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EXPERIMENTAL THERAPEUTICS

In Vivo Pharmacokinetics and Pharmacodynamics of APX001 against *Candida* spp. in a Neutropenic Disseminated Candidiasis Mouse Model

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ABSTRACT APX001 is the prodrug of APX001A, which is a first-in-class small molecule with a unique mechanism of action that inhibits the fungal enzyme Gwt1 in the glycosylphosphatidylinositol (GPI) biosynthesis pathway. The goal of the present study was to determine which pharmacokinetic/pharmacodynamic (PK/PD) index and magnitude best correlated with efficacy in the murine disseminated candidiasis model for *Candida albicans* ($n = 5$), *C. glabrata* ($n = 5$), and *C. auris* ($n = 4$). MIC values ranged from 0.002 to 0.03 mg/liter for *C. albicans*, from 0.008 to 0.06 mg/liter for *C. glabrata*, and from 0.004 to 0.03 mg/liter for *C. auris*. Plasma APX001A pharmacokinetic measurements were performed in mice after oral administration of 4, 16, 64, and 256 mg/kg of body weight APX001. Single-dose pharmacokinetic studies exhibited maximum plasma concentration (C_{max}) values of 0.46 to 15.6 mg/liter, area under the concentration-time curve (AUC) from time zero to infinity (AUC_{0-inf}) values of 0.87 to 70.0 mg · h/liter, and half-lives of 1.40 to 2.75 h. A neutropenic murine disseminated candidiasis model was utilized for all treatment studies, and drug dosing was by the oral route. Dose fractionation was performed against *C. albicans* K1, with total doses ranging from 4 to 1,024 mg/kg/day of APX001 fractionated into regimens of dosing every 3, 6, 8, and 12 h for a 24-h treatment duration. Nonlinear regression analysis was used to determine which PK/PD index best correlated with efficacy on the basis of the reduction in the number of CFU/kidney at 24 h. The 24-h free-drug AUC/MIC ratio ($fAUC_{0-24}$/MIC) was the PK/PD index that best correlated with efficacy (coefficient of determination [R^2] = 0.88). Treatment studies with the remaining strains utilized regimens of 1 to 256 mg/kg of APX001 administered every 6 h for a 24-h duration with *C. albicans* and a 96-h study duration with *C. glabrata* and *C. auris*. The dose required to achieve 50% of the maximum effect (ED_{50}) and stasis *f*AUC/MIC targets were as follows: for *C. albicans*, 3.67 ± 3.19 and 20.60 ± 6.50, respectively; for *C. glabrata*, 0.38 ± 0.21 and 1.31 ± 0.27, respectively; and for *C. auris*, 7.14 ± 4.54 and 14.67 ± 8.30, respectively. The present studies demonstrated *in vitro* and *in vivo* APX001A and APX001 potency, respectively, against *C. albicans*, *C. glabrata*, and *C. auris*. These results have potential relevance for clinical dose selection and evaluation of susceptibility breakpoints. The identification of a lower AUC/MIC ratio target for *C. glabrata* suggests that species-specific susceptibility breakpoints should be explored.

KEYWORDS APX001, *Candida*, pharmacodynamics

Invasive fungal infections have become increasingly common and remain associated with a high rate of morbidity and mortality, despite currently available antifungal therapies (1–4). *Candida albicans* is still the leading cause of candidemia, but other

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TABLE 1 *In vitro* activities of APX001A and comparators

Species	Isolate	MIC (mg/liter)		
		APX001A	Fluconazole	Micafungin
C. albicans	K1	0.008	0.5	0.016
	98-210	0.03	16	0.016
	580	0.004	0.5	0.008
	2-76	0.002	0.25	0.008
	98-17	0.03	16	0.03
C. glabrata	10956	0.016	2	0.25
	5592	0.06	32	0.016
	35315	0.016	0.25	0.06
	513	0.03	4	0.016
	5376	0.008	2	0.008
C. auris	B11104	0.03	>256	0.25
	B11221	0.008	128	1
	B11219	0.004	>256	4
	B11804 (C54007)	0.008	2	0.5

species of *Candida* (non-*albicans Candida* species) now comprise >50% of bloodstream infections in many parts of the world (5). Multidrug resistance among *C. glabrata* isolates (6) and the outbreak of infections caused by *C. auris* species further limit treatment options (7, 8). Therefore, antifungal drugs with novel mechanisms of action which lack cross-resistance to existing antifungals are desirable for the treatment of invasive fungal infections.

