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Efficacy of terbinafine compared to lanoconazole and luliconazole in the topical treatment of dermatophytosis in a guinea pig model

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The *in vivo* efficacy of terbinafine was compared to lanoconazole and luliconazole in the topical treatment of dermatophytosis caused by *Trichophyton mentagrophytes* using a guinea pig model. Topical antifungal treatment commenced three days post-infection, and each agent was applied once daily for seven consecutive days. Upon completion of the treatment period, evaluations of clinical and mycological efficacies were performed, as was scanning electron microscopy (SEM) analyses. Data showed that while all tested antifungals demonstrated significant mycological efficacy in terms of eradicating the fungi over untreated control, terbinafine and luliconazole showed superior clinical efficacy compared to lanoconazole (P-values < 0.001 & 0.003, respectively). Terbinafine demonstrated the highest clinical percent efficacy. SEM analysis revealed hairs from terbinafine and lanoconazole-treated animals had near complete clearance of fungi, while samples from luliconazole-treated animals were covered with debris and few conidia. This study demonstrates that, in general, terbinafine possessed similar efficacy to lanoconazole and luliconazole in the treatment of dermatophytosis. Terbinafine tended to have superior clinical efficacy compared to the azoles tested, although this difference was not statistically significant against luliconazole. This apparent superiority may be due to the fungicidal activity of terbinafine compared to the fungistatic effect of the other two drugs.

Keywords terbinafine, imidazoles, dermatomycoses, guinea pig, treatment outcome

Introduction

Dermatophytes such as *Trychophyton mentagrophytes* and *Trychophyton rubrum* are among the most common etiological agents of skin mycoses [1]. Physiologically, these dermatophytes have the ability to digest keratin for growth and replicate in superficial layers of the epidermis. Consequently, in clinical practice the body parts most affected by dermatophytic infection are those rich in keratin such as the hair, skin, and nails. Though most of these skin infections are not life-threatening, they are persistent and bothersome often resulting in considerable itching, pain and tissue damage. During the last decade, the development of safer and more effective antifungal agents including terbinafine and azole drugs has notably enhanced our capacity to combat cutaneous mycoses [2]. Yet, even with these advances, complete cure is often unattainable as up to 15–20% of patients experience relapses [3]. Therefore, the search for new, more effective therapies continues to be pursued.

Terbinafine, an allylamine compound, commercially available as Lamisil AT®, has demonstrated high antifungal efficacy when applied topically [4]. Additionally, lanoconazole and luliconazole, which are chemically analogous third generation azoles commercially available in Japan, have also been shown to be efficacious in the treatment of dermatophytosis [5,6]. However, a direct comparison of these agents has not been conducted. In this report, we performed a study to compare the efficacy of terbinafine to lanoconazole and luliconazole in the topical treatment of

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Trichophyton mentagrophytes-dermatophytosis using a guinea pig model.

Materials and methods

Laboratory animals

The guinea pig model employed in this experiment was optimized at our center and has previously demonstrated its utility as a screening tool for candidate antifungal agents [7]. Guinea pigs were chosen as test subjects because they are susceptible to dermatophytosis similar to humans, and their large body surface provides sufficient area to perform experiments to determine clinical and mycological efficacies.

The *in vivo* experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC). All procedures in the protocol were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Welfare. According to the protocol, male albino guinea pigs (Harlan-Sprague-Dawley, San Diego, CA) with a body weight of 450–500 g were housed in the Animal Resource Center. The environmental controls for the animal room were set to maintain a temperature of 16–22ºC, a relative humidity of 30–70%, and a 12:12 hour light:dark cycle. Experimental animals underwent an acclimation period for a minimum of 5 days prior to use.

