

In vitro assessment of the antifungal and paradoxical activity of different echinocandins against *Candida tropicalis* biofilms

Infections due to non-*albicans Candida* species are now seen with increasing frequency and in some cases their incidence exceeds that for *Candida albicans* (Bassetti *et al.*, 2006). Depending on the geographical region, *Candida tropicalis* ranks from the second to the fourth leading cause of invasive candidiasis (Bassetti *et al.*, 2006; Nucci *et al.*, 2010; Pfaller *et al.*, 2008; Tan *et al.*, 2010). Multiple studies of echinocandins against most *C. albicans* biofilms have shown them to be effective at concentrations of 2 $\mu\text{g ml}^{-1}$ or less (Bachmann *et al.*, 2002; Cocuau *et al.*, 2005; Ferreira *et al.*, 2009; Jacobson *et al.*, 2008, 2009; Kuhn *et al.*, 2002; Miceli *et al.*, 2009a). However, a paradoxical effect (PE) of echinocandins against *C. albicans* biofilms treated with high concentrations of caspofungin (CFG) has been described (Stevens *et al.*, 2004). This phenomenon has subsequently been described with other echinocandins and other *Candida* species (Chamilos *et al.*, 2007; Fleischhacker *et al.*, 2008; Melo *et al.*, 2007). This effect appears to be species-, strain- and echinocandin-specific (Chamilos *et al.*, 2007; Ferreira *et al.*, 2009; Melo *et al.*, 2007; Miceli *et al.*, 2009a, b) and associated with chitin accumulation and ultrastructural alterations (Bizerra *et al.*, 2011); however, the clinical significance of the PE remains unclear.

There are limited comparative data available on the antifungal and paradoxical activity of anidulafungin (AFG) and micafungin (MFG) on the biofilms of non-*albicans Candida* species. Since *C. tropicalis* is becoming an emerging pathogenic *Candida* species, we performed a direct comparison of the efficacy and paradoxical activity of the echinocandins CFG, AFG and MFG against mature biofilms of five *C. tropicalis* strains.

A figure showing a comparison of the antifungal and paradoxical activity of echinocandins in *Candida tropicalis* biofilms is available with the online version of this paper.

One clinical (44) and four reference (ATCC 750, ATCC 44508, ATCC 44509 and ATCC 200956) strains of *C. tropicalis* were studied. AFG (Eraxis; Pfizer), CFG (Cancidas; Merck) and MFG (Mycamine; Astellas) were purchased from the hospital pharmacy (Raymond G. Murphy VA Medical Center). Biofilm formation and the XTT-reduction assay were performed as described by Ramage & López-Ribot (2005). The antifungal activities of the echinocandins were expressed as a percentage relative to the metabolic activity of the untreated biofilms. Each experiment was performed independently three times, each in triplicate. The sessile (biofilm) MICs (SMICs) were determined by the concentration of each echinocandin needed to reduce the metabolic activity of the biofilms of each strain by 50% (SMIC₅₀) and 80% (SMIC₈₀). A PE was defined as increased metabolic activity above SMIC₅₀ despite the presence of an increasing concentration of antifungal agent.

The SMIC₅₀ of all echinocandins for all strains tested were at concentrations of $\leq 1 \mu\text{g ml}^{-1}$ (Table 1; Supplementary Fig. S1 in JMM Online). The SMIC₅₀ of CFG for all strains tested ranged from 0.25 to 1 $\mu\text{g ml}^{-1}$. AFG and MFG had SMIC₅₀ values of 0.25 $\mu\text{g ml}^{-1}$ for all strains tested. The SMIC₈₀ of CFG ranged between 1 and 2 $\mu\text{g ml}^{-1}$. AFG had an SMIC₈₀ of 0.25 $\mu\text{g ml}^{-1}$ for all strains except two: 1 $\mu\text{g ml}^{-1}$ for ATCC 200956 and 128 $\mu\text{g ml}^{-1}$ for strain 44. MFG also had an SMIC₈₀ of 0.25 $\mu\text{g ml}^{-1}$ for all strains except two: 0.5 $\mu\text{g ml}^{-1}$ for ATCC 200956 and $>128 \mu\text{g ml}^{-1}$ for strain 44.