APX001, the methyl-phosphate prodrug of the active moiety APX001A, is a first-in-class broad-spectrum antifungal agent for the treatment of invasive fungal infections, including species resistant to existing antifungal drug classes (9–11). APX001A inhibits the fungal enzyme Gwt1 that is part of the glycosylphosphatidylinositol (GPI) biosynthesis pathway (12). Both APX001A and APX001 have been evaluated extensively in preclinical *in vitro* and *in vivo* studies and exhibit broad-spectrum activity against pathogenic yeasts (e.g., *Candida* spp.) and molds (e.g., *Aspergillus* spp.), including multidrug-resistant strains (e.g., *Fusarium*, *Scedosporium*, and fungi from the *Mucorales* order) (13–16).

The current studies included pharmacokinetic (PK)/pharmacodynamic (PD) evaluation of the APX001 efficacy against *C. albicans*, *C. glabrata*, and *C. auris* in the neutropenic mouse model of disseminated candidiasis to determine the PK/PD index and the magnitude predictive of efficacy to assist with further clinical development of optimal dosing strategies.

RESULTS

***In vitro* susceptibility testing.** The MIC values of APX001A and comparators (fluconazole and micafungin) are shown in Table 1. APX001A showed potent antifungal activity against these clinical strains, with similar MIC ranges for the three *Candida* species. The APX001A MIC values varied 15-fold for the tested organisms.

Drug pharmacokinetics. Single-dose pharmacokinetics of APX001A are shown in Fig. 1. After an oral dose of APX001, the APX001A exposure increased in a dose-dependent manner across the dose range. The maximum plasma concentration (C_{max}) values ranged from 0.455 to 15.6 mg/liter. The area under the concentration-time curve (AUC) from time zero to infinity (AUC_{0-inf}) values ranged from 0.868 to 70.0 mg · h/liter and were linear across the 4- to 256-mg dosing range (coefficient of determination [R^2] = 0.97). The elimination half-life ($t_{1/2}$) ranged from 1.40 to 2.75 h.

PK/PD index determination. At the start of therapy, mice had 4.10 ± 0.16 $\log_{10}$ CFU/kidney of *C. albicans* K1, and the organism grew 3.71 ± 0.26 $\log_{10}$ CFU/kidney after 24 h in untreated control mice. Escalating doses of APX001 resulted in concentration-dependent killing. The highest doses studied reduced the organism burden from 0.75 ± 0.09 to 1.84 ± 0.24 $\log_{10}$ CFU/kidney compared to the numbers at the start of therapy. The

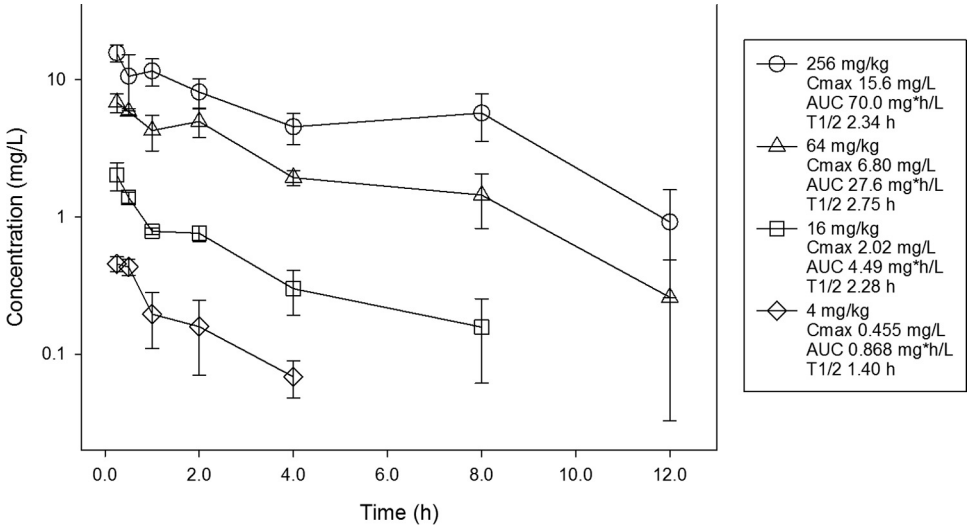

FIG 1 Single-dose plasma pharmacokinetics of APX001A. Four different doses of the prodrug APX001 that varied by 4-fold concentrations on a milligram-per-kilogram basis were administered to mice by the oral route. Groups of three mice were sampled for each time point. Each symbol represents the mean ± SD for three animals. Shown in the key is the maximum plasma concentration (C_{max}), the area under the concentration curve from time zero to infinity (AUC), and the elimination half-life ($t_{1/2}$).

dose-response relationship for the four dosing intervals against *C. albicans* K1 is shown in Fig. 2. The dose-response curves were relatively similar among the four dosing intervals.

