In vitro activity of the novel echinocandin CD101 at pH 7 and 4 against Candida spp. isolates from patients with vulvovaginal candidiasis

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Background: The novel echinocandin CD101 has stability properties amenable to topical formulation for use in the treatment of acute vulvovaginal candidiasis (VVC) and recurrent VVC (RVVC). CD101 has demonstrated potent antifungal activity at pH 7, but assessment of its activity at the physiological pH of the vaginal environment is needed.

Objectives: To evaluate the antifungal activity of CD101 against clinical VVC isolates of Candida spp., including azole-resistant strains, at pH 4.

Methods: MIC values of CD101 and comparators (fluconazole, itraconazole, micafungin, caspofungin and anidulafungin) were assessed via broth microdilution. MIC assays were conducted at pH 7 and 4 after 24 and 48 h against a 108 VVC isolate panel of Candida spp., including Candida albicans (n = 60), Candida glabrata (n = 21), Candida parapsilosis (n = 14) and Candida tropicalis (n = 13).

Results: Overall, MIC values of all drugs were slightly higher at pH 4 versus 7 and at 48 versus 24 h of incubation. CD101 MIC values typically exhibited ~4-fold shifts at pH 4 and were not affected by azole susceptibility. C. parapsilosis susceptibility was the least affected at pH 4 and did not increase for most drugs.

Conclusions: CD101 had potent activity against all Candida isolates tested, including azole-resistant strains. Although there was some reduction in activity at pH 4 versus 7, the resulting MIC values were still well below the intravaginal CD101 drug concentrations anticipated to be present following topical administration. These results support continued development of topical CD101 for the treatment of VVC/RVVC.

Introduction

Vulvovaginal candidiasis (VVC) affects most women at least once in their lifetime. The reported incidence of VVC varies widely between 12.1% and 57.3% and up to 91% in pregnant women, reflecting the lack of reliable, consistent epidemiological data to provide more definitive estimates. The incidence of recurrent VVC (RVVC), i.e. four or more episodes of VVC in 1 year, is even more difficult to evaluate. Nevertheless, the impact and morbidity of VVC (RVVC), i.e. four or more episodes of VVC in 1 year, is even more difficult to evaluate. Nevertheless, the impact and morbidity of VVC and RVVC are well recognized and warrant continued efforts to improve patient outcomes.1,2

Candida spp., are less effective against non-albicans Candida spp. and have lower activity at vaginal pH.6 Non-albicans Candida spp. are also more likely to be azole resistant and are more difficult to treat.7,8 Despite these unmet and emerging needs, there has been no novel class of agents for the treatment of VVC and RVVC for decades.

An optimal new therapeutic for VVC/RVVC would not only have strong activity against all Candida spp. and azole-resistant strains, but also maintain activity at vaginal pH (4–4.5) during VVC infections.9 Based on their intrinsic potency and efficacy against systemic Candida infections, echinocandins have many apparent advantages over azoles: fungicidal versus fungistatic, broader spectrum of activity against Candida spp., lower prevalence of resistance, fewer drug–drug interactions and better overall safety.10–12 However, little work has been done to characterize echinocandin activity at the lower pH of the vaginal environment.5

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This is ostensibly because currently available echinocandins are limited to intravenous administration, which is impractical for VVC/RVVC, and their stability/solubility are major barriers to topical dosage forms. CD101 is a novel echinocandin with demonstrated stability and solubility suitable for topical formulations. The antifungal activity of CD101 is comparable to currently marketed echinocandins against contemporary surveillance isolates as well as against panels enriched for drug-resistant Candida spp. strains. Here CD101 was evaluated against a collection of clinical VVC isolates of Candida spp., including azole-resistant strains, at pH 7 and 4.