Fungal strain

Trichophyton mentagrophytes ATCC 24953 was chosen as the test agent because of its ability to infect the skin, resulting in an inflammatory reaction and skin and hair root invasion. Moreover, MIC data for terbinafine and lanoconazole against this isolate were 0.03 μg/ml and <0.004 μg/ml, respectively. To prepare the challenge inoculum, several Petri dishes containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) were seeded with *T. mentagrophytes* and incubated at 30ºC for 7 days. At the end of this growth period, conidia were scraped from the plates with sterile cell scrapers (BD Falcon™; BD Biosciences, Bedford, MA) in normal sterile saline (0.85% NaCl). This conidial suspension was prepared fresh and used to challenge the animals in this study.

Antifungal agents

Terbinafine 1% cream (Lamisil AT®), lanoconazole1% cream, and luliconazole 1% cream were provided by Novartis Consumer Health, Inc. (Parsippany, NJ).

Animal inoculation and antifungal therapy

All animal challenge procedures were done under general anesthesia. Each guinea pig was anesthetized with a cocktail of ketamine, xylazine, and acepromazine (3:3:1; v/v/v) administered intramuscularly. Using an electric shaver, an area 2 cm to the left of midline on the animals' backs was clipped. A disposable razor was then used to obtain a closer shave of the area. Using a stencil and marking pen, a 2.5 cm × 2.5 cm square outline was drawn on the guinea pigs' skin. Marked areas were then abraded using sterile fine grit sandpaper. A cell suspension containing 1×10^7 *T. mentagrophytes* conidia in 100 μl of sterile normal saline was applied and rubbed thoroughly on the abraded skin using a sterile pipette tip. Infected guinea pigs were then randomly assigned to the following four groups (10 animals per group); (i) topical terbinafine 1% cream-treated, (ii) topical lanoconazole 1% cream-treated, (iii) topical luliconazole 1% cream-treated, and (iv) an infected-untreated control. Treatment with the topical drug preparations began 72 h post-challenge, and continued once daily for the next 7 days (see treatment schedule, Fig. 1). Each guinea pig in a treated group received 0.2 ml of the drug cream applied topically to the infected area using a sterile pipette tip. Infected-untreated control guinea pigs did not receive any form of treatment.

Clinical and mycological evaluation of treatment efficacy

Clinical and mycological endpoints were used to determine the efficacies of the drugs tested. The clinical assessment was carried out first and then hair samples were uprooted and evaluated for eradication of fungal infection by means of their inoculation onto culture media (mycological cure). Treated and control guinea pigs were examined daily throughout the course of the experiment. Final clinical and mycological assessments were performed on study day 13.

The infected area marked on the back of each animal was divided into four quadrants. The clinical assessment of local changes of the infected skin in each quadrant was

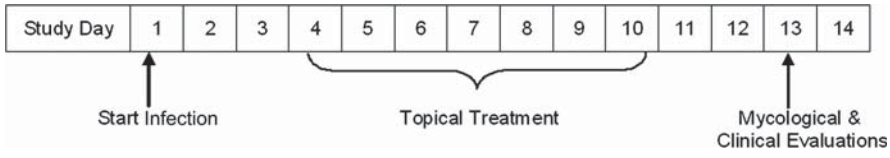

Fig. 1 Treatment and evaluation schedule.

scored on a scale of 0 to 5 as follows; 0 – no lesions, 1 – few slightly erythematous sites on the skin, 2 – well-defined redness and swelling with bristling hairs, 3 – large areas of marked redness, incrustation, scaling, bald patches, ulcerated in places, 4 – partial damage to the integument and loss of hair, and 5 – extensive damage to the integument and loss of hair (see Fig. 2). Scores from the quadrants were summed for each animal and used to determine the clinical efficacy of different treatment groups. Percent efficacy for each treatment group was expressed as a percentage relative to the infected-untreated control group using the following formula:

$$\text{Percent Efficacy} = 100 - (T \times 100/K)$$

where T = total score of the test group and K = total score of the infected-untreated control. The total score for any treatment group signifies the average clinical score from animals in the same group [2,8,9].

