

## *In Vivo* Comparison of the Pharmacodynamic Targets for Echinocandin Drugs against *Candida* Species<sup>∇</sup>

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Received 9 November 2009/Returned for modification 9 March 2010/Accepted 5 April 2010

Previous pharmacodynamic studies using *in vivo* candidiasis models have demonstrated that the 24-h area under the concentration-time curve (AUC)/MIC is a good descriptor of the echinocandin exposure-response relationship. Further studies investigating the 24-h AUC/MIC target for a stasis endpoint identified free-drug 24-h AUC/MIC against *Candida albicans* and were similar for two echinocandins, anidulafungin and micafungin. The current studies expand investigation of a third echinocandin (caspofungin) and compare the pharmacodynamic target among *C. albicans*, *Candida glabrata*, and *Candida parapsilosis*. Treatment studies were conducted with six *C. albicans*, nine *C. glabrata*, and 15 *C. parapsilosis* strains with various MICs (anidulafungin, 0.015 to 4.0 µg/ml; caspofungin, 0.03 to 4.0 µg/ml; and micafungin, 0.008 to 1.0 µg/ml). Efficacy was closely tied to MIC and the 24-h AUC/MIC. Therapy against *C. parapsilosis* required more of each echinocandin on a mg/kg basis. Caspofungin required less drug on a mg/kg basis for efficacy against all of the organisms than did the other two drugs. However, the 24-h AUC/MIC targets were similar among the echinocandins when free drug concentrations were considered, suggesting the relevance of protein binding. The targets for *C. parapsilosis* (mean, 7) and *C. glabrata* (mean, 7) were significantly lower than those for *C. albicans* (mean, 20) for each echinocandin. The results suggest that current susceptibility breakpoints and the consideration of organism species in these determinations should be reexplored.

Experimental antifungal pharmacodynamic investigations have been important for the design of optimal dosing strategies and the development of susceptibility breakpoints (1, 2, 5, 17). Several studies have begun to focus on the most recently approved antifungal drug class, the echinocandins (9, 11, 14, 21, 22, 34, 57). Results of these pharmacodynamic experiments have demonstrated concentration-dependent killing and have shown prolonged postantifungal effects. Both concentration-associated pharmacodynamic indices, the 24-h area under the concentration-time curve (AUC)/MIC and the  $C_{\max}$ /MIC, have been linked to treatment efficacy. More recently, investigations have attempted to identify the pharmacodynamic target or the drug exposure relative to the MIC needed for treatment efficacy (9, 14). Several observations were made in studies with two echinocandins in experimental models of disseminated *Candida albicans*. The pharmacodynamic targets were similar between the two echinocandins as long as free drug concentrations were considered, suggesting the relevance of protein binding. However, only a few “high” MIC organisms were available for investigation at the time, and the studies did not include the species demonstrating the least susceptibility *in vitro*, *Candida parapsilosis*. The present studies expand evaluation to include all three FDA-approved echinocandins as well as in depth exploration of the pharmacodynamic target for *C. parapsilosis*. The results from these experiments provide pharmacodynamic support for current clinical dosing regimens and

suggest that current susceptibility breakpoints based upon *Candida* species should be reconsidered.

### MATERIALS AND METHODS

**Organisms.** Thirty clinical *Candida* isolates were used for the *in vivo* treatment studies, including six *C. albicans*, nine *Candida glabrata*, and 15 *C. parapsilosis* strains (Table 1). The *C. parapsilosis* group was further characterized into *C. parapsilosis*, *Candida metapsilosis*, and *Candida orthopsilosis*, as previously described (33, 55). The organisms were chosen to include isolates with relatively similar degrees of fitness in the animal model, as determined by the amount of growth in the kidneys of untreated animals over 96 h. We also attempted to choose strains with various echinocandin susceptibilities.

**Antifungal agents.** Anidulafungin, caspofungin, and micafungin were obtained from Pfizer, Merck, and Astellas, respectively, for *in vitro* susceptibility testing. Stock solutions were prepared for susceptibility testing as described in the Clinical Laboratory Standards Institute (CLSI) standard M27-A3 (37). The same drugs were obtained from the University of Wisconsin Pharmacy for *in vivo* treatment studies and prepared according to the manufacturers' instructions.

***In vitro* susceptibility testing.** All isolates were tested in accordance with the standards in the CLSI document M27-A3, using RPMI 1640 medium, an inoculum of from  $0.5 \times 10^3$  to  $2.5 \times 10^3$  cells/ml, and incubation at 35°C (37). MICs were determined visually after 24 h of incubation as the lowest concentration of drug that caused a significant diminution ( $\geq 50\%$ ) of growth of control levels. Studies were performed on three separate occasions in duplicate. Final results were expressed as the mean of these results. Quality control was performed on each day of testing by using CLSI-recommended reference strains *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

**Animals.** Six-week-old ICR Swiss specific-pathogen-free female mice weighing 23 to 27 g purchased from Harlan Sprague-Dawley were used for all studies. Animals were maintained in accordance with the American Association for Accreditation of Laboratory Care criteria. Animal studies were approved by the University of Wisconsin Animal Care Committee.

**Infection model.** A neutropenic, murine, disseminated candidiasis model was used for the treatment studies. Mice were made neutropenic (polymorphonuclear cells,  $<100 \text{ mm}^3$ ) by injecting cyclophosphamide subcutaneously 4 days before infection (150 mg/kg of body weight), 1 day before infection (100 mg/kg), and 2 days after infection (100 mg/kg). Previous investigations have demonstrated that this regimen produces neutropenia throughout the 96-h study period

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<sup>∇</sup> Published ahead of print on 12 April 2010.

