VT-1598 inhibits the *in vitro* growth of mucosal *Candida* isolates and protects against oropharyngeal candidiasis in IL-17 deficient mice

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Background

Patients with inherited syndromes of chronic mucocutaneous candidiasis (CMC) frequently develop azole resistance, which makes treatment of their fungal infections challenging. VT-1598 belongs to a new class of antifungals that specifically target fungal cytochrome CYP51 without significant interaction with the human homolog. Drugs with similar structure, including VT-1161 and VT-1129, have been shown to be effective in the treatment of azole-resistant fungal strains both *in vitro* and *in vivo*. The long half-life, efficacy even when dosed once weekly, and decreased drug-drug interaction potential makes this new class of antifungal drugs attractive for patients with CMC who require long-term, often lifelong, antifungal prophylaxis. VT-1598 has been granted orphan drug designation and FDA Fast Track Designation for the treatment of coccidioidomycosis (Valley Fever). However, the effect of VT-1598 in the treatment of oral candidiasis has not been evaluated.

Methods

1. **In vitro susceptibility analysis:** The *in vitro* susceptibility of VT-1598 and fluconazole by CLSI broth microdilution (M27-S4) was tested against 28 mucosal *Candida* strains obtained from patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. The 24-hour minimum inhibitory concentration (MIC) at which 50% or 90% of strains were inhibited (MIC_{50} or MIC_{90}, respectively) was determined.
2. **Pharmacokinetic profiles:** *Act1*$^{-/-}$ mice (lacking IL-17 signaling) were used to determine the pharmacokinetics of VT-1598. The drug was administered via oral gavage at 20mg/kg on 4 consecutive days, and organs were harvested 1 day following the last administration. The concentration of each drug was determined using LC-MS/MS with electrospray ionization.
3. **In vivo efficacy analysis:** One oral *Candida* strain (Y72) that was found to be susceptible to both VT-1598 and fluconazole was chosen to determine the effectiveness of the drugs in the clearance of *Candida* from the oral mucosa of *Act1*$^{-/-}$ mice, and compared with another oral *Candida* strain Y37, which is fluconazole-resistant. Mice were sublingually infected with Y72 or Y37. 18 hours post-infection and once a day thereafter for 4 total administration, mice were given vehicle (1% carboxymethycellulose), fluconazole (25mg/kg), or VT-1598 (20mg/kg). One day following the last administration of the drugs (day 5 post-infection), tongues were harvested and the number of *Candida* was quantified.
4. **Dosage and washout experiments:** The *Candida* strain Y72 was used to determine the dose-dependency of VT-1598-mediated *Candida* clearance and the effectiveness of VT-1598 administration for long-term protection against the fluconazole-susceptible strain Y72. To determine the dose-dependency of VT-1598-mediated protection, mice were infected with Y72. 18 hours post-infected and once a day thereafter for 4 total administrations, mice were given vehicle, or VT-1598 (3.2mg/kg or 8mg/kg). One day following the last dose, tongues were harvested and the number of *Candida* quantified. Alternatively, for a washout experiment, mice were sublingually infected with Y72 and treated as in (3), but tongues were harvested at day 14 post-infection and *Candida* quantified.

Results

Table 1: *In vitro* activity of VT-1598 against *Candida* isolates from patients with chronic mucocutaneous candidiasis

Isolate	Species	MIC (œg/mL)		Isolate	Species	MIC (œg/mL)	
		VT1598	Fluconazole			VT1598	Fluconazole
Y31	*C. albicans*	0.12500	32	Y79	*C. albicans*	0.03125	0.5
Y37	*C. albicans*	0.12500	64	Y82	*C. utilis*	0.12500	4
Y42	*C. albicans*	0.06250	0.12	Y83	*C. albicans*	0.12500	2
Y43	*C. albicans*	0.03125	0.25	Y84	*C. albicans*	0.06250	0.5
Y46	*C. glabrata*	0.03125	8	Y88	*C. albicans*	0.06250	0.5
Y47	*C. albicans*	0.03125	0.25	Y92	*C. dubliensis*	0.06250	0.25
Y48	*C. albicans*	0.06250	0.5	Y93	*C. albicans*	0.06250	0.5
Y49	*C. glabrata*	0.06250	128	Y107	*C. albicans*	0.06250	0.5
Y51	*C. albicans*	0.12500	4	Y111	*C. albicans*	0.06250	0.5
Y54	*C. glabrata*	0.06250	128	Y125	*C. krusei*	0.125	16
Y55	*C. albicans*	0.12500	16	Y152	*C. albicans*	0.0625	0.12
Y57	*C. albicans*	0.03125	4	Y153	*C. albicans*	0.125	0.12
Y72	*C. albicans*	0.06250	0.5	Y160	*C. albicans*	0.125	1
Y75	*C. albicans*	0.06250	1	Y162	*C. albicans*	0.0625	1

