The Novel Antifungal VL-2397 Demonstrates Efficacy in an In Vivo Model of Invasive Candidiasis Caused by Wild-Type and Multi-Drug Resistant *Candida glabrata*

N.P. Wiederhold, L.K. Najvar, R. Jaramillo, M. Olivo, G. Catano, S.M. Sullivan, A.M. Hopkins, L.R. Smith, T.F. Patterson
UT Health San Antonio, Vical Incorporated,
South Texas Veterans Health Care System

Contact Information:
N.P. Wiederhold
UTHSCSA
7703 Floyd Curl Dr., MC 7750
San Antonio, TX 78229
Tel: (210) 567-4086
e-mail: wiederholdn@uthscsa.edu

ABSTRACT

Background: *Candida glabrata* is a major cause of invasive candidiasis in many institutions. Increased rates of antifungal resistance in this species have been observed in many institutions in the U.S., with many isolates demonstrating multi-drug resistance. VL-2397 is an investigational antifungal that is a structural relative of the siderophore ferrichrome with potent activity against various fungi, including *C. glabrata*. Our objective was to evaluate the in vivo efficacy of VL-2397 against invasive candidiasis caused by *C. glabrata*.
Methods: A wild-type (fluconazole [FLU] and caspofungin [CAS] MICs of 2 & 0.125 µg/ml) and resistant isolate (FLU & CAS MICs 64 & 1 µg/ml) were used. Neutropenic ICR mice (N=10/group) were inoculated intravenously, and treatment with vehicle control, VL-2397 (2 mg/kg, 4 mg/kg, or 8 mg/kg SC twice daily), FLU 20 mg/kg PO once daily, or CAS 1 mg/kg IP once daily was initiated 24 hours post-inoculation. Treatment continued for 7 days, and kidneys were collected on day 8 for analysis of fungal burden by colony-forming units (CFU/g). Differences in fungal burden were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons.
Results: VL-2397 demonstrated in vivo efficacy against invasive candidiasis caused by both the wild-type and resistant isolates. Against the wild-type isolate, each dose of VL-2397 resulted in a significant reduction in CFUs (range mean 3.62 – 4.13 $\log_{10}$ CFU/g) versus control (5.24 $\log_{10}$ CFU/g; $p \leq 0.01$). CAS also resulted in a significant reduction in CFUs (3.67 $\log_{10}$ CFU/g; $p \leq 0.01$) compared to control against the wild-type isolate, although FLU did not (4.85 $\log_{10}$ CFU/g). Against the resistant isolate, significant reductions in CFUs were also observed with each dose of VL-2397 (4.30 – 5.14 $\log_{10}$ CFU/g) compared to control (6.63 $\log_{10}$ CFU/g; $p \leq 0.01$). Treatment with CAS did not result in a significant reduction in CFUs against the resistant isolate, although treatment with FLU did (5.34 $\log_{10}$ CFU/g; $p < 0.01$). VL-2397 was also well tolerated in mice at each dose level.
Conclusions: VL-2397 demonstrated in vivo efficacy in this experimental model of invasive candidiasis caused by *C. glabrata*. Its efficacy, as measured by reductions in kidney fungal burden, was evident against infections caused by both wild-type and multi-drug resistant isolates. These results demonstrate the potential for VL-2397 as therapy against invasive infections caused by *C. glabrata*.

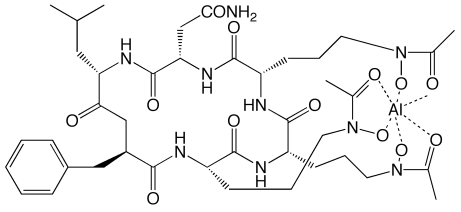
BACKGROUND & OBJECTIVE

- *Candida* species are currently the fourth most common cause of nosocomial bloodstream infections in the United States, and the second most prevalent species associated with invasive infection is *Candida glabrata*.
- Current treatment strategies for invasive infections caused by this pathogen include the use of the azoles, amphotericin B, or the echinocandins. Although effective, each of these classes has drawbacks that may limit clinical responses.
- In addition, recent epidemiologic studies have also demonstrated that fluconazole and echinocandin resistance in *C. glabrata* is increasing at many centers in the U.S.
- VL-2397 is an investigational antifungal that is a structural relative of the siderophore ferrichrome with potent activity against various fungi, including *C. glabrata*.
- Our **objective** was to evaluate the in vivo efficacy of VL-2397 as therapy against invasive candidiasis caused by *C. glabrata* in an established neutropenic murine model. The in vivo efficacy of this agent was compared to that of fluconazole (FLU) and caspofungin (CAS), and both wild-type (susceptible) and resistant isolates were used.