**Methods**

**Clinical VVC isolates**

Candida strains evaluated in this study were clinical vaginal isolates obtained from the Wayne State University Vaginitis Clinic organism bank. Isolates were obtained from VVC patients under an IRB-approved consent protocol and were anonymized prior to MIC testing. The strain panel was composed of randomly selected C. albicans (n = 60, 10 fluconazole resistant), C. glabrata (n = 21, 11 fluconazole resistant), C. parapsilosis (n = 14, 7 fluconazole resistant) and Candida tropicalis (n = 13). Isolates were plated on CHROMagar to verify purity and then plates were incubated for 48 h at 37°C. A single colony was subcultured on Sabouraud dextrose agar and incubated for 24 h at 35°C.

**Antifungal susceptibility testing**

Susceptibility testing was performed according to CLSI broth microdilution guidelines, with the exception that MIC values were read after 24 and 48 h as the lowest drug concentration to exhibit 80% reduction in growth. RPMI 1640 medium (buffered with 0.165 M MOPS) was adjusted to a final pH of 7 with NaOH or pH 4 with HCl. Assay inoculum was achieved by using spectrophotometric inoculum preparation according to CLSI guidelines. This assay involved adding 100 μL of yeast inoculum containing 0.5–2.5 × 10^6 cfu/mL in RPMI 1640 medium to each well. CD101 (Cidara Therapeutics, Inc.) was prepared in 100% DMSO. Comparator agents (flucytosine, itraconazole, caspofungin, micafungin and anidulafungin) were prepared in 100% DMSO according to CLSI guidelines. Quality control (QC) strains Candida krusei ATCC 6258 and C. parapsilosis ATCC 22019 were included in all assays.

**Results**

Table 1 presents MIC values (MIC_{90}, MIC_{50} and MIC ranges) of CD101 and comparators for 108 VVC clinical isolates at pH 7 and 4 after 24 and 48 h. MIC values of relevant drug/QC strain combinations in this study fell within CLSI ranges when called at 80% inhibition (data not shown).

**C. albicans (n = 60)**

CD101 had MIC_{90} values of 0.06/0.03 mg/L at pH 7 after 24 h, 8-fold more potent than those of caspofungin and ~8-fold less potent than those of micafungin and anidulafungin. At pH 4, CD101 activity was decreased 4-fold compared with that observed at neutral pH. CD101 maintained activity against the 10 fluconazole-resistant isolates with an MIC_{90} of 0.125/0.03 mg/L. C. albicans MIC values of itraconazole were similar to those obtained for the echinocandins, but overall much lower than those of fluconazole (MIC_{90}/MIC_{50}, 8/0.25 mg/L at pH 4).

**C. glabrata (n = 21)**

CD101 had MIC_{90} values of 0.125/0.125 mg/L at pH 7 after 24 h, ~8-fold more potent than those of caspofungin, 4-fold less potent than those of anidulafungin and 16-fold less potent than those of micafungin. As with C. albicans, at pH 4, CD101 activity versus C. glabrata decreased 4-fold compared with that observed at neutral pH. Against C. glabrata, fluconazole again had the highest MIC values, with over half of the isolates showing values >4 mg/L at 48 h at both pH levels. Similarly, itraconazole MIC values were >4 mg/L at pH 4 when read at 48 h for 10 of the 21 isolates. As was seen with C. albicans, azole resistance did not impact echinocandin MIC values for C. glabrata.

**C. parapsilosis (n = 14)**

CD101 had MIC_{90} values of 2/1 mg/L at pH 7 and 4 after 24 h, comparable to the activity observed with the comparator echinocandins. Among the seven fluconazole-resistant isolates, two had insufficient growth at 24 h at pH 7 to determine the CD101 MIC value (as did two of the fluconazole-susceptible isolates). For the remaining five fluconazole-resistant isolates, CD101 maintained activity with MIC values of 0.008-1 mg/L. Half of the 14 C. parapsilosis isolates had insufficient growth in fluconazole MIC assays after 24 h, preventing determination of MIC values, although by 48 h growth levels permitted fluconazole MIC values to be determined for all isolates. With the exception of minor MIC shifts for caspofungin and fluconazole at 48 h for pH 4, no other compound's MIC values increased at low pH versus C. parapsilosis, and most trended towards greater potency at pH 4. Itraconazole MIC values were markedly lower than those of fluconazole across all C. parapsilosis isolates at both pH values and timepoints.