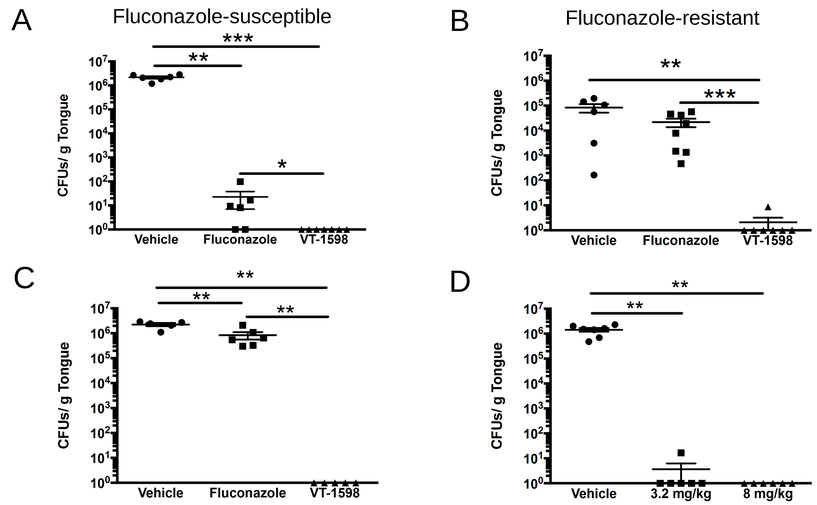
Total number of tested isolates	MIC_{50} (œg/mL)	MIC_{90} (œg/mL)
28	0.0625	0.125

Table 2: Pharmacokinetic profiles of VT-1598 and Fluconazole in *Act1*$^{-/-}$ mice

VT-1598			
Tissue Type	Concentration	SEM	Units
Plasma	11.40	1.28	µg VT-1598/mL
Fluconazole			
Tissue Type	Concentration	SEM	Units
Plasma	0.42	0.06	µg Fluconazole/mL

N= 6-7 mice per group

Figure 1: VT-1598 effectively clears *Candida* from the oral mucosa of *Act1*$^{-/-}$ mice



Act1$^{-/-}$ mice were sublingually infected with *Candida albicans* oral isolate Y72 (fluconazole-susceptible)(A, C, and D) or Y37 (fluconazole-resistant) (B) and given vehicle, fluconazole (25mg/kg), or VT-1598 (20mg/kg (A), 3.2 or 8 mg/kg (D)) via oral gavage 18 hours post-infection and every day thereafter for 4 total injections. At day 5 (A, B, and D) or 14 (C) post-infection, the total number of *Candida* in the tongues of these mice was determined. The data were analyzed using unpaired t tests or Mann-Whitney t tests, where appropriate, and an *, **, or *** indicates that the groups differ at $p < 0.05$, 0.01, or 0.001, respectively. 5-8 mice were used per group.

Summary and Future Directions

The antifungal drug VT-1598 has strong *in vitro* killing capacity, even in *Candida* isolates that have developed resistance to fluconazole. When administered *in vivo*, VT-1598 is present in circulation at higher concentrations than fluconazole, and well above the MIC for the isolates tested, thereby indicating that it may be effective in the clearance of *Candida* from the oral mucosa following infection. Using the fluconazole-susceptible oral isolate Y72, it was found that *Candida* established an infection in the tongues of vehicle-treated animals, whereas both VT-1598 and fluconazole were able to clear the vast majority of the fungus from the tongue. Using the fluconazole-resistant isolate Y37, only VT-1598 was able to significantly decrease the fungal load in the tongue, with almost complete clearance. If a 10-day washout period was used with the Y72, fluconazole-treated mice experienced regrowth of the fungus, whereas VT-1598-treated mice did not have any fungus in the tongue. Lastly, lower doses of VT-1598 were still able to effectively control *Candida* in the tongues of mice. Due to the efficacy of VT-1598 for the treatment of mice with oral candidiasis, it would be interesting to determine whether this antifungal drug is also effective in treating patients with CMC and other fungal infections.

References

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