F-752

Evaluation of CD101 with Echinocandin and Azole Comparators Against *Candida* spp. Isolated from Patients with Vulvovaginal Candidiasis (VVC)

Ken Bartizal, Ph.D.
Cidara Therapeutics, Inc.
6310 Nancy Ridge Drive, Suite 101
San Diego, CA 92121
kbartizal@cidara.com

J.D. Sobel,[1] D. Boikov,[1] K. Bartizal,[2] K. James[2]

[1]Wayne State University, Detroit, MI, USA; [2]Cidara Therapeutics, Inc., San Diego, CA, USA

ABSTRACT

Background: Fungicidal activity against *Candida* spp. makes echinocandins appealing for treatment of VVC, particularly refractory or azole-resistant infections. However, the instability and infusion delivery of approved agents are barriers to their utilization. Unlike currently marketed echinocandins, CD101 has stability suitable for topical formulations. We evaluated CD101, other echinocandins, and therapeutic azoles against VVC isolates in vitro at a vaginal pH to assess whether CD101 has sufficient potency to treat VVC, including infections caused by azole-resistant pathogens.

Methods: Susceptibility of 108 vaginal isolates of *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were evaluated using a broth microdilution method according to CLSI guidelines. Azole-susceptible and azole-resistant strains were tested. MICs for caspofungin (CAS), micafungin (MFN), anidulafungin (ANID), CD101, fluconazole (FLU), and itraconazole (ITR) were obtained after incubation at pH 7 and pH 4 for 24 and 48 h at 35ºC. MICs were read as the lowest antifungal concentrations leading to 80% growth reduction compared to non-treatment wells.

Results: From 24 to 48 h, MICs shifted upward 2- to 4-fold for each echinocandin and at least that much for the azoles. Multiple isolates from *C. albicans*, *C. glabrata*, and *C. tropicalis* had MICs >4 for the two azoles, but none had MICs >4 for the echinocandins. A summary of MIC_{90} values for azole-sensitive and azole-resistant *Candida* spp. at pH 7 and pH 4 for each test article after 48 h is presented in the table.

	MIC_{90} (µg/mL) at pH 7/4			
	C. albicans (60)	*C. glabrata* (21)	*C. parapsilosis* (14)	*C. tropicalis* (13)
CD101	0.125/0.5	0.25/1	4/2	0.125/2
FLU	16/>64	>64/>64	32/>64	64/>64
ITR	0.125/0.5	>4/>4	0.125/0.125	0.5/0.5
CAS	1/1	2/2	2/4	2/2
MFN	0.03/0.25	0.016/0.03	2/1	0.03/0.06
ANID	0.03/0.06	0.06/0.25	4/2	0.06/0.5

Conclusions: VVC isolates resistant to FLU and ITR were not cross-resistant to echinocandins. The echinocandins showed little MIC shift as a function of pH. Of the echinocandins, CAS generally had the highest MIC values; MFN had the lowest. CD101 has sufficient potency and stability at low pH to be evaluated in cream and gel formulations as a potential topical treatment for VVC, including refractory and azole-resistant infections.

INTRODUCTION

Since their introduction in 2001, the echinocandins have become increasingly important in the treatment of fungal infections, especially those caused by *Candida* spp. However, the three echinocandins currently available are all administered by daily intravenous infusion. This limitation is problematic to both doctors and patients. There are patients for whom an echinocandin would be the ideal choice in terms of activity against the invasive pathogen, but the limitations on the route and frequency of delivery preclude the utilization of current members of this class.

Two such maladies are azole-resistant and recurrent vulvovaginal candidiasis (VVC), over 90% of which are caused by *C. albicans* or *C. glabrata*.[1] Based on observations from systemic *Candida* infections, the echinocandins should have many advantages over azoles for the treatment of VVC: cidal vs. static, less development of resistance, fewer drug interactions, and better overall safety. Unfortunately, the route of delivery for the current echinocandins is not practical for treatment of VVC, and the stability/solubility properties of the currently available echinocandins are major barriers to a more suitable dosage form.

CD101 is a novel echinocandin that has demonstrated activity comparable to marketed echinocandins against candidemia isolates in a 2014 SENTRY study[2] and has displayed an uncommonly long half-life in multiple species.[3,4] It has also demonstrated stability and solubility suitable for topical formulations.[5] We evaluated CD101, other echinocandins, and therapeutic azoles against VVC isolates in vitro at a vaginal pH to assess whether CD101 has sufficient potency to treat VVC, including infections caused by azole-resistant pathogens.

METHODS

Vaginal isolates of *Candida* spp. were obtained from the Wayne State Vaginitis Clinic organism bank and were comprised of

- *C. albicans* (60 total, 10 fluconazole-resistant)
- *C. glabrata* (21 total, 11 fluconazole-resistant)
- *C. parapsilosis* (14 total, 7 fluconazole-resistant)
- *C. tropicalis* (13)

Isolates were plated on CHROMagar to verify purity; plates were incubated for 48 h at 37ºC. A single colony was resubcultured on Sabouraud Dextrose agar and incubated for 24 h at 35ºC. Susceptibility testing was performed at pH 7 and 4 using the broth microdilution method described in CLSI document M27-A3.[6] A MOPS (morpholinepropane-sulfonic acid) buffer solution was used for pH adjustment. Fluconazole was tested at a range of 0.125 – 64 µg/mL; all other antifungal agents were tested at 0.008 – 4 µg/mL. A yeast inoculum (~1.5 x 10^3 CFU/mL) in RPMI 1640 medium was added to each well, and trays were incubated for 24 h and 48 h at 35ºC. MICs were read as the lowest antifungal concentration with 80% growth reduction compared to growth in the antifungal-free growth well for all test articles.