**C. tropicalis (n = 13)**

CD101 had MIC_{90} values of 0.125/0.06 mg/L at pH 7 after 24 h, 8-fold more potent than those of caspofungin and 4- to 8-fold less potent than those of micafungin and anidulafungin. At pH 4, the 4-fold decrease in CD101 activity observed for C. albicans and C. glabrata was also observed for C. tropicalis. At pH 4, only two isolates demonstrated fluconazole resistance after 24 h although that increased to eight fluconazole-resistant isolates at 48 h. Although itraconazole MIC values shifted 4-fold from 24 to 48 h, within each timepoint there was no shift between pH 4/7, and MIC values were much lower than for fluconazole.

**Discussion**

In this study we have demonstrated that CD101 has potent activity against vaginal isolates of Candida spp. comprising the most common causative agents of VVC/RVVC at a clinically relevant pH. Furthermore, due to CD101’s intrinsic potency and lack of cross-resistance with azoles, CD101 MIC_{90} values were lower than those of fluconazole for all four Candida spp. at both timepoints and pH values.

At pH 7, the CD101 MIC_{90} data for this VVC isolate panel were consistent with values from predominantly non-vaginal Candida isolates derived from international surveillance studies that were generated using strict M27-A3 50% inhibition endpoint methodology. Overall, CD101 and comparator agents demonstrated...
Table 1. MIC\(_{90/50}\) values and MIC ranges of CD101 and comparator agents for Candida spp. VVC isolates at pH 7 and 4 following 24 or 48 h of incubation

| Incubation time (h) | Drug | pH | MIC\(_{90}\) (mg/L) | MIC\(_{50}\) (mg/L) | MIC range (mg/L) | MIC\(_{90}\) (mg/L) | MIC\(_{50}\) (mg/L) | MIC range (mg/L) | MIC\(_{90}\) (mg/L) | MIC\(_{50}\) (mg/L) | MIC range (mg/L) |
|---------------------|------|----|---------------------|---------------------|------------------|---------------------|---------------------|------------------|---------------------|---------------------|------------------|
|                     | C. albicans (n = 60) | C. glabrata (n = 21) | C. parapsilosis (n = 14) | C. tropicalis (n = 13) |
| 24                  | CD101 | 7  | 0.06 (0.03) | 0.016–0.125 | 0.125 | 0.125 | 0.06–2 | 0.25 | 0.125 | 0.06–2 | 0.125 | 0.06–2 |
|                     | FLC  | 7  | 0.25 (0.125) | 0.06–0.5 | 0.5 | 0.5 | 0.125–2 | 0.5 | 0.25 | 0.25–0.5 | 0.5 | 0.25–0.5 |
|                     | ITC  | 7  | 0.06 (0.016) | 0.008–0.02 | 0.0125 | 0.0125 | 0.03–4 | 0.0125 | 0.008–0.03 | 0.0125 | 0.008–0.03 |
|                     | CAS  | 7  | 0.125 (0.016) | 0.008–0.02 | 0.015 | 0.015 | 0.03–2 | 0.015 | 0.008–0.02 | 0.015 | 0.008–0.02 |
|                     | MCF  | 7  | 0.008 (0.008) | 0.008–0.03 | 0.008 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 |
|                     | ANF  | 7  | 0.08 (0.008) | 0.008–0.06 | 0.03 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 |
| 48                  | CD101 | 7  | 0.125 (0.03) | 0.016–0.125 | 0.25 | 0.125 | 0.06–2 | 0.4 | 0.25 | 0.05–4 | 0.125 | 0.06–0.05 |
|                     | FLC  | 7  | 0.5 (0.5) | 0.125–1 | 1 | 1 | 0.25–2 | 2 | 1 | 0.5–2 | 2 | 1 | 0.5–2 |
|                     | ITC  | 7  | 0.125 (0.016) | 0.008–0.02 | 0.5 | 0.5 | 0.03–4 | 0.125 | 0.03 | 0.016–0.25 | 0.5 | 0.125 | 0.03–0.25 |
|                     | CAS  | 7  | 0.5 (0.5) | 0.25–1 | 1 | 0.5 | 0.05–2 | 1 | 0.5 | 0.125–1 | 1 | 0.5 | 0.125–1 |
|                     | MCF  | 7  | 0.008 (0.008) | 0.008–0.03 | 0.008 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 |
|                     | ANF  | 7  | 0.008 (0.008) | 0.008–0.06 | 0.03 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 |
|                     | MCF  | 7  | 0.008 (0.008) | 0.008–0.03 | 0.008 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 | 0.008 | 0.008–0.01 |
|                     | ANF  | 7  | 0.008 (0.008) | 0.008–0.06 | 0.03 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 | 0.03 | 0.008–0.08 |

