The Clinical Antifungal Agent VT-1161 is Orally Efficacious in a Murine Model of *C. albicans* Vaginitis

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BACKGROUND

The azole class of drugs inhibits fungal CYP51. Although these agents provide significant clinical benefit, they all cause side-effects that stem from inhibition of human CYP enzymes (Nivoix et al., 2008). For example, each has numerous drug-drug interaction (DDI) warnings cited within their package inserts (see table below). VT-1161, a novel inhibitor of fungal CYP51, is currently in Phase I studies. Based on its *in vitro* antifungal potency and high selectivity, it has potential of being a best-in-class CYP51 inhibitor to treat *Candida* spp. infections.

Agent	*C. albicans* MIC90 (μg/ml)	huCYP IC_{50} Values (μM)			Sel. Ratio[1]	# DDI[2]
		2C9	2C19	3A4		
VT-1161	≤ 0.03[3]	100	72	65	≥1150	TBD
Fluconazole	0.5[3]	34	13	31	8	37
Voriconazole	0.06[4]	10	10	13	59	35
Itraconazole	0.25[4]	>60	56	0.07	<1	40
Posaconazole	0.125[5]	25	7.2	0.07	<1	38

[1]Selectivity ratio = most sensitive huCYP IC50 value divided by MIC90 value (converted to molarity). [2]Drug-drug interaction warnings cited in package insert. [3]Fothergill et al., 2010. [4]Marco et al., 1998. [5]Cacciapuoti et al., 2000.

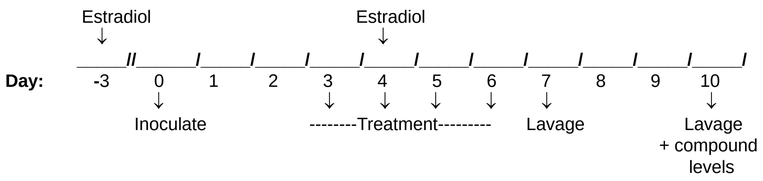
Although fluconazole (FLU) is largely efficacious in treating vulvovaginal candidiasis (Cha and Sobel, 2004), more effective treatments are needed for recurrent infections (Sobel et al., 2004), infections caused by resistant strains of *C. albicans* (Ge et al., 2010), and those caused by *C. glabrata* (Goswami et al., 2006). In addition, FLU is prone to DDIs. Based on the excellent oral efficacy demonstrated in numerous fungal infection models, VT-1161 was tested in a *Candida* vaginitis model.

MATERIALS AND METHODS

Female CBA/J mice, 8-10 weeks old and weighing 20 g were obtained from National Institutes of Health (NCI, Frederick, MD). *Candida albicans* ATCC 3153A strain was obtained from the American type Culture Collection (Rockville, MD). VT-1161-000-M and fluconazole was supplied by Viamet Pharmaceuticals, Inc. (Morrisville, NC).

Murine vaginitis model. A condition of pseudoestrus is required to obtain a persistent vaginal yeast infection in rodents. Mice were injected subcutaneously (under manual restraining) with 1.0 mg estradiol valerate dissolved in sesame oil 3 days prior to and 4 days after vaginal inoculation. On the day prior to inoculation, a blastospore culture of *C. albicans* 3153A was prepared. On the day of infection, blastospores were collected and washed once with phosphate-buffered saline (PBS) and resuspended at 2.5 x 10^6/ml in PBS for an inoculum of 5x10^4 cell/ 0.02 ml PBS for each infection. For inoculation, animals were anesthetized "to effect" by isoflurane inhalation. To anesthetized animals, 5 x 10^4 *C. albicans* 3153A blastospores in 20 μl PBS was introduced into the vagina, using a pipetman. Once-daily oral gavage treatments of 4, 10, and 25 mg/kg VT-1161, 25 mg/kg FLU, or vehicle (20% cremaphor EL) began on day 3 post-inoculation and continued through day 6 (10 animals per group). On day 7, animals were anesthetized and the vagina cavity was lavaged with 100 ul of phosphate buffered saline (PBS). The lavage fluid was examined microscopically for *Candida* and serially diluted and plated for enumeration of organisms. On day 10, the animals were bled retro-orbitally, sacrificed, and similarly lavaged with subsequent processing for evaluation of vaginal fungal burden. Plasma was prepared from collected blood and stored for drug analysis. Additionally, vaginal tissue was isolated and quick-frozen in a cryo-tube for future homogenization and drug analysis.

