Short-term therapy with luliconazole, a novel topical antifungal imidazole, in guinea pig models of tinea corporis and tinea pedis.

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Luliconazole is a novel topical antifungal imidazole with a broad spectrum of potent antifungal activity. The drug is under clinical development in the United States for management of dermatophytosis with a short-term treatment regimen. The present study was undertaken to investigate the clinical benefit of a short-term therapy with luliconazole cream in guinea pig models of tinea corporis and tinea pedis induced with *Trichophyton mentagrophytes*. The dose-dependent therapeutic efficacy of topical luliconazole cream (0.02-1%), as measured by macroscopic improvement of the skin lesions and fungal eradication determined by a culture assay, was demonstrated using a tinea corporis model. The improvement in skin lesions by luliconazole cream was observed even at a concentration of 0.02% and the efficacy of it at 0.1% was equal to that of 1% bifonazole cream. The efficacy of short-term therapy with 1% luliconazole cream, which is used for clinical management, was investigated using the tinea corporis model (4- and 8-day treatment regimens) and the tinea pedis model (7- and 14-day treatment regimens). The 1% luliconazole cream completely eradicated the fungus in half or less treatment time required for the 1% terbinafine cream and the 1% bifonazole cream, as determined by a culture assay for both models. These results clearly indicate that the 1% luliconazole cream is sufficiently potent for short-term treatment for dermatophytosis compared to existing drugs. Luliconazole is expected to be useful in the clinical management of dermatophytosis.
**Introduction**

Superficial mycoses are not fatal, but they constitute a serious problem for patients’ quality of life in view of the considerable discomfort and/or a cosmetic deformity they cause. These diseases are found worldwide and affect 20-25% of the world’s population (14). Dermatophytosis is the most common infection among the superficial mycoses (1, 6-8, 11, 13, 26). According to an epidemiological survey of ambulatory visits in the United States, the incidence of dermatophytosis, such as tinea unguium, tinea corporis and tinea pedis, was as high as 23.2%, 20.4% and 18.8%, respectively, during 1995-2004 (24). *Trichophyton rubrum*, an anthropophilic fungus, is the most prevalent causative agent of the dermatophytosis in developed countries. The incidence of this fungus has not changed in the past decades (1, 14, 24, 25, 27, 29), although many antifungal drugs with potent action against this species have been introduced into the markets during this period (3, 23). One reason for the unsuccessful antifungal management is the relapse of skin manifestations due to poor adherence to long-term treatment regimens using topical antifungal drugs (2, 11, 13).

Under these circumstances, a drug with a more potent therapeutic efficacy capable of shortening treatment duration and leading to improvement in patients’ adherence rate would be beneficial.

Luliconazole (LLCZ; (−)-(E)-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl) acetonitrile) is a novel optically active antifungal imidazole (21). The compound has
a unique chemical structure (Fig. 1), which is augmented by the introduction of an imidazole moiety into the ketene dithioacetate structure. By these modifications, an unusually high potency against filamentous fungi, including dermatophytes, was achieved while maintaining the broad antifungal spectrum of an imidazole. The *in vitro* antifungal activity of LLCZ against *Trichophyton* spp. was found to be the highest among existing topical antifungal drugs (17, 18, 30). Luliconazole was developed as a topical antifungal drug and approved in Japan in 2005. Currently, a 1% cream and 1% solution of LLCZ is available for the treatment of superficial mycoses such as dermatophytosis, candidiasis and pityriasis versicolor. Topical LLCZ has been introduced in India (approved in 2010) and, presumably, will also be available in Asian countries, such as China and Vietnam, among others. In the United States, the clinical development of LLCZ for the treatment of dermatophytosis with a short-term regimen has recently begun based on the clinical data obtained in Japan (33). The drug is now in Phase II clinical trials to evaluate its efficacy in curing tinea pedis in half the treatment time compared to commonly prescribed products. Although the excellent *in vitro* antifungal activity of LLCZ strongly suggests it would be useful for short-term therapy, preclinical evidence from an *in vivo* study using an animal model supporting this hypothesis is limited (21, 31).

