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Evaluation of the Efficacy of Different Regimens of VT-1161 in the Oral Treatment of Dermatophytosis in a Guinea Pig Model

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Introduction

Dermatophytosis causes significant personal discomfort as well as cosmetic problems globally (1). Currently available antifungal preparations often do not provide adequate cure for dermatophytosis. New, more effective therapy is needed. The purpose of this study was to evaluate antifungal agents, for the oral treatment dermatophytosis in a guinea pig model. The guinea pig model has been developed and used successfully at the Center for Medical Mycology in the pre-clinical evaluation of terbinafine (both oral and topical), an FDA-approved antifungal agent for the treatment of onychomycosis.

Materials and Methods

The experiments were performed following Institutional Animal Care and Use Committee guidelines. Male albino guinea pigs with a body weight of 450 - 500g were housed in Animal Resource Center assigned rooms under standard conditions.

•**Inoculum:** *T. mentagrophytes* ATCC 24953 was used as the infecting fungus. *T. mentagrophytes* (from frozen stock) was sub-cultured on Potato Dextrose Agar (PDA) plates and incubated at 30°C for 5 - 7 days. The colonies were scraped from the plates using sterile saline solution. Cells were washed in saline and harvested by centrifugation.

•**Inoculation:** Each animal was anesthetized with a cocktail of xylazine, ketamine and acepromazine, intramuscularly. On the left side of the back, hair was clipped and shaved. A square of 2.5 cm × 2.5 cm was marked. The area was abraded with sterile fine grit sandpaper. A cell suspension containing 10^7 conidia in 100 µl was applied.

•**Treatment Groups:** Infected guinea pigs were randomized into the following groups (5 per group): itraconazole 10 mg/kg daily, terbinafine 10 mg/kg daily, VT-1161 10 mg/kg daily, 70 mg/kg itraconazole on days 1 and 8, terbinafine 70 mg/kg on days 1 and 8, 70 mg/kg VT-1161 on days 1 and 8, daily vehicle control (Cremaphor buffer), and an infected untreated control group.

•**Treatment Schedule:** Beginning two hours post-inoculation, animals were treated orally either once-daily for a period of twelve days or on days one and eight. Untreated control guinea pigs were left untreated.

•**Mycological Evaluation:** On day-13 post inoculation, ten hair samples from each quadrant were inoculated on PDA plates and incubated at 30ºC for 2 days. The fungal growth at the hair root was examined under a stereomicroscope. The effectiveness of a compound is expressed as percentage relative to the untreated control group.

•**Clinical Evaluation:** Local changes to the area were clinically assessed and scored on day-13. The assessment of clinical efficacy for each treatment group is expressed as percentage relative to the untreated control group.

•**Percent Efficacies** = 100 – (T x 100/ K), where T = score in test group, and K = score in untreated control.

Materials and Methods (cont.)

•**Blood and Tissue Collection:** On day-13, blood was harvested via a cardiac stick, placed in EDTA tubes, for plasma separation. Post-mortem, livers were collected and individually weighed. Hair samples and skin biopsies were collected and stored at -80ºC, prior to compound level evaluation by LC/MS-MS (OpAns. LLC, Durham, NC).

•**Statistical analysis:** Statistical significance of clinical and mycological study data was determined using test(s) as appropriate. The treated groups were compared to the untreated control and to one another to determine antifungal activity.

Results

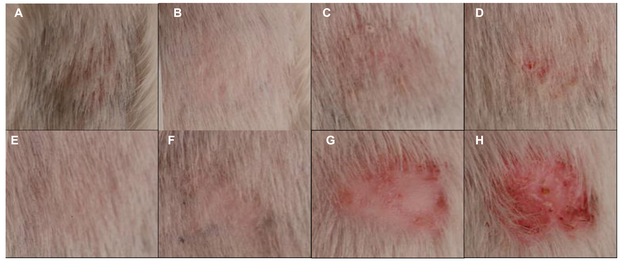
•**Clinical efficacy:** Table 1 and Figure 1 show the clinical efficacy of each treatment. As expected, the untreated control guinea pigs showed hair loss and ulcerated, scaly skin. Percent efficacies for groups dosed VT-1161 10 mg/kg daily and 70 mg/kg VT-1161 on days 1 and 8 were 68.0 and 86.6, respectively. Percent efficacies for groups dosed itraconazole 10 mg/kg daily and itraconazole 70 mg/kg on days 1 and 8 were 82.5 and 61.9, respectively. Percent efficacies for groups dosed terbinafine 10 mg/kg daily and terbinafine 70 mg/kg on days 1 and 8, were 85.6 and 84.5, respectively. The vehicle control (20% Cremophor) also behaved as expected with a percent efficacy of 4.1. All treatments showed significant efficacy when compared to the untreated and vehicle controls. VT-1161 dosed once-weekly 70 mg/kg on days 1 and 8 showed significant efficacy when compared to the once-weekly itraconazole.