FLC, fluconazole; ITC, itraconazole; CAS, caspofungin; MCF, micafungin; ANF, anidulafungin.

Due to insufficient growth for some of the C. parapsilosis isolate/drug combinations at 24 h, MIC values were only obtained for a subset of strains: \(^a^n = 10, ^b^n = 13, ^c^n = 7, ^d^n = 12, ^e^n = 11, ^f^n = 13, ^{13}n = 13, ^{19}n = 10.\)

minor increases in MIC values at longer incubation times and lower pH. An inverse correlation between MIC values and pH was also observed against a similar collection of Candida spp. VVC isolates for a variety of antifungal drug classes.\(^6\) With the exception of C. parapsilosis, the organisms in this study yielded similar results. MIC shifts at 48 versus 24 h functionally resulted in the transition from susceptible to resistant fluconazole MIC values for many isolates, as documented previously.\(^19\) Not surprisingly, many isolates resistant to fluconazole were also resistant to itraconazole (especially C. glabrata). Another study demonstrated increases in fluconazole MIC values at pH 4 (as well as for clotrimazole and miconazole); however, interestingly, itraconazole had an inverse effect against that collection of vaginal isolates tested.\(^20\) MIC shifts incurred by CD101 at pH 4 (typically 4-fold) are likely inconsequential to efficacy based on the high localized drug concentrations anticipated following topical administration of CD101 (>100 mg/L estimated).\(^21\) In the absence of in vivo data to confirm intravaginal concentrations, MIC analyses using anticipated CD101 topical formulations would be informative, but are precluded as some formulation viscosities are incompatible with CLSI broth-testing methodologies. Although the comparator echinocandins also exhibited minimal shifts at pH 4,\(^4\) their results can only provide within-class context, as these comparators are unviable for topical administration. Future studies investigating the activity of CD101 against echinocandin-resistant Candida isolates at vaginal pH would be informative and their absence is a limitation of this current dataset.

Despite their prevalent use for treatment of VVC/RVVC, theazole drug class is not adequate for infections caused by non-albicans Candida spp. andazole-resistant C. albicans. From spectrum, potency, mechanism of action and safety perspectives, echinocandins are an attractive option, but the chemical stability of currently available echinocandins has precluded topical administration and consideration as treatment of VVC/RVVC. Correspondingly, very little work has been done to characterize the antifungal activity of echinocandins at low pH. The enhanced stability properties of CD101 are highly differentiated and may enable topical administration. The susceptibility data generated in this study demonstrate that CD101 retains potent activity against VVC isolates under conditions relevant to the vaginal environment. These results support the continued clinical development of CD101 as the first topical echinocandin for the treatment of VVC/RVVC.

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