The present study investigated the clinical efficacy of short-term LLCZ treatment in the management of dermatophytosis using guinea pig models of tinea corporis and tinea pedis. *Trichophyton mentagrophytes* was used as a pathogenic agent because this zoophilic fungus is able
to produce a stable infection in guinea pigs and has previously been used to establish a tinea corporis and tinea pedis model (12, 15, 22, 26).

Materials and Methods

Antifungal drugs

Luliconazole was synthesized and prepared in a cream formulation at concentrations of 0.02, 0.1, 0.5 and 1% at the Research Center of Nihon Nohyaku Co. Ltd. (Osaka, Japan). The recipe for the cream formulation was the same as that of the commercial product sold in Japan (Pola Pharma Inc., Tokyo, Japan) and the clinical trials in the United States. The reference drugs, 1% terbinafine (TRB) cream (Novartis Pharma K.K., Tokyo, Japan) and 1% bifonazole (BFZ) cream (Bayer Yakuhin Ltd., Osaka, Japan) are commercial versions available for clinical use.

Animals

Male SPF Hartley guinea pigs were purchased from Nippon SLC Ltd (Shizuoka, Japan). The animals were acclimatized to laboratory conditions for more than 7 days, including the quarantine period, and used when they were 6 weeks old. They were housed in individual cages during the experiment at 21±2°C with a relative humidity of 50±20%. The room was ventilated 12-15 times per hour by the all fresh air system and lit 12 hours per day. The animals were allowed free access to food (Lab-G stock, Nosan Corporation, Kanagawa, Japan) and local tap water, which was filtrated twice through 5 µm pore filters, ad libitum.
The animal care and use conformed to the standards established by the animal welfare committee of the institute and complied with the legal requirements for the humane treatment and management of animals (The act on welfare and management of animals; Law Number: Act No. 105 of 1973, Amendment: Act No. 68, 2005, Japan).

**Test organisms and inoculum preparation**

*Trichophyton mentagrophytes* TIMM1189 and TIMM2789, which were obtained from Teikyo University Institute of Medical Mycology (Tokyo, Japan), were used for the tinea corporis and tinea pedis models, respectively. The MIC value of LLCZ, BFZ and TRB against these organisms, as measured by the standardized microdilution method using RPMI-1640 medium (5, 28), were 0.002, 0.5 and 0.0078 μg ml⁻¹, respectively, for *T. mentagrophytes* TIMM1189 and 0.002, 4 and 0.016 μg ml⁻¹, respectively, for TIMM2789. To prepare the inoculum for animal infections, the test organisms were precultured on a Sabouraud dextrose agar (SDA; Difco™, MD, USA) slant at 27°C for 1-2 weeks until maturity. Sterile saline containing 0.1% (v/v) Tween 80 was added to the slant, and the conidia were suspended by gently rubbing them with a loop. The suspension was filtered through sterilized gauze to remove the hyphal fragments. The number of conidia in the filtrate was measured using a Thoma hemacytometer, and the concentration was adjusted to 4.0 x 10⁷ conidia ml⁻¹ for *T. mentagrophytes* TIMM1189 and 1.0 x 10⁸ conidia ml⁻¹ for *T. mentagrophytes* TIMM 2789 with sterile saline containing 0.1% (v/v) Tween 80.
Tinea corporis was induced in the dorsal skin by the method reported by Niwano, Y. et al. (22), with slight modifications. The dorsal skin was clipped, and the skin sites (φ2 cm bilaterally) were stripped with adhesive tape three times (No. 159, Teraoka, Tokyo, Japan). The *T. mentagrophytes* TIMM1189 inoculum (0.05 ml per site) was then applied to the skin surface. Meanwhile, the animals were prepared to host the fungal infection. The drug treatment commenced 3 or 5 days after the inoculation. The infected site remained uncovered throughout the experiment.