TABLE 1. *In vitro* susceptibility of study organisms (*C. albicans*, *C. glabrata*, and *C. parapsilosis*) against anidulafungin, caspofungin, and micafungin

Organism <sup>a</sup>	MIC ( $\mu\text{g/ml}$ )		
	Anidulafungin	Caspofungin	Micafungin
CA 98-17	0.03	0.12	0.03
CA 98-210	0.015	0.02	0.016
CA 580	0.015	0.03	0.008
CA 98-234	0.015	0.03	ND
CA K1	0.015	0.03	0.016
CA 570	0.03	0.12	0.02
CG 5592	0.06	0.03	0.016
CG 513	0.06	0.03	0.016
CG 33609	0.03	0.03	0.016
CG 32930	0.06	0.03	0.008
CG 33616	1	0.25	0.25
CG 35315	0.25	0.12	0.06
CG 5376	0.03	0.03	0.008
CG 34341	0.12	0.25	0.06
CG 37661	2	ND	0.25
CP-M 20463.02	0.25	0.06	0.12
CP 20450.096	1	0.50	0.25
CP 20477.078	2	0.50	0.50
CP 20385.079	1	0.50	0.50
CP 20477.048	2	4.00	0.50
CP 20423.072	1	0.12	0.25
CP 20423.078	1	0.25	0.50
CP 20562.03	1	0.25	0.50
CP 20553.009	2	1.00	1.00
CP 20489.085	1	0.25	0.12
CP 20511.092	1	0.06	0.25
CP 20557.012	0.5	0.12	0.25
CP-O 20577.055	0.5	0.06	0.25
CP 20519.069	4	1.00	1.00
CP 20435.057	1	0.06	0.50

<sup>a</sup> CA, *C. albicans*; CG, *C. glabrata*; CP, *C. parapsilosis*; CP-M, *C. metapsilosis*; CP-O, *C. orthopsilosis*.

(4). Organisms were subcultured on Sabouraud dextrose agar (SDA) 24 h prior to infection. The inoculum was prepared by placing three to five colonies into 5 ml of sterile 0.15 M NaCl warmed to 35°C. The final inoculum was adjusted to a 0.6 transmittance at 530 nm. Fungal counts of the inoculum determined by viable counts on SDA were  $6.25 \pm 0.33 \log_{10}$  CFU/ml.

Disseminated infection with the *Candida* organisms was produced by injection of 0.1 ml of the inoculum via the lateral tail vein 2 h prior to the start of antifungal therapy. At the end of the study period, animals were sacrificed by CO<sub>2</sub> asphyxiation. After sacrifice, the kidneys of each mouse were immediately removed and placed in 0.15 M NaCl at 4°C. The organs were homogenized and then serially diluted 1:10. Aliquots were plated onto SDA for viable fungal colony counts after incubation for 24 h at 35°C. The lower limit of detection was 100 CFU/ml. The results were expressed as the mean number of CFU/kidneys for three mice.

**Pharmacokinetic studies and analysis.** The single-dose pharmacokinetics of anidulafungin and micafungin used for these studies were previously performed, analyzed, and published in this journal (9, 14). Identical experiments were undertaken using this animal model with caspofungin. Briefly, studies were performed in infected neutropenic mice by using the infection model described above. Mice were administered single doses of the echinocandin drugs by the intraperitoneal route at doses of 80, 20, and 5 mg/kg in a 0.2-ml volume. Blood samples were collected from groups of three mice per time point at each of eight time points. Serum was stored at -80°C until drug assay. Anidulafungin and micafungin concentrations were analyzed by gas chromatography-mass spectrometry (GC/MS) and microbiologic assay as previously described (8, 9). Caspofungin was analyzed using the same assay as that used for micafungin. *C. albicans* K1 was used as the assay organism. The lower limit of detection of the caspofungin in the microbiologic assay was 0.10  $\mu\text{g/ml}$ . The lower limit of quantitation was 0.4  $\mu\text{g/ml}$ . The mean intraday variation for the caspofungin assay was <5%. The assay performances for anidulafungin and micafungin were previously reported (8, 9).

A noncompartmental model was used in the pharmacokinetic analysis (Win-

Nonlin, Pharsight Corporation, Cary, NC). Pharmacokinetic parameters, including elimination half-life and concentration at time zero ( $C_0$ ) were calculated via nonlinear least-squares techniques. The AUC was calculated by the trapezoidal rule. For treatment doses in which no kinetics were determined, the pharmacokinetic index was estimated by extrapolation from higher and lower dose levels and interpolation for dose levels within the dose range studied. Protein binding for each echinocandin was based upon previous reports of binding in mice and humans (anidulafungin, 99%; caspofungin, 97%; and micafungin, 99.75%) (9, 14, 24, 26).

**Pharmacodynamic target determination.** The thirty *Candida* strains described above were used for *in vivo* treatment studies with each of the three echinocandins. Infection in neutropenic mice was produced as described above. Dosing regimens were chosen to vary the magnitude of the 24-h AUC/MIC index and to attempt to produce a range of efficacy that included no effect to maximal effect. Four to six (all but four treatment studies used six) dose levels that varied from 0.078 to 160 mg/kg were administered in a 0.2-ml volume by the intraperitoneal route. The antifungals were given every 24 h for a 4-day period. Groups of three mice were used for each dosing regimen. At the end of the treatment period, mice were euthanized, and the kidneys were immediately processed for determination of the number of CFU as described above.

The dose response results were analyzed using a sigmoid dose-effect model. To compare the *in vivo* potencies of each drug against the various *Candida* strains and species, we utilized the dose level required to produce a net static effect (no change in organism burden compared to the start of therapy) and a 1 log kill (relative to burden at the start of therapy) as previously described (9). The echinocandin exposure associated with each treatment endpoint was calculated from the following equation:  $\log_{10} D = \log_{10} (E/E_{\max} - E)N + \log_{10} ED_{50}$ , where  $D$  is the drug dose,  $E$  is the control growth in untreated animals,  $E_{\max}$  is the maximal effect,  $N$  is the slope of the dose-response relationship, and  $ED_{50}$  is the dose needed to achieve 50% of the maximal effect.  $E_{\max}$  was constrained when the true  $E_{\max}$  was not achieved. The significance of differences among for the dose levels (static dose and 1 log kill) among echinocandins and *Candida* species was determined by using analysis of variance (ANOVA) and pairwise comparisons. One-way ANOVA and the Holm-Sidak method were used for normally distributed data. ANOVA on ranks and Dunn's method were used for nonparametric analysis when the data were not normally distributed.

## RESULTS

***In vitro* susceptibility testing.** The MICs of anidulafungin, caspofungin, and micafungin for the *Candida* strains are shown in Table 1. The MICs for the entire group varied 500-fold (range, 0.008 to 4.0  $\mu\text{g/ml}$ ). The ranges were relatively similar among the three echinocandins (anidulafungin, 0.015 to 4.0  $\mu\text{g/ml}$ ; caspofungin, 0.03 to 4.0  $\mu\text{g/ml}$ ; micafungin, 0.008 to 1.0  $\mu\text{g/ml}$ ). The *C. parapsilosis* group exhibited the highest MICs among the *Candida* species. For the group of *C. parapsilosis*, the anidulafungin MICs were 2- to 3-fold higher than for caspofungin or micafungin (mean MICs, anidulafungin, 1.28  $\mu\text{g/ml}$ ; caspofungin, 0.58  $\mu\text{g/ml}$ ; micafungin, 0.43  $\mu\text{g/ml}$ ;  $P = 0.01$ ). The MICs were lower, and the range was smallest for the *C. albicans* group (0.008 to 0.12  $\mu\text{g/ml}$ ). This pattern of MIC results is similar to that reported in larger surveillance studies.