The relationships between the microbiological effect and each of the pharmacodynamic indices, 24-h free-drug AUC/MIC ratio (fAUC$_{0-24}$/MIC), free-drug C_{max}/MIC ratio (fC_{max}/MIC), and the percentage of the dosing interval during which plasma free-drug levels exceeded the MIC for each of the dosage regimens studied (T_{MIC}), against *C. albicans* K1 are shown in Fig. 3. The strongest relationship was seen when the results were correlated with the fAUC$_{0-24}$/MIC ratio, with an R^2 value of 88%. Regression with fC_{max}/MIC (R^2 = 82%) and T_{MIC} (R^2 = 7.5%) resulted in less strong relationships.

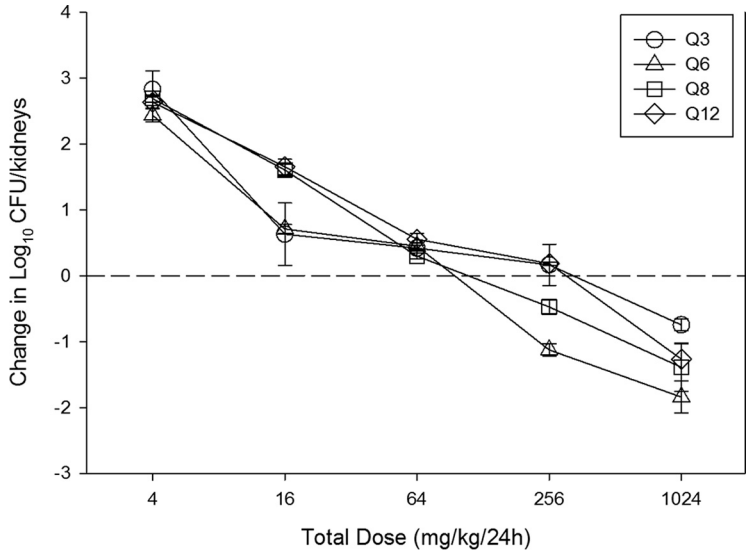

FIG 2 Relationship between the APX001 dosing interval and efficacy against *C. albicans* K1 in a murine neutropenic disseminated candidiasis model. Each symbol represents the mean ± SD for three animals. The error bars represent the standard deviations. The dashed horizontal line represents net stasis over the treatment period. Points above the line represent net growth, and points below the line represent net killing (cidal activity).

