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EXPERIMENTAL THERAPEUTICS

Pharmacodynamic Optimization for Treatment of Invasive *Candida auris* Infection

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ABSTRACT *Candida auris* is an emerging multidrug-resistant threat. The pharmacodynamics of three antifungal classes against nine *C. auris* strains was explored using a murine invasive candidiasis model. The total drug median pharmacodynamic (PD) target associated with net stasis was a fluconazole AUC/MIC (the area under the concentration-time curve over 24 h in the steady state divided by the MIC) of 26, an amphotericin B C_{max}/MIC (maximum concentration of drug in serum divided by the MIC) of 0.9, and a micafungin AUC/MIC of 54. The micafungin PD targets for *C. auris* were ≥20-fold lower than those of other *Candida* species in this animal model. Clinically relevant micafungin exposures produced the most killing among the three classes.

KEYWORDS *C. auris*, pharmacodynamics, antifungal therapy, antifungal resistance

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Candida auris is an emerging multidrug-resistant threat to human health across the globe (1, 2). The first documented clinical case of this species occurred in Japan in 2009 (3). Since then, infections due to *C. auris* have been published from numerous countries throughout the world (1, 2, 4–17). Unfortunately, antifungal therapeutic failure and mortality have been commonly reported. This is attributed in part to antifungal resistance. Many isolates exhibit high triazole and polyene MICs. This might be expected, as the species is phylogenetically related to *Candida krusei*, *Candida lusitaniae*, and *Candida haemulonii*, which are known to be less susceptible to these antifungal classes (18, 19). Variable *in vitro* susceptibility results have been noted for the echinocandins (1), rendering some isolates potentially clinically resistant to all three classes of commonly used antifungal agents. The optimal antifungal agent and dosing regimen for treatment of these infections have not been defined. As such, preliminary susceptibility breakpoints are based on limited *in vitro* data using breakpoints developed for other *Candida* species.

The goal of the present studies was to define the pharmacokinetic/pharmacodynamic (PK/PD) target for the three available antifungal drug classes against this emerging pathogen. Specifically, we designed an *in vivo* PK/PD study to compare the treatment effects of fluconazole, micafungin, and amphotericin B, using the neutropenic murine model of invasive candidiasis against nine clinical isolates of *C. auris* (Table 1). Strains were chosen to include those with variable *in vitro* susceptibility to available antifungal drug classes. Strains were screened for fitness in the animal model prior to treatment studies (Table 1). Fluconazole, micafungin, and amphotericin B deoxycholate were prepared as described in manufacturer instructions. Antifungal susceptibility testing was performed according to CLSI guidelines for fluconazole and micafungin or Etest for amphotericin B (20, 21). MICs varied by 128-fold for fluconazole (range, 2 to 256 mg/liter), 32-fold for micafungin (range, 0.125 to 4 mg/liter), and

TABLE 1 Treatment effects for nine select *Candida auris* strains used in the studies, with country of origin, antimicrobial susceptibility results, and 24-h total drug PK/PD target exposures in the murine invasive candidiasis model

Strain	Country of origin	96 h growth in untreated controls (CFU/kidney)	Fluconazole		Micafungin			Amphotericin B	
			MIC (mg/liter)	Stasis (AUC/MIC)[a]	MIC (mg/liter)	Stasis (AUC/MIC)[a]	1-log kill (AUC/MIC)[a]	MIC (mg/liter)	Stasis (C_{max}/MIC)[a]
B11804	Colombia	2.17	2	51.2	0.5	48.1	120.3	0.5	0.87
B11801	Colombia	2.86	16	26.3	1	32.9	49.9	2	NA[b]
B11799	Colombia	2.08	16	36.3	2	18.5	92.1	0.5	1.29
B11221	South Africa	1.85	128	6.3	1	47.6	140.6	0.38	0.52
B11211	India	1.97	256	NA	4	NA	NA	1.5	0.69
B11785	Colombia	2.32	8	34.1	0.5	59.4	119.2	1.5	1.50
B11220	Japan	1.04	4	5.0	0.125	286.5	674.4	0.38	NA
B11203	India	2.13	256	NA	0.25	117.0	376.4	4	0.51
B11104	Pakistan	1.71	256	4.1	0.25	134.3	536.8	1	2.13
Median				26.3		53.7	130.5		0.87
Standard deviation				18.5		87.9	235.3		0.60

[a]After 24 h.
[b]NA, not applicable (endpoint not achieved).