**Pharmacokinetics.** The pharmacokinetics of anidulafungin and micafungin in this *in vivo* model have been previously published (9, 14). The pharmacokinetic parameters following single intraperitoneal doses of 5, 20, and 80 mg/kg are reported in Table 2. The serum concentrations for each compound are shown in Fig. 1. Peak levels were observed by 1 to 4 h with each of the drugs. The elimination half-lives in mouse serum ranged from 7.9 to 24 h and were relatively similar for caspofungin and micafungin and slightly longer for anidulafungin. The  $AUC_{0-\infty}$  values ranged from 96 to 1,975  $\mu\text{g} \cdot \text{h/ml}$  for anidulafungin, 164 to 667  $\mu\text{g} \cdot \text{h/ml}$  for caspofungin, and 135 to 1,400  $\mu\text{g} \cdot \text{h/ml}$  for micafungin. The AUC values were linear ( $R^2 = 0.98$  to 0.99) over the dose range studied.

TABLE 2. Serum pharmacokinetic parameters for anidulafungin, caspofungin, and micafungin in mice

Drug	Dose (mg/kg)	C <sub>max</sub> (μg/ml)	AUC (mg · h/liter)	t <sub>1/2</sub> (h)	Protein binding (%)
Anidulafungin	80	60	1975	24	99
	20	24	382	14.7	
	5	4.3	96	14.8	
Caspofungin	80	58	667	8.75	97
	20	26	345	6.87	
	5	18	164	6.18	
Micafungin	80	53	1400	16	99.75
	20	28	520	13	
	5	7	138	7.50	

**Pharmacodynamic target determination.** To determine if the index magnitudes among *Candida* strains and echinocandins were similar, we studied the *in vivo* activities of anidulafungin, caspofungin, and micafungin by using a 24-hour dosing regimen against up to 30 isolates. At the start of therapy, mice had  $4.16 \pm 0.39$  log<sub>10</sub> CFU/kidneys. The organisms grew  $2.47 \pm 0.86$  log<sub>10</sub> CFU/kidneys after 96 h in untreated control mice, and the growth values were similar among *Candida* species. There was also no significant difference in the burden at the start of therapy among the three *Candida* species. Escalating doses of each echinocandin resulted in concentration-dependent killing of all three *Candida* species (Fig. 2A to C, 3A to C, and 4A to C). We did not observe a paradoxical effect over the dose range with the organisms utilized in this study (54).

In general, the shapes of the exposure-response curves were similar for all strains, with the exception of the anidulafungin-treated *C. parapsilosis* group. The location of the exposure-response curve in most cases was related to the MIC for the organism. The higher MIC values likely explain the lower anidulafungin *C. parapsilosis* response. The relationship between efficacy and both echinocandin dose and 24-h AUC/MIC was strong for each of the drug-organism groups (as reflected in the relatively high *R*<sup>2</sup> values), with the exception of the anidulafungin data with *C. parapsilosis*, for which the data variability was quite large.

Caspofungin dosing regimens produced a static effect and 1 log kill for all of the study organisms (Fig. 2B, 3B, and 4B). The micafungin regimens similarly achieved the stasis endpoint for

all organisms and the 1 log killing endpoint for all but two *C. parapsilosis* isolates and one *C. glabrata* isolate (Fig. 2C, 3C, and 4C). The treatment regimens for anidulafungin were similarly effective against *C. albicans*. However, the regimens were somewhat less effective against *C. glabrata* (1 log kill for five of nine strains) and exhibited reduced *in vivo* efficacy against the *C. parapsilosis* group (stasis endpoint for 7 of 15 organisms and 1 log kill for 3 of 15 organisms) (Fig. 2A, 3A, and 4A).

When one considers the dose levels (mg/kg) needed to achieve the treatment endpoints, two patterns were observed across the treatment results. First, therapy with caspofungin required less drug on a mg/kg basis (3- to 63-fold) than did that of either anidulafungin or micafungin to achieve the study endpoints. These differences were statistically significant (*P* value range of <0.001 to 0.01) for the stasis endpoint with each *Candida* species. The differences were also significant for the 1 log kill against the *C. glabrata* and *C. parapsilosis* species. The dose levels associated with these treatment goals were similar for both anidulafungin and micafungin (with the exception of the 1 log kill endpoint for *C. glabrata*, which was higher for anidulafungin). A second pattern observed throughout the study was the need for higher doses of each of the echinocandins for efficacy against *C. parapsilosis* (2.4- to 22-fold more drug on a mg/kg basis; *P* value range of <0.001 to 0.03) than those needed against either *C. albicans* or *C. glabrata*, which were relatively similar.

The 24-h AUC/MIC was used for pharmacodynamic index exploration of the exposure-response relationship (Fig. 2A to C, 3A to C, 4A to C, and 5; Table 3). Both total and free drug concentrations were considered, but only the free drug values are shown in the figures. When total drug concentrations were considered, exposures for each of the echinocandins were similar to those observed with dose alone. The total-drug 24-h AUC/MIC (tAUC/MIC) for caspofungin to achieve stasis or killing was 2- to nearly 16-fold lower than that for anidulafungin or micafungin against *C. albicans* and *C. glabrata* (*P* value range of 0.001 to 0.01). These differences were smaller and not statistically significant against *C. parapsilosis* where the data variability was greater. Although the tAUC/MIC values for anidulafungin and micafungin were similar, the anidulafungin tAUC/MIC values were slightly (but not statistically) smaller than those for micafungin (<2-fold) across the three *Candida* species. We theorize that this discrepancy is due to small differences in protein binding.