The hair root invasion test was used to assess the mycological cure rate resulting from treatment with the test antifungals. Hair samples were removed with sterile forceps from the four quadrants. Ten uprooted hairs from each quadrant were planted onto the surface of PDA plates divided into the corresponding quadrants. Plates were incubated at 30ºC for 2 days. Following incubation, the number of hairs exhibiting fungal growth at the hair root was counted using a stereomicroscope. Counts from the quadrants were summed for each animal and used to determine the mycological efficacy of different treatment groups. The effectiveness of a compound in reducing the number of fungus-positive hair samples per treatment group was expressed as a percentage relative to the infected-untreated group using the following formula:

$$\text{Percent Efficacy} = 100 - (T \times 100/K)$$

where T = total positive hairs in the test group and K = total positive hairs in the infected-untreated control. The total score for any group denotes the average count of fungus-positive hairs obtained from the animals in the same group [2,9].

Scanning electron microscopy

Guinea pig hairs were fixed in 2% glutaraldehyde and prepared for SEM examination according to a previously published protocol [10]. The fixed and dehydrated hairs were sputter coated with gold:paladium (60:40) and viewed under a Philips XL30 scanning electron microscope.

Statistical analysis

SPSS for Windows 16.0 statistical analysis software was utilized. A one-way ANOVA with a Bonferroni post-hoc test was employed to determine whether statistically

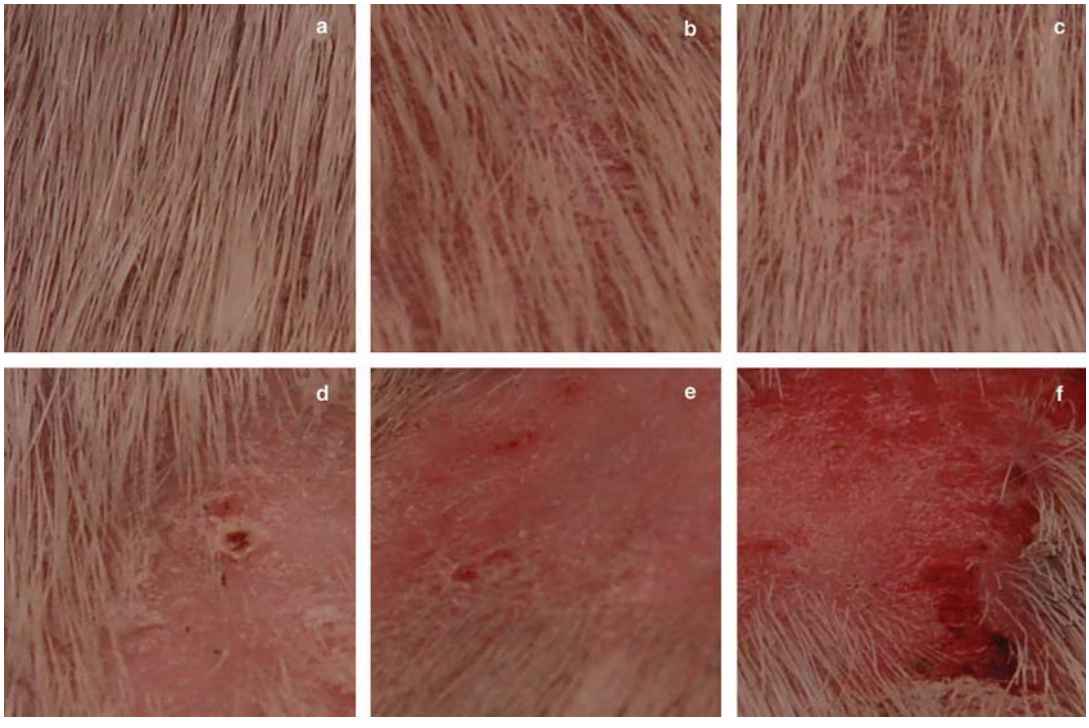

Fig. 2 Typical appearances over clinical scoring range: 0 (a), 1 (b), 2 (c), 3 (d), 4 (e), 5 (f).