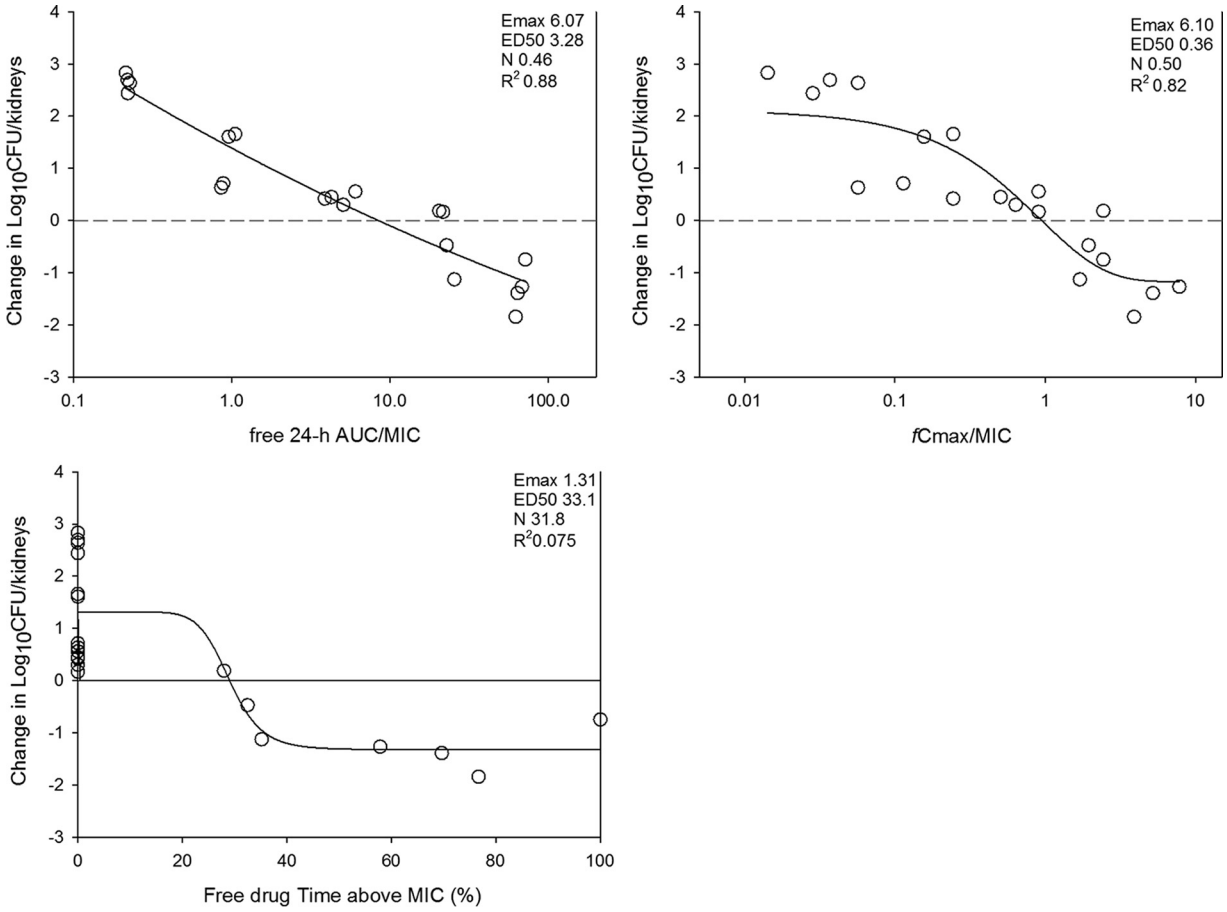

FIG 3 Pharmacodynamic regression of the *in vivo* dose fractionation study with APX001 against *C. albicans* K1. Each symbol represents the mean and standard deviation for three mice. The dose data are expressed as fAUC$_{0-24}$/MIC, fC_{max}/MIC, and the percentage of the dosing interval during which plasma free-drug levels exceeded the MIC for each of the dosage regimens studied (T_{MIC}). R^2 is the coefficient of determination. Also shown for each PD index is the maximum effect (E_{max}), the PD index value associated with 50% of the maximum effect (ED_{50}), and the slope of the relationship, or the Hill coefficient (N). The line drawn through the data points is the best-fit line based upon the sigmoid E_{max} formula.

Consideration of bound or unbound drug levels did not appreciably impact the relationships between efficacy and PK/PD indices.

Treatment efficacy and pharmacodynamic magnitude determination. At the start of therapy, mice had 4.04 ± 0.3 $\log_{10}$ CFU/kidney, and the burden increased 3.30 ± 0.4 $\log_{10}$ CFU/kidney in the untreated controls at the end of therapy. The initial burden and growth in the controls for each organism are listed in Table 2. The *in vivo* dose-response curves for each *Candida* species are shown in Fig. 4A1, B1, and C1. Dose-dependent activity was observed with all *Candida* species groups. The maximal reduction in the *C. albicans* burden in APX001-treated mice compared to the burden in the untreated controls ranged from 0.99 to −1.54 $\log_{10}$ CFU/kidney, and static efficacy was reached in three of five *C. albicans* isolates. For *C. glabrata*, the maximal reduction was −0.72 to −1.62 $\log_{10}$ CFU/kidney, stasis was observed in all five strains, and 1-log kill below stasis was reached in four of five strains. For *C. auris*, the maximal reduction was 0.21 to −1.02 $\log_{10}$ CFU/kidney, with static efficacy being achieved in three of four strains. Potency was more pronounced against *C. glabrata* on the basis of the dose-response curves. The relationship between the PK/PD index fAUC$_{0-24}$/MIC and treatment effect is shown in Fig. 4A2, B2, and C2. The coefficients of determination (R^2) were strong, ranging from 0.67 to 0.90.

Calculation of the doses necessary to achieve the 50% of the maximum effect (ED_{50}) and a static effect against multiple organisms are shown in Table 2. The ED_{50} and stasis fAUC/MIC$_{0-24}$ targets for each organism group were as follows: for *C. albicans*, 3.67 ± 3.19 and 20.60 ± 6.50, respectively; for *C. glabrata*, 0.38 ± 0.21 and 1.31 ± 0.27, respectively; and for *C. auris*, 7.14 ± 4.54 and 14.67 ± 8.30, respectively.

TABLE 2 Static doses and associated AUC/MIC values in the neutropenic disseminated candidiasis model[a]