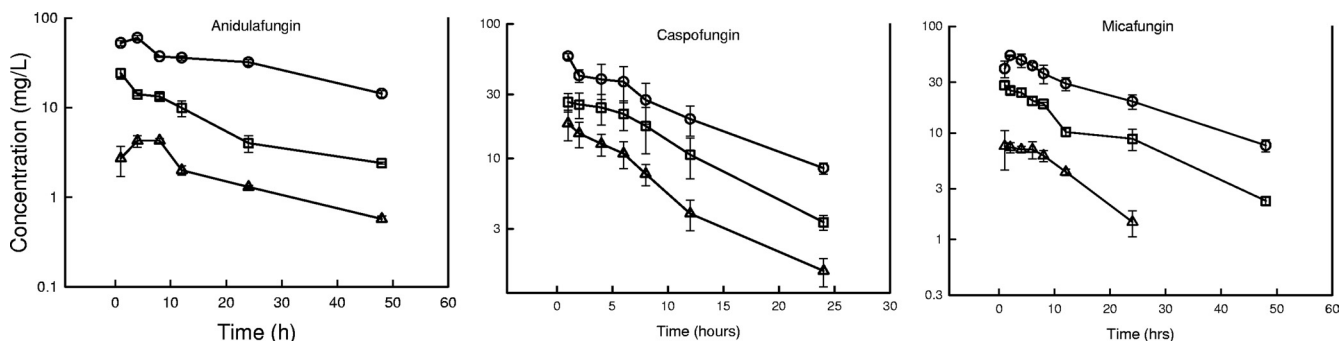


FIG. 1. Single-dose serum pharmacokinetics of anidulafungin, caspofungin, and micafungin following intraperitoneal doses of 5, 20, and 80 mg/kg. Each symbol represents the mean concentration from three mice. The error bars represent the standard deviation.

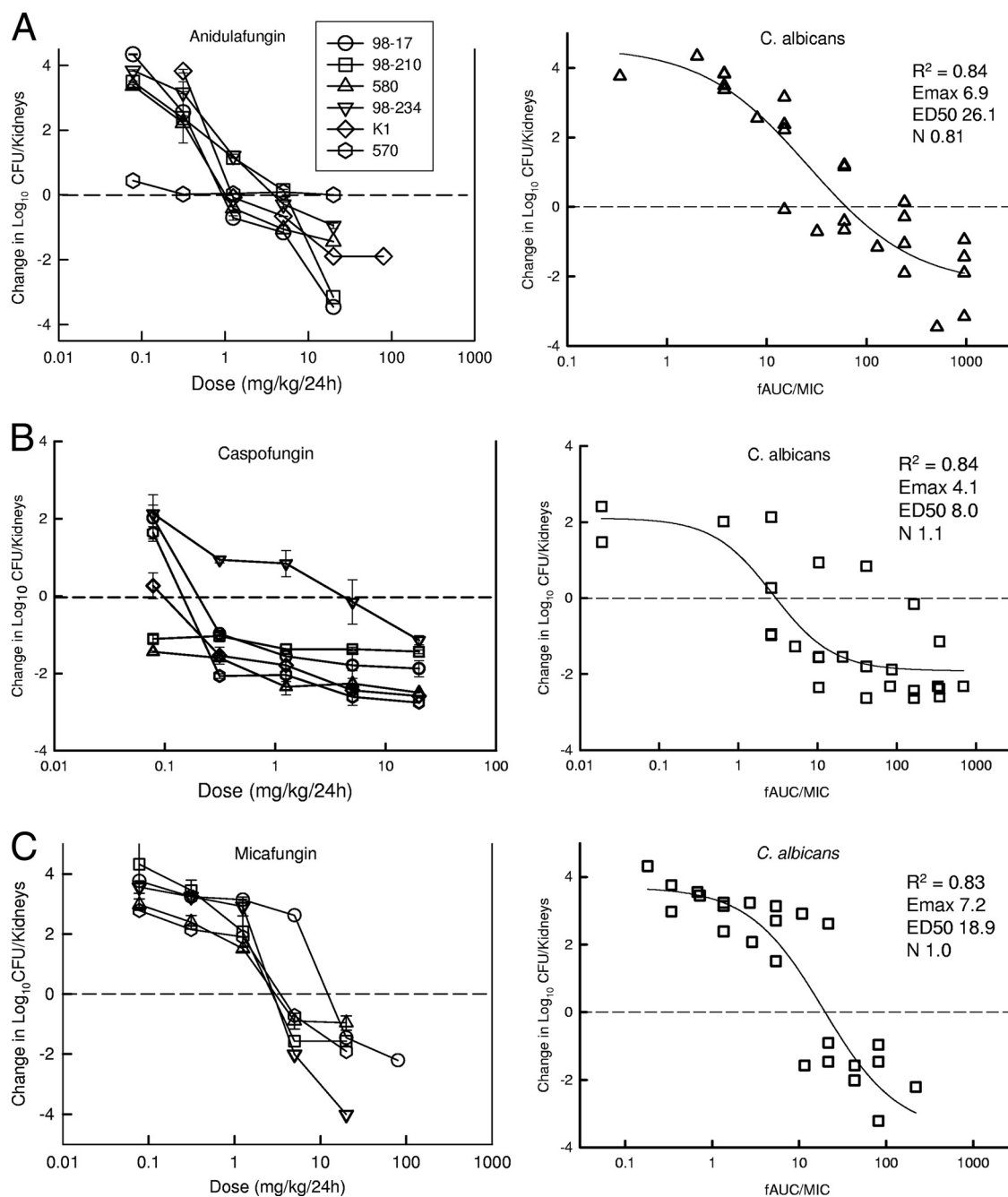


FIG. 2. Relationship between anidulafungin (A), caspofungin (B), and micafungin (C) exposure (left panel, dose [mg/kg]; right panel, fAUC/MIC) and the burden of organisms in the kidneys of mice after 96 h of therapy against *C. albicans*. Each symbol represents the mean log<sub>10</sub> CFU/kidneys of three mice. Efficacy on the y axis is expressed as the change in log<sub>10</sub> CFU/kidneys compared to organism burden at the start of therapy. The horizontal dashed line represented the burden of organisms at the start of therapy. The solid line drawn through the data points represents the best fit line. The  $R^2$  is the coefficient of determination from regression using a sigmoid  $E_{max}$  model. The  $E_{max}$  is the maximal effect, the  $ED_{50}$  is the exposure value associated with 50% of  $E_{max}$ , and  $N$  is the slope of the regression.

