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Efficacy of CD101 to Treat Echinocandin-Resistant *Candida albicans* in a Murine Model of Invasive Candidiasis

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ABSTRACT

Background: CD101 is a novel, long-acting echinocandin being developed for the treatment of invasive *Candida* infections. CD101 has demonstrated potent in vitro activity against a broad range of *Candida and Aspergillus* species including antifungal-resistant strains. Herein, we have evaluated the in vivo efficacy of CD101 against echinocandin-susceptible and -resistant *C. albicans* strains in an invasive candidiasis murine model.

Methods: Female 6-week-old BALB/c mice weighing 18-22 g were rendered neutropenic by receiving 150 mg/kg and 100 mg/kg of cyclophosphamide via IP injection on day -4 and day -1 prior to infection, respectively. Two *C. albicans* strains, ATCC 90028 (*FKS* WT) and DPL22 (*fks*/*FKS* mutant S645P/S), were used in this experiment. On day 0, mice were infected with 5×10^5 CFU WT or mutant strain via lateral tail vein injection. Groups consisting of 10 mice were given single doses of vehicle, CD101 (20, 40, or 60 mg/kg) or micafungin (MCF) (5 mg/kg), which are doses that are equivalent to their anticipated human drug exposures after a single IV dose at 3 h post-inoculation via IP injection. At 24 h and 48 h post-inoculation, 5 mice from each group were euthanized, and kidneys were aseptically removed for enumeration of fungal burdens. Data analysis was performed by using GraphPad Prism software. A *P* value of <0.05 is considered statistically significant.

Results: CD101 at all three doses exhibited activity against both WT and heterozygous *fks*/*FKS* mutant strains of *C. albicans*, as demonstrated by significant kidney burden reductions compared to infected controls in all treatment groups at both 24 h and 48 h post-inoculation time points. In WT strain infected mice, CD101 exhibited greater efficacy than MCF at 24 h post-inoculation at all three doses. Superiority relative to MCF was observed with the 60 mg/kg CD101 group at 48 h post-inoculation. In *fks/FKS* mutant infected mice, CD101 treatment significantly reduced kidney burdens by over 2 logs at 24 h post-inoculation compared to vehicle control. Superior efficacy of CD101 relative to MCF was observed for all three doses at 48 h post-inoculation.

Conclusions: Potential therapeutic doses of CD101 (20 to 60 mg/kg) were effective in treating invasive candidiasis caused by both *FKS* WT and heterozygous *fks*/*FKS* mutant *C. albicans* in mice.

INTRODUCTION

The echinocandins are recommended as first-line therapy for non-neutropenic patients with *Candida albicans*, *Candida glabrata* and suspected severe invasive candidiasis [1]. CD101 is a novel, long-acting echinocandin being developed for the treatment of invasive *Candida* infections. CD101 has demonstrated potent in vitro activity against a broad range of *Candida* and *Aspergillus* species including antifungal-resistant strains. Presently, a paucity of information exists concerning the antifungal properties of CD101 against well-defined echinocandin-resistant clinical isolates. Herein, we have evaluated the in vivo efficacy of CD101 against echinocandin-susceptible and -resistant *C. albicans* strains in an invasive candidiasis murine model.

MATERIALS AND METHODS

Strains. Two *C. albicans* strains (ATCC 90028 and DPL22) were used in this mouse study. ATCC 90028 is an *FKS* wild-type strain and sensitive to echinocandin drugs. DPL22 is a heterozygous *fks*/*FKS* mutant (S645P/S) strain with elevated minimal inhibitory concentration (MIC) and glucan synthase half maximal inhibitory concentration (IC_{50}) values for both micafungin (MCF) and CD101 (**Table 1**).

Table 1. Strains used in mouse study

Strain	Organism	Fksp Phenotype		MIC (μg/ml)		IC_{50} (ng/ml)	
		Fks1p	Fks2p	MCF	CD101	MCF	CD101
ATCC 90028	*C. albicans*	WT	WT	<0.03	<0.03	18	14
DPL22	*C. albicans*	S645P/S	WT	0.5	0.5	245	30

Animals. Female 6-week-old BALB/c mice weighing 18-22 g were used for this experiment. Mice were housed in presterilized filter-top cages and maintained in accordance with American Association for Accreditation of Laboratory Care criteria. The animal study was approved by Rutgers Institutional Animal Care and Use Committee.

Murine infection model and antifungal treatment. A well-established neutropenic disseminated candidiasis murine model was used for the treatment study [2]. The mice were rendered neutropenic by receiving 150 mg/kg and 100 mg/kg of cyclophosphamide via IP injection on day -4 and day -1 prior to infection, respectively. The organisms were subcultured in liquid yeast extract-peptone-dextrose (YPD) medium at 37°C with shaking overnight. Cells were collected by centrifugation, washed twice with sterile phosphate-buffered saline (PBS), and counted with a hemocytometer. The inoculum was adjusted to $5x10^6$ CFU/ml and 100 μl was used to infect each mouse. Actual infection dose was verified by viable counts on YPD plates spread with proper dilutions of the inoculum and incubated at 37°C for 24 h. On day 0, mice were infected with $5x10^5$ CFU of *C. albicans FKS* wild-type (ATCC 90028) (n=50) or heterozygous mutant strain (DPL22 S645P/S) (n=50) via lateral tail vein injection. Groups consisting of 10 mice were given one dose of vehicle (provided by Cidara), CD101 at 20 mg/kg, 40 mg/kg, or 60 mg/kg, or antifungal control (micafungin, 5 mg/kg, equivalent to clinical therapeutic dose) at 3 h post-infection via IP injection. At 24 h post-inoculation and at the experiment endpoint 48 h post-inoculation, 5 mice from each group were euthanized via CO_2 inhalation and kidneys were aseptically removed for enumeration of fungal burdens.