Table 1 Summary of clinical and mycological efficacies of test articles

	Clinical			Mycological		
Test compound	% Efficacy	*P*-values		% Efficacy	*P*-values	
Untreated	0	-	0.002**	0	-	<0.001**
Terbinafine	56.4	<0.001*	<0.001**	100	<0.001*	1.000**
Lanoconazole	26.2	0.002*	-	100	<0.001*	-
Luliconazole	50.8	<0.001*	0.003**	100	<0.001*	1.000**

**P*-values compared to untreated control. ***P*-values compared to lanoconazole. $P < 0.05$ was considered significant.

significant differences existed among the mean clinical scores and mean fungus-positive hair counts of the treatment groups. The alpha level was set to 0.05.

Results

Clinical assessment

Infected guinea pigs were monitored daily for signs of infection. By study day 4 (72 h post-inoculation) the animals showed scaling and redness of the affected area. At the time of evaluation (study day 13) infected-untreated control guinea pigs exhibited hair loss, marked ulceration, and occasional scabbing. In contrast, treatment with test agents overall yielded much improved clinical appearances.

Table 1 presents the clinical and mycological results of this study. Treatment with terbinafine yielded the highest clinical efficacy (56.4%) which is attributable to the regrowth of hair, absence of skin lesions, and no noted erythematous areas of skin. Guinea pigs treated with lanoconazole presented an improved clinical appearance over the infected-untreated control with near complete clearance of erythematous areas of skin, yet areas of marked hair loss persisted. This antifungal generated the lowest clinical efficacy (26.2%) of those tested. Luliconazole treatment produced a clinical efficacy somewhat below terbinafine with 50.8%. This was attributable to the persistence of a small number of slightly erythematous areas of the skin and a few bristling hairs. Refer to Fig. 3 for representative clinical appearances of the treatment groups.

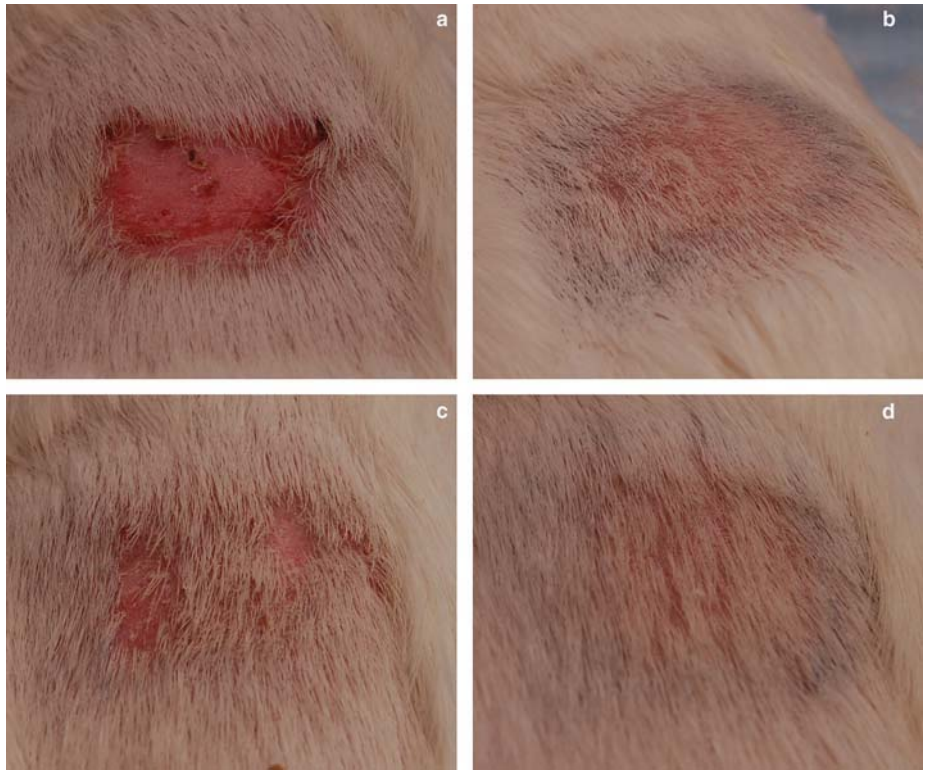

Fig. 3 Representative clinical appearances: untreated (a), terbinafine (b), lanoconazole (c), and luliconazole (d).