Several interesting observations were seen in evaluation of the impact of the infecting *Candida* species. For micafungin, the tAUC/MIC values for *C. parapsilosis* and *C. glabrata* were roughly 3-fold lower than for *C. albicans*. For anidulafungin, the exposures for *C. albicans* and *C. glabrata* were similar. The anidulafungin values for *C. parapsilosis* are more difficult to interpret due to the small number of strains for which the

endpoints were achieved and the wide variability in the available results; however, for the few strains the target was lower than for *C. albicans* and *C. glabrata*. For caspofungin, the tAUC/MIC exposure associated with stasis was a bit higher for *C. parapsilosis*. However, there were three strain outliers among the 15 strains studied. In each of the three cases, the MICs were low (0.06  $\mu$ g/ml). Mean pharmacodynamic target

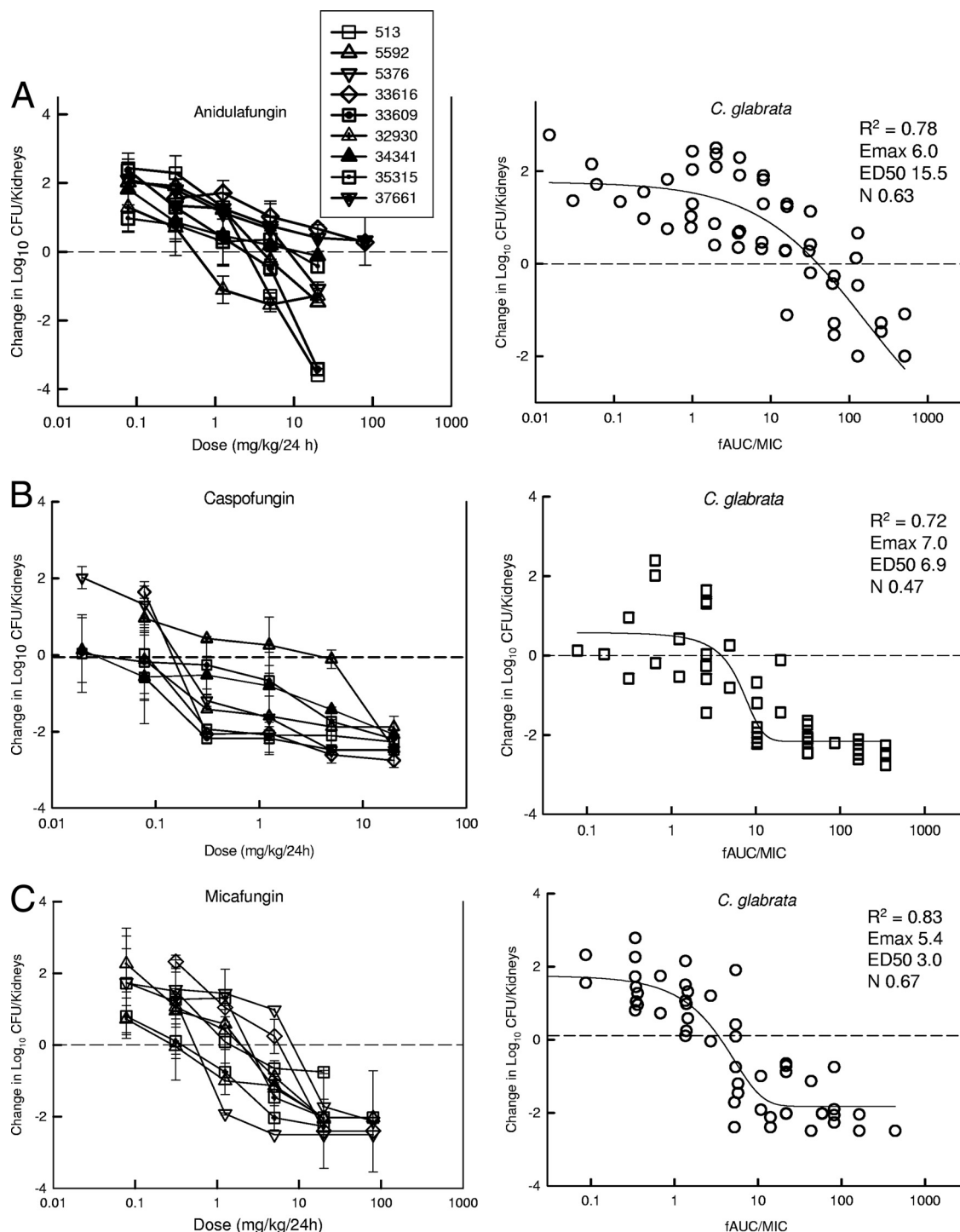


FIG. 3. Relationship between anidulafungin (A), caspofungin (B), and micafungin (C) exposure (left panel, dose [mg/kg]; right panel, fAUC/MIC) and the burden of organisms in the kidneys of mice after 96 h of therapy against *C. glabrata*. Each symbol represents the mean log<sub>10</sub> CFU/kidneys of three mice. Efficacy on the y axis is expressed as the change in log<sub>10</sub> CFU/kidneys compared to organism burden at the start of therapy. The horizontal dashed line represented the burden of organisms at the start of therapy. The solid line drawn through the data points represents the best fit line. The R<sup>2</sup> is the coefficient of determination from regression using a sigmoid E<sub>max</sub> model. The E<sub>max</sub> is the maximal effect, the ED<sub>50</sub> is the exposure value associated with 50% of E<sub>max</sub>, and N is the slope of the regression.

calculations for caspofungin in the absence of these data three points result in a *C. parapsilosis* pharmacodynamic target (free-drug AUC/MIC [fAUC/MIC], 6.0) that is nearly identical to that observed with the other two echinocandins.

Consideration of free drug exposure markedly reduced the differences among the echinocandins (differences were less than 2-fold and not statistically significant) (Table 3). The 24-h fAUC/MICs associated with the stasis endpoint for the echi-