METHODS

- Both wild-type (05-761; FLU MIC 2 µg/ml, CAS 0.125 µg/ml) and multi-drug resistant (05-62; FLU MIC 64 µg/ml, CAS 0.5 µg/ml) *C. glabrata* clinical isolates were used.
- Outbred male ICR mice were rendered neutropenic with 5-fluorouracil 150 mg/kg IV x 1 on the day prior to inoculation.
- Mice were infected intravenously via the lateral tail vein at an inoculum level of ~1.0 x 10^8 cells/animal.
- Antifungal therapy began 1 day post-inoculation and consisted of the following groups (N = 10 mice per group per arm):
  1) Untreated controls, 2) VL-2397 at 2, 4, or 8 mg/kg by SC BID, 3) FLU 20 mg/kg PO QD, 3) CAS 1 mg/kg IP QD
- Therapy continued through day 7. Kidneys were collected on day 8, and fungal burden was assessed by colony-forming units (CFU/g tissue).
- Differences in kidney and spleen fungal burden (CFU/g) among the groups were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons. A p-value ≤ 0.05 was considered statistically significant.

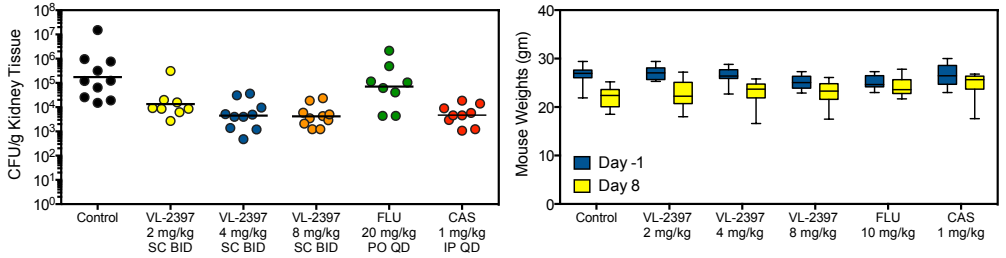
Figure 1. Structure of VL-2397



This work is funded with Federal funds from the NIH/NIAID/DMID Under Contract No. HHSN272201100038I (Task Order A93).

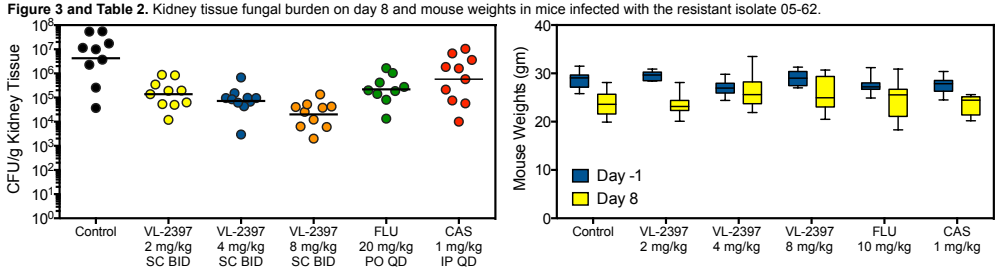
RESULTS

Figure 2 and Table 1. Kidney tissue fungal burden on day 8 and mouse weights in mice infected with the wild-type isolate 05-761.



Group	Untreated Control	VL-2397 2 mg/kg	VL-2397 4 mg/kg	VL-2397 8 mg/kg	FLU 20 mg/kg	CAS 1 mg/kg
Mean (SD) CFU/g	5.24 (0.93)	4.13 (0.61) *p = 0.0142	3.65 (0.59) *p < 0.0001	3.62 (0.43) *p < 0.0001	4.85 (0.92)	3.67 (0.43) *p = 0.0001
Mean (SD) Weight Day 1	26.6 (1.93)	27.1 (1.35)	26.5 (1.67)	25.1 (1.38)	25.0 (1.41)	26.5 (2.25)
Mean (SD) Weight Day 8	22.0 (2.26) **p = 0.0001	22.7 (2.87) **p = 0.0003	22.94 (2.62) **p = 0.002	23.0 (2.46) **p = 0.037	21.2 (1.85)	24.6 (2.83)

Figure 3 and Table 2. Kidney tissue fungal burden on day 8 and mouse weights in mice infected with the resistant isolate 05-62.



Group	Untreated Control	VL-2397 2 mg/kg	VL-2397 4 mg/kg	VL-2397 8 mg/kg	FLU 20 mg/kg	CAS 1 mg/kg
Mean (SD) CFU/g	6.63 (1.06)	5.14 (0.58) *p = 0.0011	4.86 (0.58) *p < 0.0001	4.30 (0.55) *p < 0.0001	5.34 (0.61) *p = 0.0082	5.76 (0.99)
Mean (SD) Weight Day 1	28.6 (1.68)	29.5 (0.95)	26.9 (1.59)	29.0 (1.57)	27.5 (1.67)	27.6 (1.69)
Mean (SD) Weight Day 8	23.8 (2.62) **p = 0.0001	23.5 (2.16) **p < 0.0001	26.2 (3.35)	25.8 (3.68)	24.7 (3.75)	23.6 (2.09) **p = 0.0002

CONCLUSIONS & FUTURE DIRECTIONS

- VL-2397 demonstrated in vivo efficacy in this experimental model of invasive candidiasis caused by *C. glabrata*.
- Its efficacy, as measured by reductions in kidney fungal burden, was evident against infections caused by both wild-type and multi-drug resistant isolates. VL-2397 was also well tolerated by the mice at each dosage level.
- These results demonstrate the potential for VL-2397 as therapy against invasive infections caused by *C. glabrata*.