10-fold for amphotericin B (range, 0.38 to4 mg/liter). The neutropenic disseminated candidiasis model was used for all experiments. Three mice per treatment or control group were included. Mice were inoculated with 6.34 $\pm$ 0.08 $\log_{10}$ CFU/ml via the lateral tail vein with each of the nine strains. Antifungal treatment began 2 h after inoculation and continued for 96 h, at which time mice were euthanized for CFU determination in the kidneys. Drug dosing consisted of 0.78, 3.125, 12.5, 50, and 200 mg/kg fluconazole every 12 h by subcutaneous (s.c.) administration, 0.3125, 1.25, 5, 20, and 80 mg/kg micafungin every 24 h by intraperitoneal (i.p.) administration, or 0.078, 0.3125, 1.25, 5, and 20 mg/kg amphotericin B deoxycholate every 24 h by i.p. administration. The treatment studies were designed to include clinically relevant exposures. Organism burden in mouse kidneys after 4 days (96 h) of therapy was compared to the *Candida* quantity at the start of therapy. The treatment results were analyzed using a sigmoidal maximum effect (E_{max}) model (22). Pharmacokinetic exposures were obtained from our lab in this mouse model (23–25). The PK exposures were plotted relative to MIC and the previously defined PK/PD driver. Specifically, AUC/MIC (area under the concentration-time curve over 24 h in the steady state divided by the MIC) was used for fluconazole and micafungin, and C_{max}/MIC (the maximum concentration of drug in serum divided by the MIC) was used for amphotericin B (26, 27). The magnitude of the PK/PD index (AUC/MIC or C_{max}/MIC) associated with net stasis and 1-log kill (when achieved) for each strain was calculated with the equation $\log_{10} D = \log_{10} (E/[E_{max} - E])/(N + \log_{10} ED_{50})$, where E is the control growth for the static dose (D), $E + 1$ is the control growth for the 1-log kill dose (D), and ED_{50} is the 50% effective dose.

The results of the dose-ranging studies with the nine *C. auris* isolates for each drug are shown in Fig. 1A through C. Dose-dependent activity was observed with each strain. Net stasis was achieved against 7 of 9 strains for fluconazole. The two strains that did not achieve stasis over the dose range had an MIC of 256 mg/liter. Fluconazole therapy resulted in a 1-log kill for only one of the strains. In micafungin experiments, stasis and 1- and 2-log kill endpoints were achieved against 8 of 9 strains. The single strain in which these endpoints were not met (B11211) had the highest micafungin MIC of the group at 4 mg/liter. Finally, treatment of amphotericin B resulted in stasis with 8 of 9 strains. The single strain for which stasis was not observed with amphotericin B had an elevated MIC at 2 mg/liter. However, only 3 of 9 strains achieved 1-log kill endpoints for amphotericin B. Thus, for each drug, the dose-effect relationship against *C. auris* was proportional to the MIC.