Tinea pedis was induced in the planta of the hind leg using a modified version of the method pioneered by Fujita, S. and T. Matsumiya (12). The planta was cleaned with a cotton swab moistened with sterile saline, and then the surface of the skin was lightly abraded with sandpaper. A sterile adhesive bandage (Band-Aid®, Johnson & Johnson Co., Ltd., Tokyo, Japan), soaked with 0.15 ml of the *T. mentagrophytes* TIMM2789 inoculum, was applied to the planta and covered with a film (Saran Wrap®, Asahi Kasei Corporation, Tokyo, Japan). To avoid excessive pressure to the infected site, a form pad (Reston™ 1560M, Sumitomo 3M Ltd., Tokyo, Japan) was placed on the site with adhesive elastic tape (Elastplast®, Smith & Nephew Wound Management KK, Tokyo, Japan). The bandage was removed 7 days after the commencement of the inoculation, and the animals were maintained for another 21 days to establish the infection.

**Experimental design**
A dose-response study of the LLCZ cream was performed using the tinea corporis guinea pig model. A total of forty guinea pigs were used. Three days after inoculation, the animals without any accidental skin damage (e.g., by self-scratching) were selected and assigned into 7 groups of 5 animals each. Different concentrations of LLCZ cream [0 (base), 0.02, 0.1, 0.5 and 1%] or 1% BFZ cream were applied to the skin surface (0.2 ml per site) once daily, without covering the surface, for 7 consecutive days. Animals in the non-treated group received no drug treatment. The animals were macroscopically observed throughout the experiment, and a culture assay for the infected skin sites was performed on day 14 post-inoculation (5 days after completion of the drug treatment).

A short-term study of the 1% LLCZ cream treatment was conducted on both the tinea corporis and tinea pedis guinea pig models. Both 1% TRB cream and 1% BFZ cream were used for comparison. In the tinea corporis guinea pig model, a total of forty five guinea pigs were used. Five days after the inoculation, the animals with developing skin lesion (score:1) without any accidental skin damage were selected and assigned into one of the following 7 groups of 5 animals each: the non-treated group, cream base group (8-day treatment), 1% LLCZ cream groups (4- and 8-day treatments), 1% TRB cream groups (4- and 8-day treatments) and the 1% BFZ cream group (8-day treatment). The drugs were applied topically to the skin (0.2 ml per site) once daily, without covering the surface, for 4 or 8 consecutive days. The animals were macroscopically observed throughout the experiment, and a culture assay was performed on day 17 post-inoculation on skin.
from the site of infection (5 and 9 days after completion of the drug treatment). In the tinea pedis model, a total of forty guinea pigs were used. The animal presenting with skin lesion (scaling) were assigned to one of the following 8 groups of 4 animals each (4 groups each for 7- and 14-day treatments): the non-treated group, 1% LLCZ cream groups, 1% TRB cream groups and 1% BFZ cream groups. In total, 0.1 ml of each drug was applied topically to the planta once daily for 7 and 14 consecutive days. A culture assay of the planta was performed 14 days after the completion of each treatment. Another experiment was performed using tinea pedis model with the same treatment regimen but using a deactivator-supplemented medium, which is able to prevent any carry-over effect in the culture assay.

**Macroscopic observation**

The skin lesions caused by tinea corporis were macroscopically observed daily throughout the experiment and was assessed using the following score: 0 (no sign or normal), 1 (a small number of distinct erythematous papules at the site), 2 (moderate erythema spread over the entire site accompanied partly by an inflammatory response or abrasion of the epidermis in some parts), 3 (patches of intense erythema with scaling and crusting) and 4 (severe erythema with extensive and intense crusting). The average score of each group was calculated to determine the average lesion score.