RESULTS

Table 1. MIC_{90} values for CD101 and comparators against 108 VVC clinical isolates at pH 7/4 read at 24 h.

	MIC_{90} (µg/mL) at pH 7/4 at 24 h			
	C. albicans (60)	*C. glabrata* (21)	*C. parapsilosis* (14)	*C. tropicalis* (13)
CD101	0.06/0.25	0.125/0.5	2/2	0.125/0.5
FLU	8/8	16/64	i.g.*/8	2/8
ITR	0.06/0.125	1/1	0.03/0.016	0.125/0.125
CAS	0.5/0.5	1/1	1/1	1/1
MFN	0.008/0.125	0.008/0.016	1/0.5	0.016/0.06
ANID	0.008/0.03	0.03/0.125	2/1	0.03/0.125

* i.g. = Insufficient growth. Only 7 of the 14 organisms exhibited sufficient growth to obtain MIC values. The range was from 0.125 to 16 µg/mL.

RESULTS (cont'd)

Table 2. MIC_{90} values for CD101 and comparators against 108 VVC clinical isolates at pH 7/4 read at 48 h.

	MIC_{90} (µg/mL) at pH 7/4 at 48 h			
	C. albicans (60)	*C. glabrata* (21)	*C. parapsilosis* (14)	*C. tropicalis* (13)
CD101	0.125/0.5	0.25/1	4/2	0.125/2
FLU	16/>64	>64/>64	32/>64	64/>64
ITR	0.125/0.5	>4/>4	0.125/0.125	0.5/0.5
CAS	1/1	2/2	2/4	2/2
MFN	0.03/0.25	0.016/0.03	2/1	0.03/0.06
ANID	0.03/0.06	0.06/0.25	4/2	0.06/0.5

Table 3. Distribution of MIC values for CD101 and approved azole antifungals against 60 *C. albicans* and 21 *C. glabrata* VVC clinical isolates at pH 7 and 4 read at 24 h and 48 h.

MIC (µg/mL)	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	>4
***C. albicans* 24 h**											
CD101 pH = 7		5	43	10	2						
pH = 4				1	33	23	3				
FLU pH = 7					25	19	7	1	2		6
pH = 4						35	13	2	2	1	7
ITR pH = 7	21	20	9	3		2	2		1		
pH = 4	8	24	13	8	2	2	2		1		
***C. albicans* 48 h**											
CD101 pH = 7		2	29	16	9	1	1	2			
pH = 4					6	16	34	4			
FLU pH = 7					3	37	8	2	1		9
pH = 4							27	13	5	1	14
ITR pH = 7	6	34	8		6	2	2		1		1
pH = 4		10	23	10	6	3	3	1			4
***C. glabrata* 24 h**											
CD101 pH = 7				3	17				1		
pH = 4					2	3	15		1		
FLU pH = 7							4	2	5	2	8
pH = 4									5	3	13
ITR pH = 7			4	2	6	3	3	2		1	
pH = 4			1	2		2	9	6	1		
***C. glabrata* 48 h**											
CD101 pH = 7				2	16	1	1		1		
pH = 4						2	8	9	2		
FLU pH = 7								3	4	2	12
pH = 4									1	2	18
ITR pH = 7			1	1	1	5	8		2		3
pH = 4			1	1	1		2	4	2	2	8

RESULTS (cont'd)

Table 4. MIC values for CD101 and comparators against 10 VVC clinical isolates of fluconazole-resistant *C. albicans* at pH 7/4 read at 48 h.

Isolate	MIC (µg/mL) at pH 7/4 at 48 h					
	CD101	FLU	ITR	CAS	MFN	ANID
1	0.03/0.5	16/>64	0.125/0.125	0.5/0.25	0.008/0.016	0.008/0.03
2	0.25/0.125	16/32	0.25/0.25	0.5/1.0	0.016/0.016	0.03/0.03
3	0.03/0.5	16/>64	0.5/0.5	0.5/0.5	0.008/0.016	0.008/0.03
4	0.06/0.25	8/>64	0.03/0.125	0.5/1.0	0.008/0.03	0.008/0.06
5	0.125/0.25	>64/>64	0.25/>4	2/4	0.06/0.016	0.016/0.125
6	0.03/0.5	0.25/>64	0.016/>4	1/1	0.008/0.03	0.008/0.06
7	0.03/0.5	16/32	0.5/0.5	0.5/1	0.06/0.25	0.008/0.06
8	0.125/0.25	32/>64	2/>4	0.5/0.5	0.008/0.06	0.008/0.06
9	1/1	>64/>64	>4/>4	2/1	0.06/0.125	0.125/0.25
10	0.125/0.25	16/64	0.125/0.25	0.5/1	0.008/0.06	0.008/0.06

CONCLUSIONS

- VVC isolates resistant to FLU were often resistant to ITR. This is particularly evident with *C. glabrata* but also evident in some *C. albicans* isolates.
- VVC isolates resistant to FLU and ITR were not resistant to the echinocandins.
- Among the echinocandins, MFN typically had the lowest MIC values; CAS had the highest.
- Any MIC shift as a function of pH was slight, if present.
- The potency and stability of CD101 at low pH are sufficient for testing of topical formulations for treatment of VVC, including azole-resistant infections.

REFERENCES

1. Sobel, JD. *Drugs*. 2003;63:1059-1066.
2. ICAAC 2015, Poster M-849.
3. ICAAC 2014, Poster A-693.
4. ICAAC 2014, Poster A-694.
5. ICAAC 2014, Poster F-1592.
6. CLSI. CLSI document M27–A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

ACKNOWLEDGEMENTS

This work was funded by a research grant from Cidara Therapeutics, Inc.