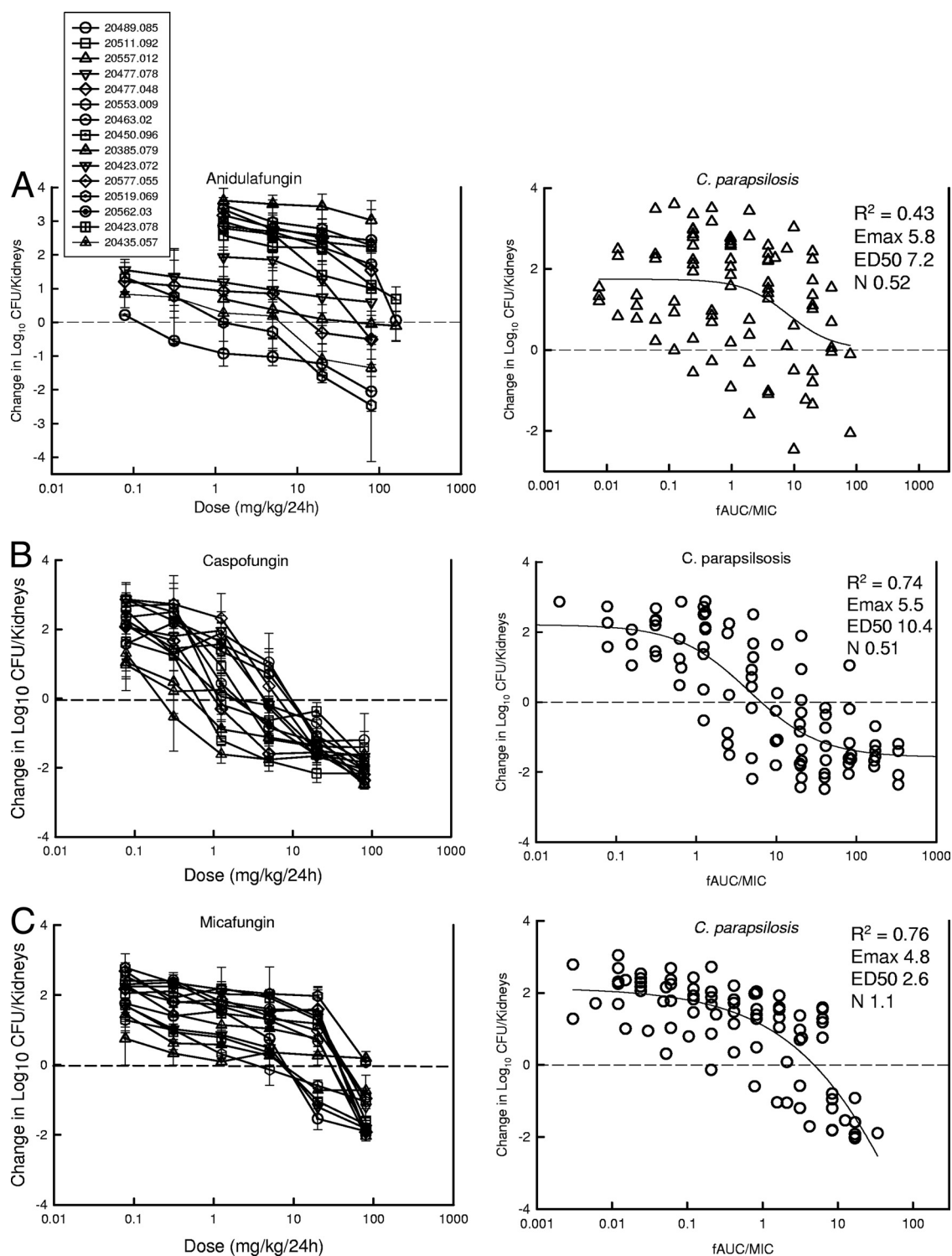


FIG. 4. Relationship between anidulafungin (A), caspofungin (B), and micafungin (C) exposure (left panel, dose [mg/kg]; right panel, fAUC/MIC) and the burden of organisms in the kidneys of mice after 96 h of therapy against *C. parapsilosis*. Each symbol represents the mean  $\log_{10}$  CFU/kidneys of three mice. Efficacy on the y axis is expressed as the change in  $\log_{10}$  CFU/kidneys compared to organism burden at the start of therapy. The horizontal dashed line represented the burden of organisms at the start of therapy. The solid line drawn through the data points represents the best fit line. The  $R^2$  is the coefficient of determination from regression using a sigmoid  $E_{max}$  model. The  $E_{max}$  is the maximal effect, the  $ED_{50}$  is the exposure value associated with 50% of  $E_{max}$ , and  $N$  is the slope of the regression.

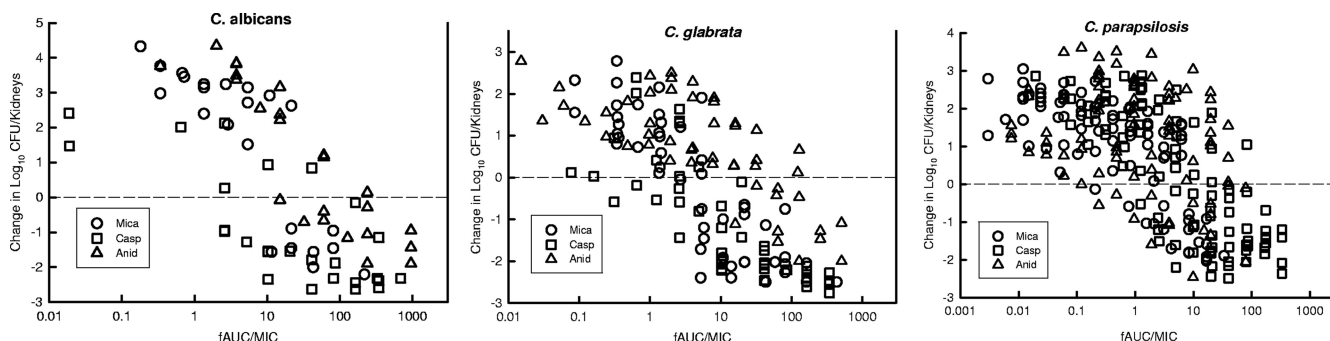


FIG. 5. Relationship between anidulafungin (Anid), caspofungin (Casp), and micafungin (Mica) exposure (24-h fAUC/MIC and effect on the burden of organisms in the kidneys of mice after 96 h of therapy against *C. albicans* [left panel], *C. glabrata* [middle panel], and *C. parapsilosis* [right panel]). Each symbol represents the mean log<sub>10</sub> CFU/kidneys of three mice. Efficacy on the y axis is expressed as the change in log<sub>10</sub> CFU/kidneys compared to organism burden at the start of therapy. The horizontal dashed line represented the burden of organisms at the start of therapy.

nocandin group against *C. albicans* was 20.6 ± 32 (mean ± standard deviation). Similar values for *C. glabrata* and *C. parapsilosis* were 7.0 ± 8.3 and 7.6 ± 7.1, respectively. The values for *C. glabrata* and *C. parapsilosis* were statistically lower than those for *C. albicans* (*P* < 0.001). The dose-response relationships were fairly steep for each organism group, and the exposures needed for a 1 log<sub>10</sub> kill were only 1.2- to 4.5-fold higher (mean ± standard deviation, 2.3 ± 0.92) than that needed for the stasis endpoint.