**Culture assay**
The animals were euthanized with ether, and the skin tissue specimens, including the epidermis and dermis, were collected from the infected dorsal skin or planta using sterile scissors. The tissues were rinsed with 0.1% (v/v) benzalkonium chloride followed by sterile saline. The skin tissues were cut into small pieces (approximately 5 mm square), and 10 blocks of the samples were randomly selected for culture. These samples were implanted on an SDA plate containing cycloheximide (500 μg ml⁻¹), chloramphenicol (50 μg ml⁻¹) and sisomicine (50 μg ml⁻¹). For the culture study using the deactivator-supplemented medium (20), skin blocks were implanted on an SDA plate containing the following deactivators; 1% egg lecithin (Kanto Chemical Co., Inc., Tokyo, Japan) and 0.7% Tween 80 (polyoxyethylene sorbitan monooleate, Kanto Chemical Co., Inc., Tokyo, Japan). The plates were incubated at 27°C for 14 days, and the formation of colonies on the plates was observed. A skin block yielding a colony was regarded as fungus-positive. The percentage of fungus-positive feet in each group was calculated as the culture-positive rate. To evaluate the amount of fungus associate with residual infections, the number of culture-positive skin blocks was rated 0 to 10, according to the corresponding number of culture-positive skin tissues among the 10 skin tissues implanted per plate. The average score of each group was calculated to determine the fungal burden score.

Statistical analysis

The SAS® system for Windows (Release 8.02, SAS institute Inc., Cary, NC, USA) was used for
statistical analysis. The average lesion score on the last day of macroscopic observation and the fungal burden score in the culture assay were analyzed by Dunnett’s multiple comparison test or by Steel’s multiple comparison rank sum test (nonparametric Dunnett’s multiple comparison test) based on the homogeneity of variance tested by Bartlett’s test (p<0.01). The culture-positive rate in the culture assay was analyzed by Fisher’s exact probability test. The difference of the mean between the groups compared with that of the non-treated group and/or cream base group was considered significant when the p value was below 0.05.

Results

Dose-response study

Figure 2 shows the results of the macroscopic observation of the tinea corporis model. Luliconazole cream prepared at various concentrations improved skin lesions in a dose-dependent manner. Compared to the non-treated group and/or cream base group, there was a statistically significance improvement in lesions treated with 0.02% LLCZ cream, and 0.1% LLCZ was comparable in efficacy to the 1% BFZ cream. The results of the culture assay performed on day 14 post-inoculation are shown in Table 1. The culture-positive rate and the fungal burden score of the non-treated group reached maximum values of 100% and 10.0, respectively. The LLCZ cream eliminated the fungi in a dose-dependent manner, and statistically significant differences in the
fungal burden were observed even after treatment with a concentration of 0.02% LLCZ compared to the non-treated and/or cream base groups. The LLCZ cream completely cured the mycological infection at concentrations of 0.5% and above.

Short-term treatment study

The treatment duration of 1% LLCZ cream required to cure a fungal infection was assessed in the tinea corporis and tinea pedis models. In the tinea corporis model, the commencement of the drug treatment was changed from day 3 to day 5 post-inoculation to generate a more severe lesion, because the 1% LLCZ cream was found to be extremely potent in the dose-response study. The results of the macroscopic observation of the tinea corporis model are shown in Fig 3. In both the 1% LLCZ cream and 1% TRB cream treatment groups, the average lesion score in the 8-day treatment groups was higher than in the 4-day treatment groups after day 11 due to accumulated cream vehicle materials on the surface of the treated skin, not inflammation caused by the microorganisms. The 1% LLCZ cream significantly improved the skin lesions in the 4-day treatment and the 8-day treatment compared to those in controls, and these efficacy ratings were similar to those of the 1% TRB cream groups (4- and 8-day treatments). In the culture assay (Table 1), the 1% LLCZ cream eliminated the fungi from the skin at the site of infection with the 4-day treatment regimen and completely cured the infection with the 8-day treatment regimen with regard to the culture positive rate and fungal burden score. In comparison, the 1% TRB cream showed moderate
efficacy, and the 1% BFZ cream showed only slight efficacy, even with an 8-day treatment. The efficacy of short-term therapy with different drugs was also compared in the tinea pedis model, as shown in Table 2. The treatment with 1% LLCZ cream resulted in a complete mycological cure with a 7-day treatment regimen, with regard to the culture positive rate and fungal burden score. The 1% TRB cream significantly deduced the amount of fungi with a 7-day treatment regimen, however the complete eradication of the fungi by this drug required more than 14 days. The 1% BFZ cream did not show significant antifungal effects even after 14 days of treatment. The results of the culture assay using the deactivator-supplemented medium are also shown in Table 2. The culture positive rates and the fungal burden scores of the 1% LLCZ cream and reference drugs using the deactivator-supplemented medium were similar to that of the conventional SDA medium (upper column).