Schedule of Treatment



Quantification of drug levels. Compound levels were measured in plasma and vaginal tissue by LC/MS-MS using internal standards and standard curves (OpAns, LLC, Durham, NC).

RESULTS

Figure 1. Vaginal fungal burden on days 7 and 10 post-infection.

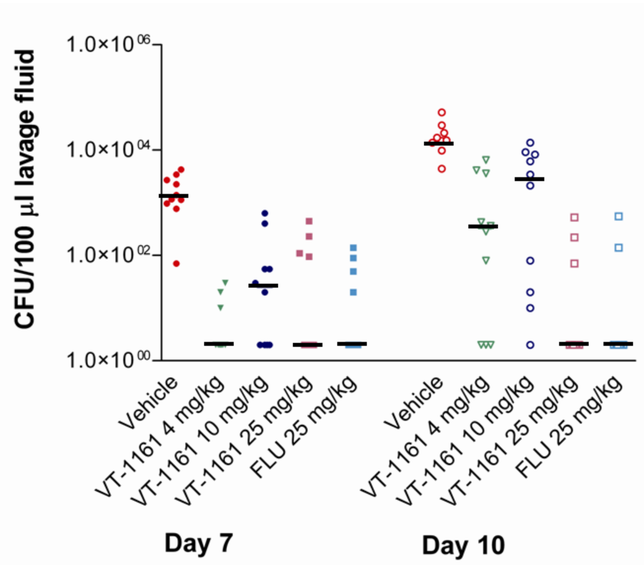


Figure 1. Colony forming units (CFU) in vaginal lavage samples from all mice in each treatment group measured either 7 or 10 days post-infection (1 or 4 days after last dose). Hashes represent the median value of a particular dose group. All treatments resulted in median CFU values that were statistically less than vehicle-treated groups ($p < 0.0001$, except for 10 mg/kg VT-1161 group vs. control, $p = 0.0002$). No treatment group was statistically different from any other on day 7. The 25 mg/kg groups of both VT-1161 and FLU were statistically different than 10 mg/kg VT-1161 group on day 10 ($p = 0.004$); neither was different than 4 mg/kg VT-1161 ($p = 0.054$).

Table 1. Clearance of vaginal fungal burden.

Treatment	Median CFU/100 μl lavage fluid		# Animals w/ undetectable fungus/Total # animals	
	Day 7	Day 10	Day 7	Day 10
Vehicle	1290	16125	0/10	0/10
4 mg/kg VT-1161	0	325	7/10	3/10
10 mg/kg VT-1161	25	2790	4/10	1/10
25 mg/kg VT-1161	0	0	6/10	7/10
25 mg/kg FLU	0	0	5/9	7/9

Table 2. Drug levels in plasma and vaginal tissue.

Plasma and tissue samples were taken on day 10, 4 days after last dose. VT-1161 levels were dose proportional in both plasma and vaginal tissue samples. Levels in both tissues indicated that VT-1161 fully equilibrated from blood into vaginal tissue.

Treatment	Plasma, μM (SD)	Vagina, μM[1] (SD)
4 mg/kg VT-1161	1.6 (0.3)	2.9 (0.6)
10 mg/kg VT-1161	7.6 (2.5)	11 (2)
25 mg/kg VT-1161	22 (7)	30 (6)
25 mg/kg Fluconazole	0.13 (0.07)	0.16 (0.9)

[1]nmol/g tissue ~ μM, assuming 1 g ~ 1 ml.

CONCLUSIONS

- Oral VT-1161 was highly efficacious in this stringent murine vaginitis model (with treatment beginning 3 days after infection).
  - At day 7, low dose (4 mg/kg) VT-1161 was as efficacious as 25 mg/kg VT-1161 or FLU in the reduction of vaginal fungal burden and number of mice cleared of *C. albicans*.
  - VT-1161 at 25 mg/kg was as effective as standard-of-care FLU in the reduction of vaginal fungal burden on day 7 or 10.
  - Oral VT-1161 provided plasma and vaginal tissue levels that were greater than FLU when measured 4 days after last dose.
- The clinical agent VT-1161 holds promise as a vulvovaginal candidiasis treatment given its good oral *in vivo* efficacy and potential for fewer drug-drug interactions compared to the azole class of drugs.

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