Species	Isolate or parameter	Log_{10} no. of CFU/kidneys			ED_{50}			Stasis		
		Start	Growth	Maximum kill	24-h dose (mg/kg)	24-h *t*AUC/MIC	24-h *f*AUC/MIC	24-h dose (mg/kg)	24-h *t*AUC/MIC	24-h *f*AUC/MIC
C. albicans	K1	4.16	3.40	−1.37	87.3	3,413	6.83	265.5	12,952	25.91
	98-210	3.36	4.05	0.99	43.1	371	0.742	NA		
	580	4.24	3.09	−0.93	51.3	3,361	6.72	86.0	6,678	13.35
	2-76	3.54	3.78	−1.54	17.7	1,999	4.00	76.5	11,270	22.54
	98-17	4.39	2.90	0.11	5.71	41.9	0.084	NA		
	Mean	3.94	3.44	−0.55	41.0	1,837	3.67	142.5	10,300	20.60
	Median	4.16	3.40	−0.93	43.1	1,999	4.00	86.0	11,270	22.54
	SD	0.46	0.48	1.07	31.8	1,597	3.19	106.7	3,248	6.50
C. glabrata	10956	3.85	3.64	−1.22	4.7	65	0.13	28.1	432.8	0.866
	5592	3.91	3.13	−0.72	29.9	124	0.247	125.2	734.2	1.47
	35315	4.07	3.15	−1.41	20.9	307	0.61	41.9	675.5	1.35
	513	3.86	3.56	−1.15	22.5	178	0.36	69.4	647.2	1.29
	5376	4.17	3.15	−1.62	10.32	284.1	0.568	26.0	792.3	1.59
	Mean	3.97	3.33	−1.22	17.7	192	0.38	58.1	656.4	1.31
	Median	3.91	3.15	−1.22	20.9	178	0.36	41.9	675.5	1.35
	SD	0.14	0.25	0.34	10.1	103	0.21	41.3	136.9	0.27
C. auris	B11104	4.18	3.31	0.21	24.8	682.0	1.36	NA		
	B11221	4.32	3.24	−0.94	47.5	3,095.1	6.19	100.2	4,213.0	8.25
	B11219	4.31	2.36	−0.99	106.4	4,469.2	8.94	78.6	5,864.2	11.73
	B11804	4.18	3.46	−1.02	671.3	6,034.5	12.07	243.3	12,022	24.04
	Mean	4.25	3.09	−0.69	212.5	3,570.2	7.14	140.7	7,336.4	14.67
	Median	4.25	3.28	−0.97	77.0	3,782.2	7.56	100.2	5,864.2	11.73
	SD	0.08	0.50	0.60	307.8	2,269.2	4.54	89.5	4,150.2	8.30

[a]NA, not achieved; *t*AUC, total drug AUC; *f*AUC, free (non-protein-bound) drug AUC.

DISCUSSION

Candida spp. are the most frequent organisms encountered in invasive fungal infections among hospitalized patients (3). Immunocompromised or immunosuppressed patients, including solid-organ or hematopoietic stem cell transplant recipients and individuals who are on immunosuppressive drug regimens, are especially at risk for invasive fungal infection, which is associated with a high rate of morbidity and mortality (5). Although *C. albicans* remains the most common pathogen, the species distribution of *Candida* spp. has changed considerably over the past decade, with a substantial emergence of non-*albicans Candida* spp. being seen in different health care settings worldwide (6). More recently, multidrug-resistant *C. auris* has emerged as an important health care-associated fungal pathogen associated with high rates of clinical treatment failure (7, 8). However, current available antifungal drugs have limitations in terms of spectrum of activity, toxicity, pharmacokinetic variability, and drug interactions. These drug limitations, along with the steady emergence of strains with resistance, prompted an expanded search for new antifungal agents with novel mechanisms of action.

APX001 is a first-in-class, intravenous (i.v.) and oral (p.o.) broad-spectrum antifungal agent in clinical development for the treatment of invasive fungal infections (9, 12). The active moiety APX001A inhibits the inositol acyltransferase in fungal GPI biosynthesis but not the human homolog, Pig-W (12). The goal of the present study was to determine the PK/PD index for this new antifungal and also the amount of drug relative to the MIC or the magnitude of the predictive pharmacodynamic index required for the treatment efficacy of APX001. In addition, we wanted to determine whether the pharmacodynamic target was similar among *Candida* species.

Our studies demonstrated APX001 to be active against *C. albicans*, *C. glabrata*, and *C. auris*, including against isolates resistant to fluconazole and micafungin. This potent activity has been observed in previous *in vitro* studies, with MIC values ranging from ≤0.008 to 0.016 mg/liter for *C. albicans* and from ≤0.008 to 0.06 mg/liter for *C. glabrata*. APX001A has also shown equally potent activities against fluconazole-resistant (14, 16) and caspofungin-resistant (16) *Candida* strains.