Discussion

The two animal models employed in the present study are highly reproducible and are used for studying the pathogenesis and antifungal treatment of dermatophytosis (4, 15, 26). The tinea corporis model reliably results in infection after only one inoculation and has proven to be time-saving when testing the antifungal activity of drugs. One is able to select the severity of the model used by choosing the appropriate number of days after inoculation for analysis because the...
inflammatory responses at the infected site increases daily after the inoculation; e.g., the skin lesions at day 5 after the inoculation employed in the short-term treatment study were more severe than those at day 3 in the dose-response study. In contrast to the tinea corporis model, the tinea pedis model in the guinea pig requires more skilled techniques for inoculation and a longer time period to develop an infection (a total of 4 weeks), although it is a stable and substantiated model that closely mimics human tinea pedis. We used the conventional tinea corporis model for testing the dose-response of LLCZ creams and the efficacy of short-term treatment of the 1% LLCZ cream, and then adopted the true tinea pedis model for further confirmation of the efficacy of the short-term therapy. The reference drugs used in this study were 1% BFZ cream and 1% TRB cream. Bifonazole is the first drug to be used in a once-daily treatment of superficial mycoses and has been the drug of choice for topical antifungal infections for the last two decades in Japan (19, 32). Terbinafine is another potent antifungal agent with proven short-term efficacy for the treatment of tinea (9, 10).

The LLCZ cream showed a dose-dependent efficacy in the tinea corporis model. Significant improvement of lesions was observed after treatment with a concentration of 0.02%, and the efficacy of LLCZ at 0.1% was equal to that of 1% BFZ cream. These data are in accordance with results obtained in randomized clinical trials for dose optimization, in which LLCZ cream (0.1-1%) showed significant antimycotic activity against tinea pedis at doses as low as at 0.1% (34). Additionally, the efficacy of short-term treatment with 1% LLCZ cream was examined and
compared with that of 1% TRB cream and 1% BFZ cream using the tinea corporis and tinea pedis models. In both models, treatment with the 1% LLCZ cream resulted in a complete mycological cure in half or less treatment time period required for the 1% TRB cream and the 1% BFZ cream. The strong antymycotic activity of the 1% LLCZ cream against tinea pedis in the guinea pig has also been reported by Uchida et al. (31), in which study complete eradication of the fungi was demonstrated after a 7-day treatment regimen with a culture assay performed at up to 16 weeks after the completion of treatment.

Although *T. mentagrophytes* was used as the pathogenic agent in the study, the susceptibility of *T. rubrum*, the major causative agent of dermatophytosis, to LLCZ is higher than that of *T. mentagrophytes* (17, 18). Therefore, 1% LLCZ cream would be expected to be more potent in clinical use than that exhibited in the present study. In fact, the mycological efficacy of a 2-week treatment of tinea pedis with 1% LLCZ cream was comparable to or superior to that of a 4-week treatment with 1% BFZ in a clinical trial in Japan (33).