The degree to which MIC influences outcome in relation to pharmacokinetic exposures is the basis of PK/PD analyses. The results of these analyses are shown in Fig. 1D

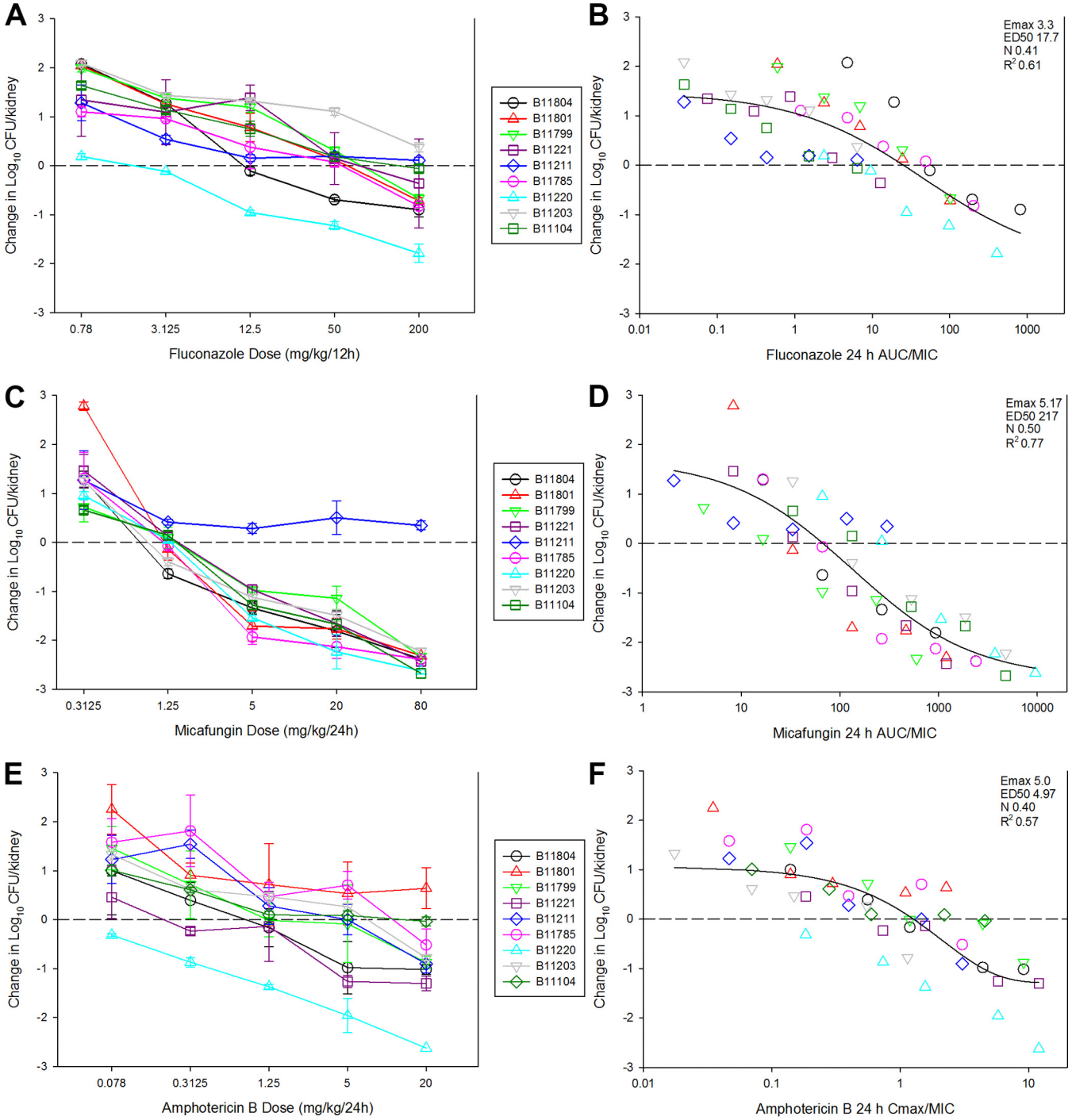

FIG 1 *In vivo* dose-response curves for 9 *C. auris* strains against fluconazole (A), micafungin (B), and amphotericin B (C). Each symbol represents the mean and standard deviation (error bars) of burden in the kidneys of three mice. The horizontal dashed line represents the burden at the start of therapy. The relationship between PK/PD index (AUC/MIC or C_{max}/MIC) and efficacy for fluconazole (D), micafungin (E), and amphotericin B (F) are also shown. Each symbol represents the mean burden from three mice, and the horizontal dashed line is the burden at the start of therapy. A best-fit line based on the Hill equation is shown, as are the PD parameters maximum effect (E_{max}), ED_{50}, slope (N), and the coefficient of determination (R^2).

