodies can then cause further fungal damage.^{126,127} In addition, both caspofungin and micafungin have data to support additional monocyte and macrophage activity with *A. fumigatus* if the patient has been previously exposed to an echinocandin.^{128,129} Although these are interesting concepts, further research is still warranted in this area.

Clinical implications

Clinical studies in patients with oropharyngeal and/or oesophageal candidiasis suggest that higher doses of echinocandins (specifically micafungin and anidulafungin) may improve treatment outcomes.^{130–133} In a randomized, double-blind, parallel group study, adult HIV-positive patients ($n=245$) with oesophageal candidiasis received once-daily doses of either micafungin (50, 100 or 150 mg) intravenously or fluconazole 200 mg orally for 14–21 days.¹³¹ A dose-dependent endoscopic cure rate was observed for micafungin-treated patients in the intent-to-treat group (68.8%, 77.4% and 89.8%, respectively). Cure rates between the 50 and 150 mg groups were significantly different ($P=0.007$) whereas no clinically significant differences regarding treatment-related side effects were noted among different dosage groups. These results were consistent with an earlier study in which higher doses of micafungin (50–100 mg) demonstrated an increase in resolution or improvement in HIV-positive patients with oesophageal candidiasis when compared with lower doses (i.e. 12.5–25 mg).¹³² Cure rates ranging from 84% to 97% were observed with the higher doses compared with 33% to 54% with the lower doses with once again no differences noted in adverse drug events among the dosage groups ($P=0.001$). In contrast, a dose–response relationship could not be detected for micafungin in a multicentre, multinational, double-blind, randomized, non-inferiority study of patients ($n=452$) with oesophageal candidiasis.¹³⁰ Although no between-group statistical comparisons were reported and mean daily doses of micafungin were similar, response rates of 93%, 91% and 91%, respectively, were reported in patients receiving either micafungin 300 mg every other day, micafungin 150 mg daily or caspofungin 50 mg daily. For the treatment of oesophageal candidiasis and/or oropharyngeal candidiasis with anidulafungin, endoscopic improvement (85% versus 81%) and clinical improvement scores (81.8% versus 68.8%) were reported in patients receiving 35 mg/day (following a 75 mg loading dose) and 25 mg/day (following a 50 mg loading dose), respectively.¹³³ However, no between-group statistical comparison was reported in this study.

In contrast to the treatment of oropharyngeal and/or oesophageal candidiasis, the impact of dose escalation of echinocandins in patients with invasive candidiasis is less clear.^{11,134–137} The safety (a primary endpoint) and efficacy of caspofungin in adult patients with invasive candidiasis was evaluated in a multicentre, double-blind trial.¹³⁴ No significant differences were observed in the rate of either adverse drug events (2% versus 3% incidence) {difference 1.1% [95% confidence interval (CI) –4.1% to 6.8%]} or success (78% versus 72%) [difference 6.3% (95% CI –5.9% to 18.4%)] in patients receiving ‘traditional-dosed’

caspofungin (a single 70 mg loading dose followed by 50 mg/day) or higher dose caspofungin (150 mg/day), respectively. However, the trial was not adequately powered to detect significant differences in clinical efficacy.

