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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 and RVVC are well recognized and warrant continued efforts toward improving patient outcomes.[1,2] *Candida albicans* is the most common aetiological agent of VVC and RVVC. Non-*albicans Candida* spp., particularly *Candida glabrata*, account for 5%–15% of infections and are more prevalent in certain populations (e.g. patients with type 2 diabetes[3] or RVVC[4,5]). VVC is commonly treated with azole antifungals, which are fungistatic against *Candida* spp., are less effective against non-*albicans Candida* spp. and have lower activity at vaginal pH.[6] Non-*albicans Candida* spp. also are 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.[6]

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.[13] The antifungal activity of CD101 is comparable to currently marketed echinocandins against contemporary surveillance isolates[14] as well as against panels enriched for drug-resistant *Candida* spp. strains.[15] 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), *Candida 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,[16,17] 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.[16] This assay involved adding 100 μL of yeast inoculum containing $0.5–2.5 \times 10^3$ cfu/mL in RPMI 1640 medium to each well. CD101 (Cidara Therapeutics, Inc.) was prepared in 100% DMSO. Comparator agents (fluconazole, itraconazole, caspofungin, micafungin and anidulafungin) were prepared in 100% DMSO according to CLSI guidelines.[17] 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}s, MIC_{50}s 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).[17]

C. albicans (n = 60)

CD101 had $MIC_{90/50}$ 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 $MIC_{90/50}$ 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 ($MIC_{90/50}$, 8/0.25 mg/L at pH 4).

C. glabrata (n = 21)

CD101 had $MIC_{90/50}$ 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 $MIC_{90/50}$ 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_{90} 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 $MIC_{90/50}$ 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.[14,18] 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

			MIC (mg/L)											
			C. albicans (n = 60)			*C. glabrata* (n = 21)			*C. parapsilosis* (n=14)			*C. tropicalis* (n = 13)		
Incubation time (h)	Drug	pH	MIC_{90}	MIC_{50}	MIC range	MIC_{90}	MIC_{50}	MIC range	MIC_{90}	MIC_{50}	MIC range	MIC_{90}	MIC_{50}	MIC range
24	CD101	7	0.06	0.03	0.016–0.125	0.125	0.125	0.06–2	2[a]	1	0.008–2	0.125	0.06	0.03–0.125
		4	0.25	0.125	0.06–0.5	0.5	0.5	0.125–2	2[b]	1	0.008–2	0.5	0.25	0.25–0.5
	FLC	7	2	0.25	0.125–64	16	2	0.5–64	16[c]	0.5	0.125–16	2	1	0.25–16
		4	8	0.25	0.25 to >64	64	8	2 to >64	8[d]	2	0.125–32	8	1	1–8
	ITC	7	0.06	0.016	0.008–2	1	0.125	0.03–4	0.03[e]	0.03	0.008–0.03	0.125	0.06	0.016–1
		4	0.125	0.016	0.008–2	1	0.5	0.03–2	0.016[f]	0.016	0.008–0.016	0.125	0.125	0.03–0.25
	CAS	7	0.5	0.25	0.25–1	1	0.5	0.5–2	1[g]	0.5	0.008–2	1	0.5	0.125–1
		4	0.5	0.5	0.25–0.5	1	0.5	0.125–1	1	1	0.008–1	1	0.5	0.125–1
	MCF	7	0.008	0.008	0.008–0.03	0.008	0.008	0.008–1	1	0.5	0.008–1	0.016	0.008	0.008–0.03
		4	0.125	0.03	0.008–0.25	0.016	0.008	0.008–0.5	0.5	0.5	0.008–0.5	0.06	0.03	0.008–0.06
	ANF	7	0.008	0.008	0.008–0.06	0.03	0.03	0.008–1	2[h]	0.5	0.008–2	0.03	0.008	0.008–0.06
		4	0.03	0.016	0.016–0.125	0.125	0.06	0.03–1	1	0.5	0.008–1	0.125	0.06	0.008–0.125
48	CD101	7	0.125	0.03	0.016–1	0.25	0.125	0.06–2	4	2	0.5–4	0.125	0.125	0.06–0.5
		4	0.5	0.5	0.125–1	1	1	0.25–2	2	2	1–2	2	0.5	0.5–2
	FLC	7	16	0.25	0.25 to >64	>64	8	1 to >64	32	2	0.5–64	64	2	1 to >64
		4	>64	1	0.5 to >64	>64	>64	2 to >64	>64	4	2 to >64	>64	8	2 to >64
	ITC	7	0.125	0.016	0.008 to >4	>4	0.5	0.03 to >4	0.125	0.03	0.016–0.25	0.5	0.125	0.03 to >4
		4	0.5	0.03	0.016 to >4	>4	2	0.03 to >4	0.125	0.03	0.016–0.25	0.5	0.125	0.125 to >4
	CAS	7	1	0.5	0.25–2	2	1	0.5–2	2	2	0.5–2	2	1	0.25–2
		4	1	1	0.25–4	2	1	1–4	4	2	1–4	2	1	0.5–4
	MCF	7	0.03	0.008	0.008–0.125	0.016	0.008	0.008–1	2	1	0.016–2	0.03	0.016	0.008–0.06
		4	0.25	0.03	0.008–0.5	0.03	0.008	0.008–1	1	0.5	0.008–2	0.06	0.03	0.03–0.5
	ANF	7	0.03	0.008	0.008–0.125	0.06	0.03	0.016–2	4	2	0.125–4	0.06	0.016	0.008–0.125
		4	0.06	0.06	0.016–0.5	0.25	0.125	0.03–2	2	1	0.125–2	0.5	0.125	0.03–1