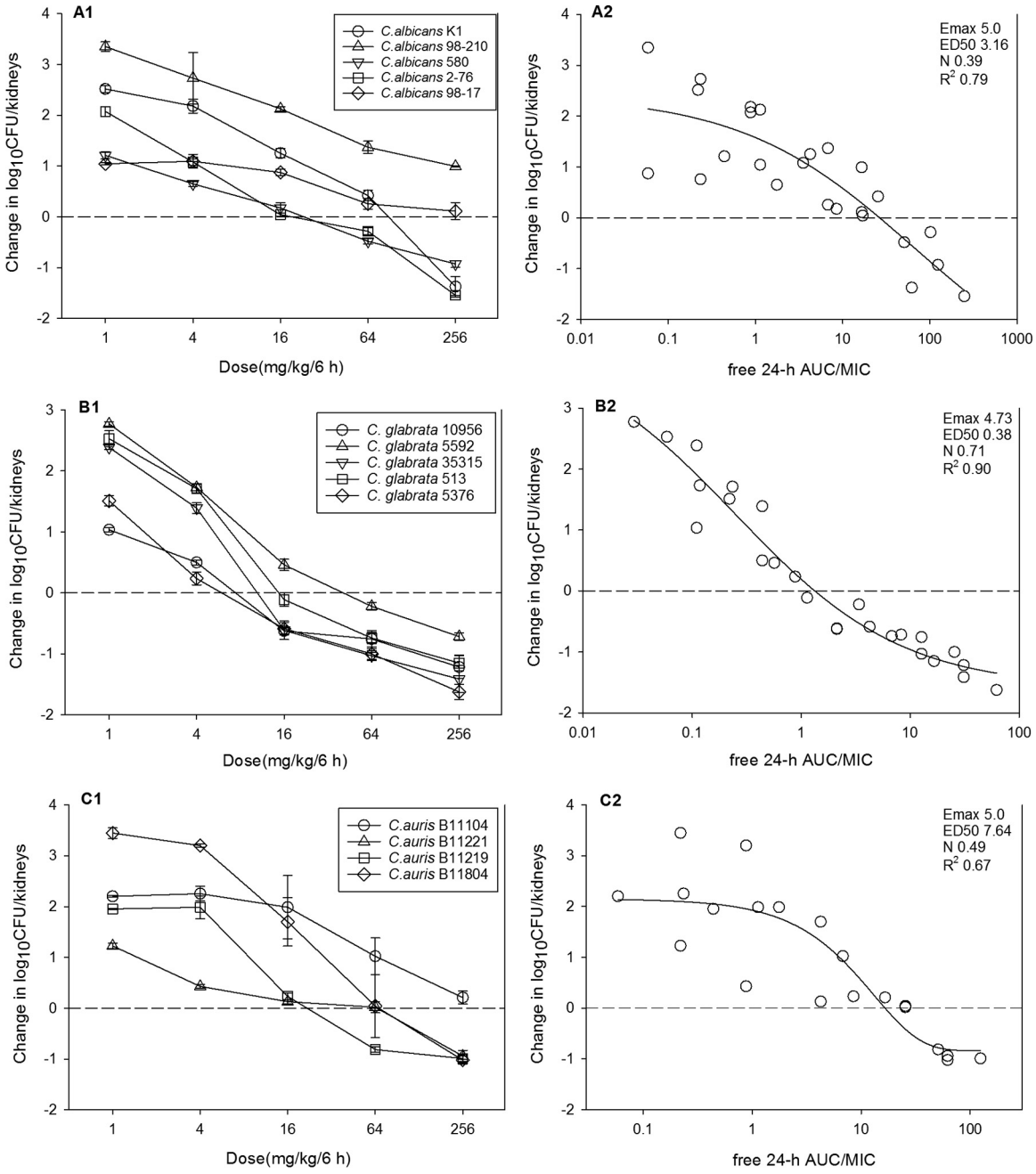

FIG 4 *In vivo* dose effect of APX001 against select *C. albicans* (A1 and A2), *C. glabrata* (B1 and B2), and *C. auris* (C1 and C2) strains in the mouse disseminated candidiasis model. Each symbol represents the mean and standard deviation for three mice. Five total drug dose levels were fractionated into a regimen of administration every 6 h. The burden of organisms was measured at the start and the end of therapy. The study period was 24 h for *C. albicans* and 96 h for *C. glabrata* and *C. auris*. The horizontal dashed line at 0 represents the burden of organisms in the kidneys of the mice at the start of therapy. Data points below the line represent killing, and points above the line represent growth.

In vivo pharmacodynamic evaluation of antimicrobial activity requires the integration of the aforementioned *in vitro* potency (MIC), drug pharmacokinetics, and antimicrobial activity over time (17). A previous *in vivo* pharmacokinetic study with APX001 demonstrated a short half-life of 2.2 h in mice, but only at the 1-mg/kg of body weight dosage (13). Our study expanded the dose range (4 to 256 mg/kg), and a similar half-life (1.40 to 2.75 h) was observed with a linear PK profile ($R^2 = 0.97$) across the dose range. Concentration-dependent activity has been shown in both the previous *in vivo* study (13) and our current study. The PK/PD indexes associated with the efficacy of antibiotics

characterized by this pattern of activity are AUC/MIC and C_{max}/MIC. The present study demonstrated a stronger correlation with the AUC/MIC index.