A PE, i.e. increased metabolic activity at concentrations above SMIC₅₀, was seen in three of the five strains tested. Two strains, ATCC 750 and ATCC 44508, exhibited a PE with CFG at concentrations of 32, and 16 to $>128 \mu\text{g ml}^{-1}$, respectively (Table 1; Supplementary Fig. S1). AFG produced a PE in one strain (ATCC 200956) at

concentrations from 16 to 64 $\mu\text{g ml}^{-1}$. MFG did not exhibit a PE in any of the strains tested. No strains exhibited a PE with more than one echinocandin. While not meeting our definition of a PE, increasing concentrations of AFG and CFG resulted in increased metabolic activity (reduced antifungal activity) above SMIC₈₀ in all strains tested except for strain 44 for AFG and ATCC 44509 for CFG. Increasing concentrations of MFG resulted in increased metabolic activity in two strains, ATCC 44508 and ATCC 44509. Thus, despite not meeting a strict definition of PE, all strains exhibited increased metabolic activity in response to increasing concentrations of at least one echinocandin. Two strains, ATCC 750 and ATCC 220956, had increased metabolic activity with higher concentrations of all three echinocandins.

In this study, all three echinocandins were effective against *C. tropicalis* biofilms with the SMIC₅₀ of all three echinocandins $\leq 1 \mu\text{g ml}^{-1}$. There was a wide range of SMIC₅₀ values for CFG among the strains tested, compared to the SMIC₅₀ of both AFG and MFG, which is similar to results reported by Choi *et al.* (2007). The SMIC₅₀ of CFG tended to be higher than that of MFG and AFG: 1 $\mu\text{g ml}^{-1}$ versus 0.25 $\mu\text{g ml}^{-1}$. Although our results might suggest that MFG and AFG may be more potent than CFG, it should be pointed out that the addition of human serum to AFG and MFG during *in vitro* susceptibility testing of *Candida* species sharply increased their MIC while modestly increasing the MIC of CFG (Garcia-Effron *et al.*, 2009; Odabasi *et al.*, 2007; Wiederhold *et al.*, 2008). It would be of clinical interest to validate these findings within *C. tropicalis* biofilms.

As seen in previous studies as well as ours, CFG appears the most likely echinocandin to produce a PE whereas MFG was least likely to produce a PE in *C. tropicalis* biofilms. We did not see a correlation in the presence of PE of one echinocandin

Table 1. SMIC₅₀, SMIC₈₀ and PE of the three echinocandins for all strains tested

	SMIC ₅₀					SMIC ₈₀					PE (drug concentration)				
	Strain	ATCC	ATCC	ATCC	ATCC	Strain	ATCC	ATCC	ATCC	ATCC	Strain	ATCC	ATCC	ATCC	ATCC
	44	750	44508	44509	200956	44	750	44508	44509	200956	44	750	44508	44509	200956
AFG	<0.25	<0.25	<0.25	<0.25	<0.25	0.5	<0.25	<0.25	0.25	<0.25	*	*	*	*	≥16
CFG	1	0.5	<0.25	<0.25	0.5	2	1	1	1	1	*	32	≥16	—	*
MFG	<0.25	<0.25	<0.25	<0.25	<0.25	4	0.25	<0.25	0.25	<0.25	*	*	—	—	*

*These strains did not meet our definition of a PE; however, biofilm metabolic activity increased at higher concentrations of the drug tested.

with another in any of the strains tested, or a correlation between SMIC₅₀ and the presence of PE. However, in all the strains that exhibited a PE with one echinocandin, there was increased activity above the SMIC₈₀ with another echinocandin. Although the clinical significance of PE is not clear, this could theoretically have an impact on the potential use of echinocandins as a part of antimicrobial lock therapy (ALT) for the management of infected intravascular catheters. Because of the lack of adequate data, no recommendations can be made on the use of ALT in the management of catheter-related bloodstream infections due to *Candida* species at this time but further studies are warranted.

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