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# Post-antifungal effects and time-kill studies of anidulafungin, caspofungin, and micafungin against *Candida glabrata* and *Candida parapsilosis*☆

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**Abstract**

*Candida glabrata* (*Cgl*) and *Candida parapsilosis* (*Cpa*) can cause serious infections and can be resistant to some antifungal drugs. In treating infections caused by these organisms, killing rates and post-antifungal effects (PAFE) are important factors in both dose interval choice and outcome. Two strains each of *Cgl* and *Cpa* were studied. For PAFE studies, each organism was exposed to micafungin (MCF), anidulafungin (ANF), or caspofungin (CAS) for 1 h at concentrations ranging from 0.25 to 16×MIC. Cell suspensions were then washed 3 times and resuspended in fresh broth. Time 0 was immediately after resuspension of the yeast. Time-kill experiments were done using similar drug concentrations. Samples were removed at each time point (0-120 h) and viable counts determined. PAFE of ANF and CAS were generally very long, were markedly longer than those of MCF, and increased with increased drug concentration. For ANF and CAS, PAFE for *Cgl* were greater than those for *Cpa* only at 0.5 to 2 × MIC. Time-kill experiments showed that ANF, CAS, and MCF were fungicidal at 8 to 16 × MIC up to 120 h. CAS had the greatest activity against *Cgl*, while ANF and MCF were more active than CAS against *Cpa*. Because of the prolonged PAFE of these echinocandins, especially ANF and CAS, less frequent dosing during therapy of *Cpa* and *Cgl* infections could be considered. Further studies are needed to determine the clinical efficacy of longer dosing intervals.
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*Keywords:* PAFE; Echinocandin effects; *C. glabrata*; *C. parapsilosis*

## 1. Introduction

*Candida glabrata* and *Candida parapsilosis* are emerging causes of nosocomial blood stream infection in both adults and infants, and their resistance to azole antifungals is an increasing problem (Pfaller et al., 2010, 2011a,b; Horn et al., 2009). Echinocandins are now recommended in high-risk clinical situations when Candida is suspected as a cause of serious infection (Pappas et al., 2009). Anidulafungin (ANF), caspofungin (CAS), and micafungin (MCF) are antifungal agents of the echinocandin class and have similar spectra of antifungal activity (Cappelletty and Eiselstein-McKitrict, 2007; Pound et al., 2010).

Because the use of echinocandins to treat serious *Candida* infections is increasing, and because duration of anti-infective activity after drug clears from the site of infection is important, both in dose interval choice and in outcome of infection (Gumbo et al., 2007), we compared the antifungal activities and the post-antifungal effects (PAFE) of ANF, CAS, and MCF against *C. glabrata* and *C. parapsilosis*. Based on the reports of extended PAFE by others (Ernst et al., 2000, 2002) and the observations of apparent prolonged antifungal efficacy of echinocandins in animal models (Louie et al., 2005; Gumbo et al., 2007), we postulated that echinocandin PAFE might actually be extremely long and that different *Candida* species might respond differently to treatment in vitro with different echinocandins.

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Table 1
MICs of ANF, CAS, and MCF against 2 strains of *C. glabrata* and 2 strains of *C. parapsilosis*

| Strain | MIC (μg/mL) | | |
|---|---|---|---|
| | ANF | CAS | MCF |
| *C. glabrata* 12 | 0.06 | 0.5 | 0.03 |
| C. glabrata 14 | 0.06 | 0.5 | 0.03 |
| *C. parapsilosis* 29 | 0.5 | 1 | 1 |
| *C. parapsilosis* 30 | 1 | 0.5 | 1 |

## 2. Materials and methods

### 2.1. Microorganisms

Two clinical isolates of *C. glabrata* (*Cgl* 12 and *Cgl* 14) and 2 of *C. parapsilosis* complex (*Cpa* 29 and *Cpa* 30) were obtained from the laboratory of Dr. Vishnu Chaturvedi (Wadsworth Center, NY State Dept. of Health, Albany, NY).