DISCUSSION

*Candida* species are the most common systemic fungal pathogens in humans and exhibit a disease spectrum ranging from mucosal to systemic infections. Invasive candidiasis has

high attributable mortality, especially in immunosuppressed patients (41, 42). The addition of the echinocandin class of antifungals marks a significant advance in therapy of these diseases, with enhanced safety and efficacy compared to previously available antifungal therapies (31, 36, 43, 52). However, as with the other available antifungal therapies, the echinocandins exhibit variable potencies against different fungal species, and accumulating case reports have illustrated the potential for resistance development (18–20, 25, 27, 28, 30, 32, 35, 44, 45, 49). Thus, it is clear that there are *Candida* infections for which echinocandin potency is insufficient for effective therapy. Understanding the limits of echinocandin therapy in relation to the MIC should aid in development of susceptibility breakpoints and guide optimal antifungal treatment strategies.

TABLE 3. *In vivo* pharmacodynamic target identification of anidulafungin, caspofungin, and micafungin against *C. albicans*, *C. glabrata*, and *C. parapsilosis* in a neutropenic murine disseminated candidiasis model

Drug	Organism	Mean ± SD <sup>a</sup>					
		Static dose (mg/kg/24 h)	Static dose 24-h AUC/MIC	Static dose 24-h fAUC/MIC	1 log kill (mg/kg/24 h)	1 log kill 24-h AUC/MIC	1 log kill 24-h fAUC/MIC
Anidulafungin	<i>C. albicans</i>	2.32 ± 1.48	2,782 ± 2116	27.8 ± 21.1	4.25 ± 8.17	9,125 ± 8,144	91 ± 81
	<i>C. glabrata</i>	21.1 ± 33.5	1,366 ± 1100	13.7 ± 11.0	39 ± 39	3,188 ± 2,256	32 ± 23
	<i>C. parapsilosis</i>	51.0 ± 35	1,154 ± 1091	11.5 ± 10.9	47 ± 46	2,244 ± 2,384	22 ± 24
Caspofungin	<i>C. albicans</i>	0.72 ± 1.51	748 ± 1,675	22.4 ± 50.2	2.91 ± 6.66	865 ± 1,831	25.9 ± 54.9
	<i>C. glabrata</i>	0.33 ± 0.71	96.2 ± 96	2.9 ± 2.8	1.16 ± 2.15	450 ± 448	13.5 ± 13.4
	<i>C. parapsilosis</i>	3.56 ± 3.08	559 ± 879	16.8 ± 26.4	9.84 ± 7.40	1,185 ± 1,385	35.5 ± 41.5
Micafungin	<i>C. albicans</i>	2.79 ± 0.65	5,299 ± 1,860	12.7 ± 4.8	5.63 ± 3.08	10,088 ± 5,416	25.2 ± 13.5
	<i>C. glabrata</i>	2.47 ± 2.79	1,542 ± 1,252	3.90 ± 3.1	5.88 ± 6.33	3,778 ± 3,003	9.45 ± 8.24
	<i>C. parapsilosis</i>	32.3 ± 24.0	1,981 ± 1,439	4.95 ± 3.6	55.8 ± 23.9	3,090 ± 1,781	7.73 ± 4.45

<sup>a</sup> Comparisons were statistically significant as follows. Static dose *C. albicans* caspofungin versus micafungin and anidulafungin, *P* = 0.01 and 0.04, respectively. Static dose *C. glabrata* caspofungin versus micafungin and anidulafungin, *P* < 0.001 and *P* = 0.05, respectively. Static dose *C. parapsilosis* caspofungin versus micafungin and anidulafungin, *P* = 0.0001 and *P* = 0.04, respectively. 1 Log kill dose *C. glabrata* caspofungin versus micafungin and anidulafungin, *P* = 0.001 and 0.005, respectively. 1 Log kill dose *C. parapsilosis* caspofungin versus micafungin and anidulafungin, *P* = 0.0001 and 0.001, respectively. Caspofungin static dose *C. parapsilosis* versus *C. albicans* and *C. glabrata*, *P* = 0.01 and 0.003, respectively. Caspofungin 1 log kill dose *C. parapsilosis* versus *C. albicans* and *C. glabrata*, *P* = 0.03 and 0.002, respectively. Anidulafungin static dose *C. parapsilosis* versus *C. albicans* and *C. glabrata*, *P* = 0.002 and 0.03, respectively. Micafungin static dose *C. albicans* versus *C. parapsilosis* and *C. glabrata*, *P* = 0.001 and *P* < 0.001, respectively. Micafungin 1 log kill dose *C. albicans* versus *C. parapsilosis* and *C. glabrata*, *P* < 0.001 and *P* < 0.001, respectively. tAUC/MIC static dose *C. albicans* anidulafungin versus caspofungin, *P* = 0.03; micafungin versus caspofungin, *P* < 0.001. tAUC/MIC static dose *C. glabrata* anidulafungin versus caspofungin, *P* = 0.01; micafungin versus caspofungin, *P* = 0.001. tAUC/MIC static dose *C. parapsilosis* micafungin versus caspofungin, *P* = 0.001. tAUC/MIC anidulafungin *C. albicans* versus *C. parapsilosis*, *P* = 0.01. tAUC/MIC 1 log kill anidulafungin *C. albicans* versus *C. glabrata* and *C. parapsilosis*, *P* = 0.01 and 0.001, respectively. fAUC static dose *C. glabrata* anidulafungin versus caspofungin and micafungin, *P* = 0.002 and 0.005, respectively. fAUC/MIC 1 log kill dose *C. glabrata* anidulafungin versus caspofungin and micafungin, *P* = 0.007 and 0.02, respectively. fAUC/MIC 1 log kill dose *C. parapsilosis* caspofungin versus micafungin, *P* = 0.009. fAUC/MIC static dose micafungin *C. albicans* versus *C. glabrata* and *C. parapsilosis*, *P* < 0.001 and *P* < 0.001, respectively. fAUC/MIC 1 log kill micafungin *C. albicans* versus *C. glabrata* and *C. parapsilosis*, *P* < 0.001 and *P* < 0.001, respectively.

The MIC distributions of the echinocandin class are relatively similar against most *Candida* species (33, 45–48). The species for which potency is reduced *in vitro* is *C. parapsilosis*. Most isolates are 50- to 100-fold less susceptible to the echinocandins than other common *Candida* species. However, clinical trials have demonstrated effectiveness for management of both mucosal and systemic candidiasis against each of the common *Candida* species, including *C. parapsilosis* (36, 43, 52). Reports from these large trials do not detect a relationship between *in vitro* susceptibility and treatment efficacy (29). However, the ability of these trials to discern this relationship has been limited due to the narrow range of MICs for study isolates and there being very few isolates with higher MIC values and the complexity of the disease state.