Statistical analysis of clinical scoring supported these observations. All treatment groups were significantly improved in comparison to the infected-untreated control group with *P*-values for terbinafine, lanoconazole, and luliconazole of < 0.001, 0.002, and < 0.001, respectively. Furthermore, terbinafine was significantly more efficacious than lanoconazole (*P*-value of < 0.001) and trended to being more efficacious than luliconazole based on clinical percent efficacies, albeit this difference did not reach statistical significance. Our data also showed luliconazole was significantly more efficacious than lanoconazole (*P*-value of 0.003).

Mycological assessment

The hair root invasion assay demonstrated that hairs harvested from untreated control guinea pigs yielded a large number of fungus-positive hairs, indicating a successful *T. mentagrophytes* infection. Groups treated with terbinafine, lanoconazole, and luliconazole each failed to produce any fungus-positive hairs, therefore demonstrating 100% mycological efficacy and clearance of the dermatophyte from the test area. Relative to the infected-untreated control, all test articles' mean positive hair counts were statistically significant with *P*-values of <0.001 each.

Scanning electron microscopy

Scanning electron microscopy analysis revealed that hairs acquired from infected-untreated control guinea pigs were heavily colonized with *T. mentagrophytes* conidia and hyphae. The hair shafts of terbinafine and lanoconazole treated animals had very few conidia on them with virtually no debris. In contrast, hairs from luliconazole treated guinea pigs were covered with amorphous debris and a small number of conidia were detected. Scanning electron micrographs are displayed in Fig. 4.

Discussion

In this study, our group assessed through the use of a guinea pig model the clinical and mycological efficacies of terbinafine compared to two imidazole antifungal compounds commercially available in Japan, lanoconazole and luliconazole, and an untreated control group in the topical treatment of *T. mentagrophytes* dermatophytosis. Although lanoconazole and luliconazole have been shown to be efficacious against dermatophytes [5,6], *in vivo* comparisons of these compounds to terbinafine are lacking.

Our data shows that all treated groups demonstrated statistically significant clinical and mycological improvement