It is generally recognized that azoles are more fungistatic than allylamine (e.g., TRB) or thiocarbamate (e.g., liranaftate). However, the LLCZ has exceptionally strong antifungal activity. The MIC of LLCZ against *T. mentagrophytes* TIMM1189 and TIMM2789 was 4 and 8 times lower than that of TRB, respectively, and 250 and 2000 times lower than that of BFZ, respectively. In addition, LLCZ has been shown to have preferable pharmacokinetic properties in the skin compared
In short, the drug concentration in the stratum corneum of the tinea skin of guinea pigs treated with 1% LCZ cream was significantly higher than that found in those treated with the 1% TBF cream. Luliconazole was also significantly easier to release from the stratum corneum than TRB. Thus, the strong therapeutic efficacy of LLCZ cream as a topical antifungal drug can be attributed not only to its excellent *in vitro* antifungal activity, but also to its optimum pharmacokinetic properties in the skin.

In a culture study of topical antifungal drugs, carry-over effects should be taken into account when evaluating drugs showing high retention in the skin (20). To confirm there was no carry-over effect of LLCZ in this study, we performed a culture study using drug deactivators with the tinea pedis model, in which the infected site has a thick stratum corneum. A carry-over effect was not observed for LLCZ or the reference drugs under the test conditions used in this study. The skin samples were collected two weeks after the completion of drug treatment, and therefore most of the drug retained in the stratum corneum may have been eliminated by the turnover of the horny layer.

In conclusion, the results of the present study clearly indicate that the 1% LLCZ cream is sufficiently potent for short-term treatment of dermatophytosis compared to existing drugs. The use of 1% LLCZ cream for short-term treatment of dermatophytosis may encourage patients’ adherence to the treatment regimen and contribute to reducing the relapse rate.
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

FIG. 1. Chemical structure of luliconazole.

FIG. 2. Macroscopic improvement of skin lesions in experimental tinea corporis in guinea pigs treated with different concentrations of luliconazole cream and 1% bifonazole cream (Dose response study). Topical treatment (†) was started on day 3 post-inoculation and continued once daily for 7 consecutive days. * p<0.05 versus non-treated and # p<0.05 versus cream base on day 14 post-inoculation by Steel’s multiple comparison rank sum test (n=10).

FIG. 3. Macroscopic improvement of skin lesion in experimental tinea corporis in guinea pigs treated with 1% luliconazole cream, 1% terbinafine cream and 1% bifonazole cream in a 4- and 8-day treatment regimens (short-term treatment study). Topical treatment (†) was started on day 5 post-inoculation and continued once daily for 4 or 8 consecutive days. * p<0.05 versus non-treated and # p<0.05 versus cream base on day 17 post-inoculation by Steel’s multiple comparison rank sum test (n=10).
TABLE 1.

1: Topical treatment was started on day 3 post-inoculation and continued once daily for 7 consecutive days. The culture assay was performed on day 14 post-inoculation. 2: Topical treatment was started on day 5 post-inoculation and continued once daily for 4 or 8 consecutive days. The culture assay was performed on day 17 post-inoculation. *p<0.05 versus comparative non-treated and \(^{b}p<0.05\) versus comparative cream base by Fisher’s exact probability test (n=10). \(^{c}p<0.05\) versus comparative non-treated and \(^{d}p<0.05\) versus comparative cream base by Steel’s multiple comparison rank sum test (n=10).

TABLE 2.

Drug was topically applied once daily for 7 or 14 consecutive days. The culture assay was performed on 14 days after the completion of the drug treatment with or without a deactivators-supplemented medium. *p<0.05 versus comparative non-treated by Fisher’s exact probability test (n=8). \(^{#}p<0.05\) versus comparative non-treated by Steel’s multiple comparison rank sum test (n=8).