In the pharmacodynamic analyses, we considered both the protein-bound and the unbound drug concentrations, since the majority of antibacterial and antifungal pharmacodynamic experiments have shown that free-drug concentrations are more relevant. We considered the fAUC$_{0-24}$/MIC to be the PK/PD index most closely correlated with treatment efficacy, based on the results of the dose fractionation experiments performed. As a first-in-class antifungal, the nonclinical PK/PD target endpoint for APX001 that correlates with the clinical response is unknown. Previous studies in this model for the triazole class have shown the relevance of the ED_{50} endpoint for forecasting outcomes in patients (18–21). Conversely, the net stasis endpoint in the neutropenic murine disseminated candidiasis model was predictive of the clinical outcome for the echinocandin class (22). In current study, we reported both ED_{50} and stasis endpoints. The fAUC$_{0-24}$/MIC targets required for ED_{50} and stasis for *C. albicans* were 3.67 ± 3.19 and 20.60 ± 6.50, respectively. The targets for *C. auris* were relatively similar at 7.14 ± 4.54 and 14.65 ± 8.79, respectively. However, the targets for *C. glabrata* were significantly lower at 0.38 ± 0.21 and 1.24 ± 0.26, respectively. A similar species-specific PK/PD target difference has been observed for the echinocandins (23, 24). The stasis targets for APX001A in the current study were also comparable to those for micafungin and anidulafungin, which were near 20 (23, 25).

In summary, the present studies demonstrate that APX001 has concentration-dependent *in vivo* efficacy against *C. albicans*, *C. glabrata*, and *C. auris*. The fAUC$_{0-24}$/MIC ratios were very highly associated with *in vivo* APX001 activity. These PD characteristics support a dosing strategy that maximizes the AUC. The identification of a lower AUC/MIC ratio target for *C. glabrata* than for other *Candida* species suggests that species-specific susceptibility breakpoints should be explored. The clinical relevance of these PK/PD targets should be considered in future clinical development of this promising antifungal.

MATERIALS AND METHODS

Organisms, media, and antibiotic. Fourteen clinical *Candida* isolates were used for the *in vivo* treatment studies, including five *C. albicans*, five *C. glabrata*, and four *C. auris* isolates (Table 1), all of which belong to biosafety level 2. The organisms were chosen on the basis of their similar fitness in the animal model, as defined by the amount of growth in control animals over 24 h for *C. albicans* or 96 h for *C. glabrata* and *C. auris* (Table 2). We also attempted to choose strains with various susceptibilities to the study drug as well as other antifungal drug classes. The organisms were grown, subcultured, and quantified on Sabouraud's dextrose agar (SDA) plates. APX001A for *in vitro* studies and APX001 for *in vivo* studies were supplied by the study sponsor (Amplyx Pharmaceuticals, Inc., San Diego, CA). APX001A was prepared on the day of use by dissolving it with 700 mM HCl and subsequent dilution in RPMI to the required concentrations. APX001 was prepared in 0.21 M NaOH at a concentration of 100 mg/ml and then diluted with sterile 5% glucose solution to the required concentrations.

***In vitro* susceptibility testing.** The MICs of APX001A for the various isolates were determined using a broth microdilution method in accordance with the guidelines presented in Clinical and Laboratory Standards Institute (CLSI) document M27-A3 (26). All MIC assays were performed in duplicate on three separate occasions. The MIC values of APX001A were defined as the lowest concentration at which a prominent decrease in growth turbidity (that is, a 50% reduction in growth determined spectrophotometrically) relative to the turbidity of the compound-free control at 600 nm was observed. The median MIC from replicate assays is reported and was utilized in PK/PD analyses.

Murine disseminated candidiasis model. Six-week-old, specific-pathogen-free, female ICR/Swiss mice weighing 23 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). The animals for the present studies were maintained in accordance with the criteria of the Association for Assessment and Accreditation of Laboratory Animal Care. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial Veterans Hospital.

Mice were rendered neutropenic (neutrophils, $<$100/mm^3) by injecting them with cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) subcutaneously 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection and 2 days after infection (100 mg/kg). Previous studies have shown that this regimen produces neutropenia (neutrophils, $<$100/mm^3) in this model for the 96-h study period (27). Organisms were subcultured on SDA 24 h prior to infection. The inoculum was prepared by placing three to five colonies into 5 ml of sterile pyrogen-free 0.9% saline that had been warmed to 35°C. The final inoculum was adjusted to a 0.6 transmittance at 530 nm. The fungal counts of the inoculum determined by viable counts of *C. albicans*, *C. glabrata*, and *C. auris* on SDA were 6.29 ± 0.03, 6.15 ± 0.10, and 6.30 ± 0.07 $\log_{10}$ CFU/ml, respectively.