through F. There was a strong relationship between the PK/PD parameter (AUC/MIC or C_{max}/MIC) and treatment outcome for each drug (R^2, 0.61 for fluconazole, 0.77 for micafungin, and 0.57 for amphotericin). The stasis and 1-log kill target exposures (for micafungin only) are shown in Table 1. In the case of fluconazole, the stasis and ED_{50} targets (data not shown) were similar at 26 and 19, respectively. These values are consistent with prior fluconazole studies against *Candida* species demonstrating that

AUC/MIC values of approximately 25 are associated with success in the animal model and in clinical studies in patients with candidemia (27). For amphotericin B, the data for *C. auris* were also remarkably congruent with prior studies, which have shown stasis to occur at C_{max}/MIC exposures of 1 to 2 (27). The stasis C_{max}/MIC for the groups of *C. auris* strains in this study was near 1. In contrast, micafungin efficacy differed in comparison to prior data in the invasive candidiasis model with other *Candida* species. Despite elevated MICs (range 0.125 to 4 mg/liter), micafungin drug exposures resulted in killing activity at relatively low drug exposures. The median total drug AUC/MIC associated with net stasis was only 53.7. Based on protein binding levels of 99.8% (28), this would translate into a free drug AUC/MIC target of 0.18. Previous micafungin studies demonstrated free drug AUC/MIC targets of 12, 4, and 5 for *C. albicans*, *C. glabrata*, and *C. parapsilosis*, respectively (29). Thus, the PD targets observed for micafungin against *C. auris* were ≥20-fold lower than those for other *Candida* species. 1-log kill exposures were also relatively low at a total drug AUC/MIC of 131 (free drug AUC/MIC of 0.26). The reasons for the enhanced efficacy observed for micafungin against *C. auris* compared to those of fluconazole and amphotericin B are unknown and an important area for future investigation.

Importantly, targets identified in this model with triazoles and echinocandins have correlated well with clinical outcomes in patients with invasive candidiasis (27). Thus, the present findings in this PK/PD study should be useful for forecasting effective treatment regimens for patients and in the development of preliminary susceptibility breakpoints. For example, a common daily dose of fluconazole in humans (400 mg) results in an AUC of nearly 400 mg · h/liter (30, 31). Therefore, using an AUC/MIC target exposure of 26, the MIC ceiling for which success would be predicted is approximately 16 mg/liter. Using the same approach, the MIC ceiling for amphotericin B would be 1 to 1.5 mg/liter. These are similar to PK/PD based breakpoints for other *Candida* species and these antifungals. Finally, a micafungin dosing regimen of 100 mg daily results in free drug exposures of approximately 0.3 to 0.4 mg · h/liter (32). Using the stasis PK/PD target data for micafungin, the PK/PD breakpoint could be as high as 2 to 4 mg/liter, with standard dosing of 100 mg/day.

In sum, the current animal model PK/PD study suggests that echinocandins are likely to be the most efficacious drug class for most *C. auris* isolates. The results suggest that traditional MIC breakpoints are likely to be relevant for fluconazole and amphotericin B, as the drug exposures associated with optimal outcome for *C. auris* were similar to those of previous *Candida* species studies. However, micafungin demonstrated a potent cidal effect against almost all strains with an MIC of <4 mg/liter, and the drug exposure targets (AUC/MIC) were significantly lower than those for other *Candida* species. Based on these data, echinocandins should be considered first-line therapy for patients with *C. auris* infection with regimen tailoring based on susceptibility results.

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