### 2.2. Antimicrobial agents and susceptibility testing

ANF, CAS, and MCF were obtained from Pfizer Pharmaceuticals (Groton, CT), Merck (Rahway, NJ), and Astellas Pharmaceuticals (Deerfield, IL), respectively, and solutions were prepared according to the manufacturers' directions. MICs, determined using the CLSI-approved macrodilution method as described in CLSI document M27-A3 (Clinical and Laboratory Standards Institute, 2008), are shown in Table 1.

### 2.3. Post-antifungal effects

The methods used to determine the PAFE of all test isolates were those of Ernst et al. (2000). One milliliter of fungal suspension was added to each tube containing culture medium alone or with drug at concentrations ranging from 0.25 to 16 × MIC. Following a 1-h incubation, the drug was removed by washing 3 times. After the final wash, the fungal pellet was resuspended in 9 mL of RPMI 1640. The resuspended samples were then incubated at 35 °C with agitation. Colony counts were obtained after the final wash at indicated times by removing a 100-μL sample from each culture, serially diluting with sterile water, then plating 30-μL aliquots in duplicate on Sabouraud dextrose agar (SDA). All experimental conditions were conducted in duplicate, all 3 antifungals were tested in each experiment, and each experiment was performed at least 2 times. PAFE were determined using the following equation: PAFE = $T - C$, where $T$ = time required for counts in treated cultures to increase by 1 $\log_{10}$ unit above that seen following drug removal and $C$ = time required for counts in the untreated control to increase by 1 $\log_{10}$ unit above that following the last wash (Craig and Gudmundsson, 1996).

### 2.4. Time-kill procedures

Time-kill experiments were performed as described by Klepser et al. (1998, 2001) and Bopp et al. (2006). Yeast were obtained from previously stored samples and subcultured twice on SDA plates prior to testing. Fungal suspensions were prepared in sterile water by touching 3 to 5 colonies from a 24- to 48-h SDA culture and adjusting the turbidity of the resulting suspension to that of a 0.5 McFarland unit standard (approx. $5 \times 10^5$–$1 \times 10^6$ CFU/mL) by spectrophotometric methods. One milliliter of the fungal suspension was added to 9 mL of RPMI 1640 medium buffered with 3-(*N*-morpholino) propanesulfonic acid (pH 7.0), with or without drug, providing the starting inoculum of approx. $5 \times 10^4$–$1 \times 10^5$ CFU/mL. The range of echinocandin concentrations tested was 0.5 to 16 × MIC. The culture tubes were incubated with agitation at 35 °C. At predetermined time points following addition of the antifungal compound, a 0.1-mL sample was removed from each culture vial, serially diluted with sterile $H_2O$, and 30-μL aliquots were plated in duplicate on SDA. Colony counts were determined after incubation of the plates at 35 °C for 48 h. The lower limit of detection by these methods was 33 CFU/mL. In each experiment, each experimental condition was conducted in duplicate. Each experiment was performed at least 2 times.

### 2.5. Drug carryover

We utilized the method of Klepser et al. (1998) except that we performed subcultures using SDA rather than potato-dextrose agar for subcultures. Test tubes were set up containing 900 μL of sterile water and drugs at concentrations ranging from 0.25 to 16 × MIC (0.06 to 16 × MIC for CAS). Yeast (100 μL of a suspension containing $5 \times 10^3$ CFU/mL) were then added to each tube. A 30-μL aliquot

Table 2
PAFE of ANF, CAS, and MCF at 0.5, 2, and 16 × MIC for 2 strains of *C. glabrata* and 2 strains of *C. parapsilosis*

| Strain | PAFE (h)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5 × MIC | | | 2 × MIC | | | 16 × MIC | | |
| | ANF | CAS | MCF | ANF | CAS | MCF | ANF | CAS | MCF |
| *C. glabrata* 12 | >24 | >24 | 0.3 | >24 | >24 | 7 | >24 | >24 | >24 |
| *C. glabrata* 14 | >24 | >24 | -0.8 | >24 | >24 | >24 | >24 | >24 | >24 |
| *C. parapsilosis* 29 | 9 | 12 | 0.2 | >24 | >24 | 11 | >24 | >24 | >24 |
| *C. parapsilosis* 30 | 17 | 17 | 3 | >24 | >24 | 10 | >24 | >24 | >24 |

* Not all assays were continued for greater than 24 h.