Recent suggestions have been made by the CLSI to define susceptibility breakpoints for the echinocandins and *Candida* species (50). The rationale for these recommendations is based upon a “blueprint” procedure which has been effective for other antifungal compounds (50, 51, 53). The process evaluates clinical outcome relative to MIC, considers pharmacodynamic analyses from experimental and clinical data sets, and assesses the MIC distribution with a preference to avoid setting a breakpoint that would split a wild-type MIC range (51, 53).

The pharmacodynamic analysis component of this process is the focus of the current investigation. The study of antimicrobial pharmacodynamics explores the relationships among pharmacokinetics, MIC, and treatment outcome (1, 17). Application of these tools has been utilized to define the limits of treatment relative to the MIC and to subsequently guide susceptibility breakpoints. The goal of the present study was to determine the amount of drug relative to the MIC required for efficacy of three echinocandins against the three most common *Candida* species, including the species with higher MICs, *C. parapsilosis*. With this information, we then extrapolated the experimental findings to patients and asked, with clinically relevant echinocandin pharmacokinetics, what is the highest *Candida* MIC for which treatment would be expected to remain effective?

Previous studies using this model and analyses have explored the pharmacodynamic target for select echinocandins against *C. albicans* and *C. glabrata* (9, 14). Prior attempts to examine the target for the high-MIC *C. parapsilosis* group have been difficult due to the reduced fitness of this species in the mouse infection model (15). In designing the current experiments, we strove to include organisms with similar fitness levels in the infection to avoid potentially confounding differences in outcome associated with differences in organism virulence. The organisms used in the present study, were screened for fitness in the model, and all were shown to grow well in the kidneys of mice over the treatment period. The *C. parapsilosis* collection of organisms was identified through a laborious screening process in which nearly 80 individual isolates were tested in the animal model to find the group of 15 organisms sufficient for the goals of this study (data not shown).

In general, the amount of drug required to produce these treatment endpoints in the current study was related to the organism MIC. The organism group for which each of the echinocandins demonstrated the least *in vivo* activity was *C. parapsilosis*. This discrepancy was most evident for anidulafun-

gin. These study findings are not surprising, given the higher MICs for this collection of strains.

In the pharmacodynamic analyses, we considered both protein-bound and unbound drug concentrations, since numerous antibacterial and antifungal pharmacodynamic experiments have suggested the relevance of protein binding (2, 10, 12, 13, 38, 40). The majority of these investigations have shown that free drug concentrations are most relevant. Findings from the current studies suggest the relevance of this pharmacokinetic phenomenon as well. If one considers only total drug concentrations, it would appear that caspofungin requires much less drug than does either anidulafungin or micafungin. However, the degree of binding of caspofungin is lower (97%) than that of these echinocandins (anidulafungin, 99%, and micafungin, 99.75%). When unbound concentrations are considered, the amounts of drug needed for efficacy are very similar among the three compounds. Interestingly, even though the differences in binding between anidulafungin and micafungin are small, across the majority of study isolates, anidulafungin required lower total drug exposures than did micafungin, suggesting the relevance of even small differences in protein binding.

One unexpected finding from these studies was the apparent difference in the pharmacodynamic target for echinocandins among *Candida* species. The target for *C. albicans* was larger than that for either *C. glabrata* or *C. parapsilosis*. Perhaps these observations should not be surprising given the demonstrated relevance of species-specific susceptibility breakpoints for antibacterial agents (1, 3, 6, 7). For example, the 24-h AUC/MIC quinolone target for *Streptococcus pneumoniae* is near 25, while the target for Gram-negative bacilli is nearly 4-fold higher. Clinical pharmacodynamic analyses with large case numbers from each of the *Candida* species should be undertaken to further explore this phenomenon for the echinocandins.

The ability to translate these mouse pharmacodynamic results to patients lies with the premise that the drug target is in the organism and thus the host handling of the drug is of minimal consequence. The pharmacodynamic targets or amounts of drug relative to the MIC needed for effect should be similar for mice and men. Human pharmacokinetics with each of these echinocandins demonstrates protein binding values that are essentially the same as those observed in mice. The approved steady-state regimens for treatment of invasive candidiasis with these drugs include 100 mg/day of both anidulafungin and micafungin and 50 mg/day of caspofungin. These regimens produce total- and free-drug 24-h AUC values in healthy volunteers of 112 mg · h/ml and 1.12 mg · h/ml for anidulafungin, 98 mg · h/ml and 2.94 mg · h/ml for caspofungin, and 126 mg · h/ml and 0.38 mg · h/ml for micafungin (16, 23, 26, 56). If one considers these pharmacokinetics with these echinocandins and the presented pharmacodynamic targets, one can then estimate the highest MICs for the three *Candida* species that would allow the pharmacodynamic 24-h fAUC/MIC to be met. The MIC ceiling based on fAUC/MIC ranging from 5 to 20 would place the susceptibility breakpoint lower than the current CLSI value of 2 µg/ml for each of the drugs. However, the MICs for nearly all of the wild-type strains from surveillance studies would be expected to fall within the “pharmacodynamically susceptible” category based upon the fAUC/MIC targets reported in this study, with the exception of a subset of *C. parapsilosis* isolates (39, 45).

Previous animal model and clinical epidemiologic studies



have suggested that *C. parapsilosis* is a less fit or virulent species than are other commonly encountered *Candida* species. A limitation of the current study was the inability to include *C. parapsilosis* with various levels of and specifically reduced *in vivo* fitness. One may speculate that the pharmacodynamic target may be lower in organisms that are “less fit.” Thus, it is possible that the echinocandin exposure target for the *C. parapsilosis* group may be artificially elevated in the current study, as the organisms we were able to study represent a highly fit subset of the wild-type population. The impact of organism fitness on pharmacodynamic relationships is difficult but an important area for future investigation.

In summary, these studies demonstrate a relationship between echinocandin dose, MIC, and efficacy. More specifically, the results identify the pharmacodynamic target for clinically available echinocandins against the three most commonly encountered *Candida* species and suggest differences among species. Future studies should examine the impact of even higher MICs and explore the mechanistic basis for the subtle differences in echinocandin-*Candida* species targets identified in this study.

#### ACKNOWLEDGMENTS

This research was funded by grants from Merck, Astellas, and Pfizer.

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