Table 1. Percent Efficacy

Test Compound	Dose (mg/kg)	Percent Efficacy	
		Clinical	Mycological
VT-1161	10 QD	68.0 % *	96.6 % *
	70 QW	86.6 % **	94.0 % *
Itraconazole	10 QD	82.5 % *	96.6 % *
	70 QW	61.9 % *	94.0 % *
Terbinafine	10 QD	85.6 % *	99.1 % *
	70 QW	84.5 % *	95.7 % *
Vehicle	N/A	4.1 %	4.3 %

* *P*-value of < 0.001 when compared to the untreated control
** *P*-value of < 0.05 when compared to itraconazole 70 mg/kg on days 1 and 8

Figure 1. Clinical Appearance on day-13.



A. VT-1161 10 mg/kg, daily **B.** VT-1161 70 mg/kg, days 1 & 8, **C.** Itraconazole 10 mg/kg, daily, **D.** Itraconazole 70 mg, days 1 & 8, **E.** Terbinafine 10 mg/kg, daily, **F.** Terbinafine 70 mg/kg, days 1 & 8, **G.** Vehicle Control, **H.** Untreated Control

Table 2. Compound concentrations in various tissues on day-13.

Guinea Pig Plasma and Tissue Level Data

Test Compound	Dose (mg/kg)	Plasma (µg/ml)*	Hair (ppm)*	Skin (ppm)*
VT-1161	10 QD	1.8 (0.3)	7.6 (2.6)	27 (5)
	70 QW	1.3 (0.2)	9.5 (10.5)	24 (6)
Itraconazole	10 QD	0.11 (0.08)	12.4 (8.0)	0.83 (0.25)
	70 QW	< LLD**	9.7 (6.0)	0.26 (0.09)
Terbinafine	10 QD	< LLD	7.3 (2.8)	0.11 (0.04)
	70 QW	< LLD	8.9 (3.4)	0.07 (0.02)

* Average of values from five animals (standard deviation).
** Lower limit of detection, 0.01 µg/ml.

Results (cont.)

•**Mycological efficacy:** Table 1 shows the mycological efficacy of each test compound as compared to the untreated control. The controls behaved as expected. Percent efficacies for VT-1161 10 mg/kg daily and 70 mg/kg on days 1 and 8 were 96.6 and 94.0, respectively. Percent efficacies for itraconazole 10 mg/kg daily and 70 mg/kg on days 1 and 8 were 96.6 and 94.0, respectively. Percent efficacies for terbinafine 10 mg/kg daily and 70 mg/kg on days 1 and 8, were 99.1 and 95.7, respectively. All treatment groups showed significant efficacy when compared to the untreated and vehicle controls (*P*-values of < 0.05). There was no statistical difference in the mycological efficacy between the treated groups.

•**Compound Levels in Plasma, Skin, and Hair Samples:** Table 2 shows that both dosing regimens of VT-1161 had appreciably higher compound levels in plasma and skin than daily and weekly doses of itraconazole or terbinafine.

•**Liver Weights:** There was no significant difference in liver weights between controls and any of the treatment groups (*P*-values of > 0.05) (data not shown).

•**Histopathology:** On day-13, fungal elements were detectable in skin sections from untreated and vehicle-treated guinea pigs infected with *T. mentagrophytes*, indicating successful and persistent infection. No fungal elements were noted in skin sections obtained from other treatment groups.

Conclusions

Both VT-1161-treated groups showed significant clinical and mycological efficacy when compared to the untreated and vehicle controls. Daily treatments of VT-1161, itraconazole or terbinafine showed equivalent clinical and mycological efficacy. VT-1161 given once-weekly was clinically superior to the same dose of itraconazole given once-weekly. There was no significant difference between once-daily and once-weekly VT-1161 treatments. These data show that effective and convenient dosing regimens of oral VT-1161 may represent new onychomycosis treatment options.

References

1. Ghannoum MA, Hossain MA, Long LA, Mohamed S, Reyes G, and Mukherjee PK. Evaluation of Antifungal Efficacy in an Optimized Animal Model of *Trichophyton mentagrophytes*-Dermatophytosis. *J Chemother.* 16(2): 139-144 (2004).

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