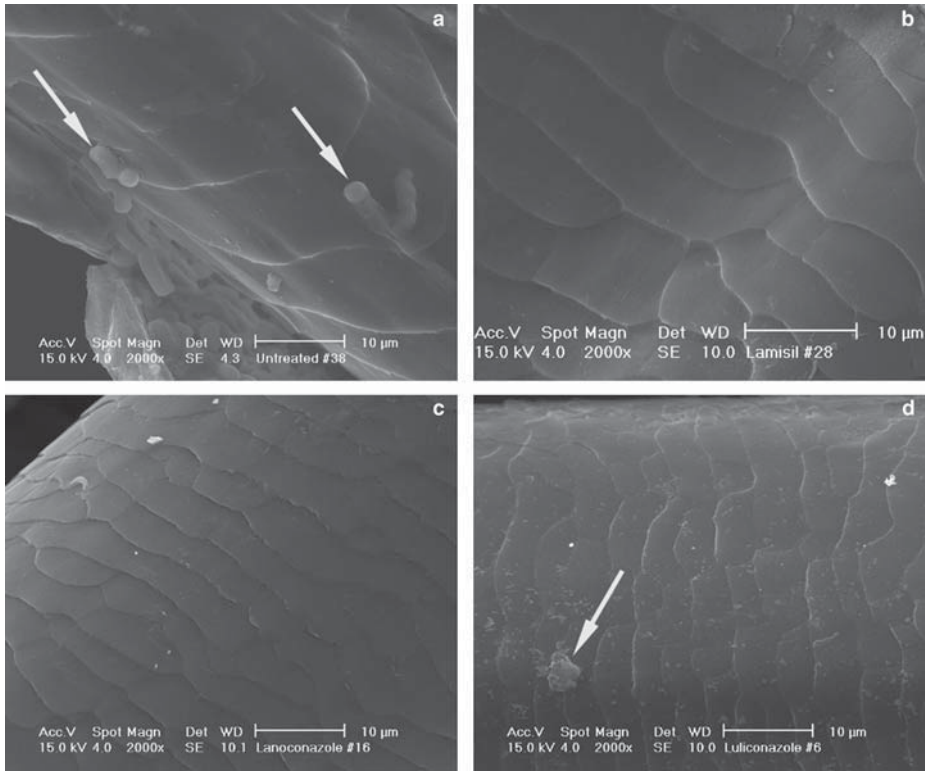

Fig. 4 Scanning electron micrographs of hair shafts (2000×): untreated (a), terbinafine (b), lanoconazole (c), and luliconazole (d). Note: Arrows indicate conidia (untreated) and debris (luliconazole).

compared to the infected-untreated control (see Table 1). Yet, while all three test drugs showed similar mycological efficacies (mycological cure), the terbinafine- and luliconazole-treated groups achieved statistically significant improvements in clinical symptomology relative to lanoconazole. When comparing clinical percent efficacies, terbinafine was more effective than luliconazole. The improved efficacy of luliconazole over lanoconazole is likely due to luliconazole being strictly an *R*-enantiomer while lanoconazole is a racemic mixture. It should be noted that the *S*-enantiomers of both compounds have no antifungal activity [6]. Added scrutiny gained from scanning electron microscopy analysis may provide insight as to why the clinical scoring of terbinafine was higher than luliconazole's, as luliconazole-treated hairs were shown to be covered with amorphous debris and contained some fungal conidia. It is possible that this debris, unlike that noted with the other two antifungals, may represent residual breakdown of the hair shaft.

The data our group has gathered using this guinea pig model proves the clinical superiority of terbinafine compared to the imidazole antifungal agents lanoconazole and luliconazole. This supports *in vitro* data gathered at our center using the recently approved Clinical and Laboratory Standard Institutes reference method (M38-A2) that compared the susceptibility of dermatophytes to terbinafine and lanoconazole. This *in vitro* testing showed that terbinafine demonstrates fungicidal antifungal activity, while lanoconazole exhibits fungistatic antifungal activity. The differences in the antifungal activity of these agents may be a result of their dissimilar mechanisms of action [11]. Although allylamines (such as terbinafine) and azoles (i.e., lanoconazole and luliconazole) inhibit fungal ergosterol synthesis, they do so at different steps in its biosynthesis. Terbinafine inhibits at the earlier squalene epoxide step while azoles do so downstream at the lanosterol demethylation step. These actions result in the accumulation of squalene and lanosterol, respectively [12,13]. Of the two intermediates, squalene has been shown to be more toxic to fungal cells while lanosterol accumulation inhibits fungal growth. The difference in the mode of action of terbinafine and its fungicidal activity compared to the static activity of the other two agents may explain the differences seen in the superior clinical efficacy of terbinafine. Moreover, these findings may have important implications regarding relapse of infection. A long-term effectiveness study comparing terbinafine to the azole itraconazole, demonstrated that terbinafine provided significantly higher long-term clinical and mycological cure rates than the azole for the treatment of onychomycosis [14].

In conclusion, this head-on study shows that overall the efficacies of terbinafine, lanoconazole, and luliconazole were similar in the topical treatment of dermatophytosis. Mycologically, each of the agents cleared the test area of fungus positive hairs upon culture. However, it is important to note that terbinafine tended to have a superior clinical efficacy compared to the azole test articles, although this difference was not statistically significant against luliconazole. This apparent superiority may be due to the fungicidal activity of terbinafine compared to the fungistatic activity of the azole drugs.

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