Data analysis. All graphic data are expressed as means ± SD and were statistically analyzed by analysis of variance (ANOVA) using computer Prism software (Prism 5; GraphPad Software, Inc., San Diego, CA). A P value of <0.05 is considered statistically significant.

RESULTS

CD101 at all three doses exhibited strong activities against both wild-type and *fks/FKS* mutant strains of *C. albicans*, as demonstrated by significant kidney burden reduction in all treatment groups at both 24 h and 48 h post-inoculation time points (P<0.05) (**Figure 1, 2**). In wild-type strain infected mice, CD101 exhibited better efficacy than micafungin at 24 h post-inoculation at all three doses. Although the superiority of CD101 relative to micafungin was only seen with the highest dose (60 mg/kg) at 48 h post-inoculation, the efficacy of CD101 at 20 mg/kg and 40 mg/kg was still comparable with micafungin at 5 mg/kg (**Figure 1**).

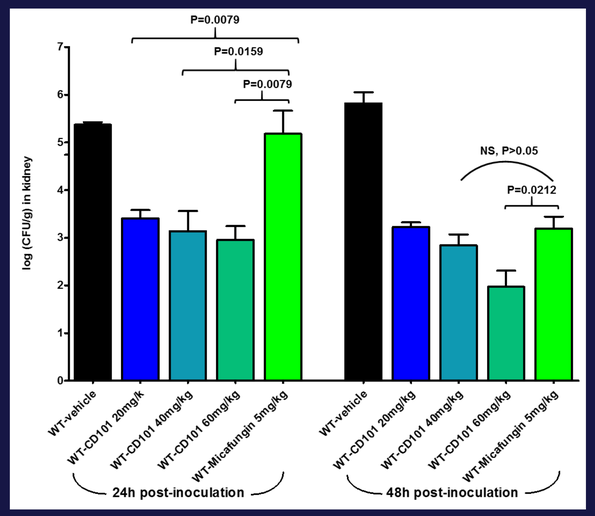

Figure 1. Kidney burdens in mice infected with *C. albicans* WT strain ATCC 90028 at 24 h and 48 h post-inoculation.

Regarding the *fks/FKS* mutant strain infected mice, CD101 treatment significantly reduced kidney burdens by over 2 logs at 24 h post-inoculation compared to vehicle control (P<0.05). The 24 h burden reduction was not significantly different among three CD101 dosage groups or micafungin treatment group. However, better efficacy of CD101 compared to micafungin at 5 mg/kg was observed for all three doses at 48 h post-inoculation. Burden reduction was comparable in three CD101 groups (**Figure 2**).

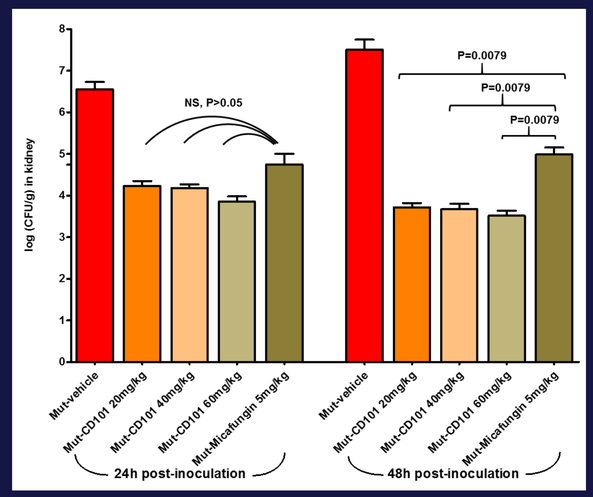

Figure 2. Kidney burdens in mice infected with *C. albicans* mutant strain DPL22 S645P/S at 24 h and 48 h post-inoculation.

CONCLUSIONS

- CD101 at the dose range of 20 to 60 mg/kg was effective to treat invasive candidiasis caused by both *FKS* wild-type and a heterozygous *fks/FKS* mutant *C. albicans* in neutropenic mice.
- Compared to a standard 5 mg/kg micafungin regimen, better efficacy of CD101 was observed for all three studied doses at 24 h post-inoculation in wild-type infected mice. The highest dose (60 mg/kg) of CD101 was better than micafungin at 48 h post-inoculation.
- For *fks/FKS* mutant caused infection, better efficacy of CD101 relative to micafungin was observed at 48 h post-inoculation for all three studied doses.

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