Disseminated infection with the *Candida* organisms was achieved by injection of 0.1 ml of inoculum via the lateral tail vein 2 h prior to the start of drug therapy. We utilized a treatment period of 24 h for

C. albicans and 96 h for *C. glabrata* and *C. auris* to allow comparison of the effects of other antifungals with those of APX001 against these pathogens in this model. At the end of the study period, the animals were sacrificed by CO_2 asphyxiation. After sacrifice, the kidneys of each mouse were removed and placed in sterile 0.9% saline at 4°C. The homogenate was then serially diluted 1:10, and aliquots were plated on SDA for viable fungal colony counts after incubation for 24 h at 35°C. The lower limit of detection was 100 CFU/ml. Results were expressed as the mean number of CFU per kidney for three mice. No-treatment and zero-hour controls were included in all experiments.

Pharmacokinetics. Single-dose plasma pharmacokinetics of APX001A were performed in mice administered a single oral dose (0.2 ml/dose) of APX001 at dose levels of 4, 16, 64, and 256 mg/kg. Groups of three mice were sampled at each time point (seven time points, consisting of 0.25, 0.5, 1, 2, 4, 8, and 12 h) and dose level. Samples were then centrifuged for 5 min at 4,000 rpm, and plasma was removed and frozen at −20°C until assay. Plasma concentrations of APX001A (the active moiety) were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by the sponsor. The lower limit of detection of the LC-MS/MS assay was 10 ng/ml. Pharmacokinetic measures, including elimination half-life ($t_{1/2}$), area under the concentration-time curve (AUC), and maximum plasma concentration (C_{max}), were calculated using a noncompartmental model. $t_{1/2}$ was determined by linear least-squares regression. AUC was calculated from the mean concentrations using the trapezoidal rule. Pharmacokinetic estimates for dose levels that were not directly measured were calculated using linear interpolation for dose levels between those with measured kinetics (e.g., between 4 and 256 mg/kg) and linear extrapolation for dose levels above or below the highest and lowest dose levels with kinetic measurements. Protein binding of 99.8% was used for estimation of free-drug concentrations, based on a report from the sponsor.

PK/PD index determination. A dose fractionation study design was undertaken to determine the PK/PD index (AUC/MIC, C_{max}/MIC, or time above the MIC) that was predictive of efficacy for APX001. Fourfold increasing doses (range, 4 to 1,024 mg/kg) of APX001 were fractionated into dosing regimens in which the drug was administered every 3 h (q3h), every 6 h (q6h), every 8 h (q8h), and every 12 h (q12h). Mice were infected with isolate *C. albicans* K1 as described above and administered APX001 by oral gavage. After 24 h the mice were euthanized and the CFU count in the kidneys was determined. To determine which PK/PD index was most closely linked with efficacy, the number of yeast cells in the kidneys at the end of 24 h of therapy was correlated with (i) the 24-h free-drug AUC/MIC ratio (*f*AUC/MIC), (ii) the free-drug C_{max}/MIC ratio (fC_{max}/MIC), and (iii) the percentage of the dosing interval during which plasma free-drug levels exceeded the MIC for each of the dosage regimens studied (T_{MIC}). The correlation between efficacy and each of the three PK/PD indices was determined by nonlinear regression based upon the Hill equation: $E = (E_{max} \times D^N)/(ED_{50}{}^N + D^N)$, where E is the effector, in this case, the $\log_{10}$ change in the number of CFU per kidney between treated mice and untreated controls after the 24-h period of study; E_{max} is the maximum effect; D is the 24-h total dose; ED_{50} is the dose required to achieve 50% of the E_{max}; and N is the slope of the dose-effect curve. The values for the pharmacodynamic parameters E_{max}, ED_{50}, and N were calculated using nonlinear least-squares regression. The coefficient of determination (R^2) was used to estimate the variance that might be due to regression with each of the PK/PD indices.

PK/PD index magnitude studies. Dose-response experiments using the disseminated candidiasis model were performed for five *C. albicans*, five *C. glabrata*, and four *C. auris* isolates as described above. The dose range consisted of 4-fold increases (range, 1 to 256 mg/kg/6 h) in drug concentration with administration by the oral route. The treatment duration was 24 h for *C. albicans* and 96 h for *C. glabrata* and *C. auris*. The dose-response relationships were quantified, and the relationship between the PK/PD index *f*AUC/MIC and treatment efficacy was determined using the sigmoid E_{max} model. These PK/PD relationships were examined utilizing the plasma total and free-drug concentrations from pharmacokinetic studies. The coefficient of determination (R^2) from this model was used to assess the strength of this relationship. The doses required to produce 50% of the maximal effect (ED_{50}) and a net static effect (static dose) compared to the number of CFU at the start of therapy for multiple pathogens in the disseminated candidiasis model were determined utilizing the plasma total and free-drug concentrations and the following equation: $\log_{10} D = \{\log_{10}[E/(E_{max} - E)]/N\} + \log ED_{50}$, where E is the control growth for dose (D) and E is the control. The associated 24-h total and free-drug AUC/MIC targets were calculated.

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