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, [g]n=13 and [h]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,[6] 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, the azole drug class is not adequate for infections caused by non-*albicans Candida* spp. and azole-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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Transparency declarations

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References

1 Foxman B, Muraglia R, Dietz JP *et al*. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis* 2013; **17**: 340–5.

2 Goncalves B, Ferreira C, Alves CT *et al*. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. *Crit Rev Microbiol* 2016; **42**: 905–27.

3 de Leon EM, Jacober SJ, Sobel JD *et al*. Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *BMC Infect Dis* 2002; **2**: 1.

4 Richter SS, Galask RP, Messer SA *et al*. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol* 2005; **43**: 2155–62.

5 Sobel JD. Recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* 2016; **214**: 15–21.

6 Danby CS, Boikov D, Rautemaa-Richardson R *et al*. Effect of pH on *in vitro* susceptibility of *Candida glabrata* and *Candida albicans* to 11 antifungal agents and implications for clinical use. *Antimicrob Agents Chemother* 2012; **56**: 1403–6.

7 Guaschino S, De Seta F, Sartore A *et al*. Efficacy of maintenance therapy with topical boric acid in comparison with oral itraconazole in the treatment of recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* 2001; **184**: 598–602.

8 Sobel JD, Chaim W, Nagappan V *et al*. Treatment of vaginitis caused by *Candida glabrata*: use of topical boric acid and flucytosine. *Am J Obstet Gynecol* 2003; **189**: 1297–300.

9 Linhares IM, Summers PR, Larsen B *et al*. Contemporary perspectives on vaginal pH and lactobacilli. *Am J Obstet Gynecol* 2011; **204**: 120.e1–5.

10 Perlin DS. Echinocandin resistance, susceptibility testing and prophylaxis: implications for patient management. *Drugs* 2014; **74**: 1573–85.

11 Wang JL, Chang CH, Young-Xu Y *et al*. Systematic review and meta-analysis of the tolerability and hepatotoxicity of antifungals in empirical and definitive therapy for invasive fungal infection. *Antimicrob Agents Chemother* 2010; **54**: 2409–19.

12 Mølgaard-Nielsen D, Svanström H, Melbye M *et al*. Association between use of oral fluconazole during pregnancy and risk of spontaneous abortion and stillbirth. *JAMA* 2016; **315**: 58–67.

13 Krishnan BR, James KD, Polowy K *et al*. CD101, a novel echinocandin with exceptional stability properties and enhanced aqueous solubility. *J Antibiot (Tokyo)* 2016; doi:10.1038/ja.2016.89.

14 Castanheira M, Messer SA, Rhomberg PR *et al*. Activity of a long-acting echinocandin (CD101) and comparator antifungal agents tested against contemporary invasive fungal isolates. In: *Abstracts of the Fifty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2015*. M–849. American Society for Microbiology, Washington, DC, USA.

15 Pfaller MA, Messer SA, Rhomberg PR *et al*. Activity of a long-acting echinocandin, CD101, determined using CLSI and EUCAST reference methods, against *Candida* and *Aspergillus* spp., including echinocandin- and azole-resistant isolates. *J Antimicrob Chemother* 2016; **71**: 2868–73.

16 Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts—Third Edition: Approved Standard M27-A3*. CLSI, Wayne, PA, USA, 2008.

17 Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Fourth Informational Supplement M27-S4*. CLSI, Wayne, PA, USA, 2012.

18 Pfaller M, Messer SA, Rhomberg PR *et al*. Activity of a long-acting echinocandin (CD101) and comparator antifungal agents tested against contemporary worldwide invasive fungal isolates. In: *Abstracts of IDWeek 2016, New Orleans, LA, USA, 2016. Open Forum Infect Dis* 2016; **3** Suppl 1: 1625.

19 Marr KA, Rustad TR, Rex JH *et al*. The trailing end point phenotype in antifungal susceptibility testing is pH dependent. *Antimicrob Agents Chemother* 1999; **43**: 1383–6.

20 Liu W, Zhang X, Liu Z *et al*. Impact of pH on the antifungal susceptibility of vaginal *Candida albicans*. *Int J Gynaecol Obstet* 2011; **114**: 278–80.

21 Miesel L, Huang H-H, You W-T *et al*. Novel echinocandin CD101 gel formulation is highly effective in eradicating *Candida albicans* in a rat model of vulvovaginal candidiasis. In: *Abstracts of the Fifty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2015*. F-755. American Society for Microbiology, Washington, DC, USA.