Studies evaluating the potential impact of dose escalation on treatment outcomes with micafungin in the management of invasive candidiasis have also been reported.^{11,136,137} Micafungin was evaluated in an international, open-label, non-comparative study, alone or in combination with other antifungals, in 126 adult, neonatal or paediatric patients with newly diagnosed or refractory candidaemia.¹³⁶ Patients received either 50 mg/day (if ≥ 40 kg) or 1 mg/kg (if < 40 kg) for *C. albicans* infections or 100 mg/day (if ≥ 40 kg) or 1 mg/kg (if < 40 kg) of micafungin for non-*albicans* or germ tube-negative infections. Doses could be escalated in 50 mg increments (1 mg/kg increments if < 40 kg) at the investigator’s discretion if the patient after 5 days of therapy had stable or progressive disease. Patients in the dosing range of 76–150 mg ($n=43$) had overall response rates that were $>90\%$, which was higher in comparison with those receiving doses of < 50 –75 mg/day (response rate 75%–87%) ($n=71$). While there were few patients in the >150 –200 mg ($n=9$) and >200 mg ($n=3$) arms, the response rates were lower in these groups [55.6% (95% CI 21%–86%) and 66.7% (95% CI 9%–99%), respectively]. These results should be interpreted with caution, since higher doses were typically utilized in patients with refractory disease, and no between-group statistical comparisons were reported. In another study, patients receiving once-daily doses of micafungin 100 or 150 mg were compared with patients receiving caspofungin 50 mg (following a single 70 mg loading dose) in a randomized, double-blind study of adult patients with either candidaemia (85% of patients) or invasive candidiasis (15% of patients).¹¹ Just over half of the isolates recovered were non-*albicans* spp. Treatment success in the modified intent-to-treat population occurred in 76.4%, 71.4% and 72.3% of patients, respectively. Overall mortality was not different between groups (29%, 33.2% and 26.4%, respectively). Relative to caspofungin, treatment differences were 4.1% (95% CI, –4.4% to 12.3%) and –1.0% (95% CI, –9.3% to 7.8%) in the micafungin 100 and 150 mg treatment groups, respectively. Contrary to these two prospective randomized studies, a retrospective analysis of patients with candidaemia suggested that patients may benefit from micafungin dose escalation.¹³⁷ In patients receiving < 2.25 mg/kg/day (low dose) ($n=13$) or ≥ 2.25 mg/kg/day (high dose) ($n=15$) of micafungin for 7 days, there was no significant difference between groups with regard to 30 day clinical response (77% low dose versus 93% high dose; $P=0.244$) and 30 day mortality (15% low dose versus 7% high dose; $P=0.583$). However, days until clinical response were fewer in the high-dose group (21.0 ± 4.6 days low dose versus 16.9 ± 3.6 days high dose; $P=0.021$). Additionally, the low-dose group received a longer duration of therapy with micafungin in comparison with the group that received the high dose ($P=0.043$). Adverse drug reactions among the treatment groups were not reported in this study.¹³⁷ Finally, anidulafungin was investigated in a Phase II, randomized, dose-ranging study in 123 patients with invasive candidiasis and candidaemia.¹³⁵ In 68 evaluable patients receiving anidulafungin 50, 75 or 100 mg once-daily dosing (following a single loading dose of twice the randomized daily dose) for up to 14 days after resolution of infection, global response rates for

the 50, 75 and 100 mg arms were 84%, 90% and 89%, respectively (no statistical information reported).

Data regarding the impact of dose escalation on the efficacy of echinocandins in the treatment of invasive aspergillosis are sparse. To date, there are no published trials in humans evaluating different caspofungin dosing regimens targeted for *Aspergillus* spp. One case report involving treatment of a 29-year-old neutropenic male with myelodysplastic syndrome and pulmonary aspergillosis treated with caspofungin describes a possible Eagle effect based on increasing concentrations of galactomannan following initiation of therapy.¹³⁸ A dose-dependent response to therapy was suggested for micafungin in one case report in a patient with acute lymphoblastic leukaemia and IPA unresponsive to 7 days of 75 mg/day of micafungin who later responded to 50 mg/day.¹³⁹ While a Japanese multi-centre, open-label study of micafungin 25–150 mg/day (up to 56 days) reported a favourable clinical response of 57% (24/42) in the aspergillosis group (6/10 for IPA, 6/9 for chronic necrotizing pulmonary aspergillosis and 12/22 for pulmonary aspergilloma), optimal doses for each infection type could not be determined.¹⁴⁰ However, no paradoxical effect was noted with patients receiving the higher doses.

Conclusions

While published *in vitro* and animal model data describe concentration-dependent activity of echinocandins against select *Candida* and *Aspergillus* spp., data for other fungal pathogens are sparse. Concerns have been raised regarding the potential for paradoxical growth, increased toxicity and cost with higher doses. Although no major safety concerns have been raised to date, the role of dose escalation in improving treatment outcomes in the treatment of IFIs is unclear, and further research in this area is warranted before use in the clinical setting can be recommended.

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