34. Watanabe, S., H. Takahashi, T. Nishikawa, I. Takiuchi, N. Higashi, K. Nishimoto, S. Kagawa, H. Yamaguchi, and H. Ogawa. 2007. Dose-finding comparative study of 2 weeks of luliconazole cream treatment for tinea pedis – comparison between three groups (1%, 0.5%, 0.1%) by a multi-center randomised double-blind study. Mycoses. 50:35-40.
FIG. 2. Macromeric improvement of skin lesions in experimental fowlpox in guinea pigs treated with different concentrations of bacitracin and 0.5% bifenazate cream (Dose response study). Topical treatment (T) was started on day 3 post-inoculation and continued once daily for 7 consecutive days. *p<0.05 versus non-treated and Tp<0.05 versus cream base on day 14 post-inoculation by Steel's multiple comparison rank sum test (n=10).
Fig. 3. Macrophagic improvement of skin lesion in experimental mice corpos in guinea pigs treated with 1% bicinecarinic ointment, 1% terbinafine cream, and 1% bicinecarinic cream in a 4- and 8-day treatment regimen (short-term treatment study). Topical treatment (T) was started on day 5 post-inoculation and continued once daily for 4 or 8 consecutive days. *p<0.05 versus non-treated and **p<0.05 versus cream base on day 17 post-inoculation by Steel’s multiple comparison rank sum test (n=10).
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Culture-positive rate (%)</th>
<th>Fungal burden score (mean ± SE)</th>
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<td><strong>Dose response study</strong> 1)</td>
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<tr>
<td>Non-treated</td>
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<td>Cream base</td>
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<td>Luliconazole cream</td>
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<td>0.02%</td>
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<td>0.1%</td>
<td>60 (^{ab})</td>
<td>1.2 ± 0.4 (^{cd})</td>
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<td>0.5%</td>
<td>0 (^{ab})</td>
<td>0 (^{cd})</td>
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<tr>
<td>1.0%</td>
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<td>0 (^{cd})</td>
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<tr>
<td>1.0% bifonazole cream</td>
<td>90</td>
<td>3.0 ± 1.0 (^{c})</td>
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<td><strong>Short-term treatment study</strong> 2)</td>
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<td>Non-treated</td>
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<td>Cream base</td>
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<td>1% terbinafine cream</td>
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<td>0 (^{cd})</td>
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<tr>
<td>1% terbinafine cream</td>
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<td>1.4 ± 0.5 (^{c})</td>
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<tr>
<td>1% bifonazole cream</td>
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1): Topical treatment was started on day 3 post-inoculation and continued once daily for 7 consecutive days. The culture assay was performed on day 14 post-inoculation. 2): Topical treatment was started on day 5 post-inoculation and continued once daily for 4 or 8 consecutive days. The culture assay was performed on day 17 post-inoculation. \(^{a}\) p<0.05 versus comparative non-treated and \(^{b}\) p<0.05 versus comparative cream base by Fisher’s exact probability test (n=10). \(^{c}\) p<0.05 versus comparative non-treated and \(^{d}\) p<0.05 versus comparative cream base by Steel’s multiple comparison rank sum test (n=10).
### Table 2

**TABLE 2.** Mycological efficacies of 1% luliconazole cream and reference drugs in experimental tinea pedis in guinea pigs.

<table>
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<th>Treatment</th>
<th>Culture-positive rate (%)</th>
<th>Fungal burden score (mean ±SE)</th>
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<tr>
<td>(7 days treatment)</td>
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<td>Non-treated</td>
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<td>9.9 ± 0.1</td>
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<td>1% luliconazole cream</td>
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<td>1% terbinafine cream</td>
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<td>1% bifonazole cream</td>
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<td>(14 days treatment)</td>
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<tr>
<td>Non-treated</td>
<td>100</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td>1% luliconazole cream</td>
<td>0*</td>
<td>0#</td>
</tr>
<tr>
<td>1% terbinafine cream</td>
<td>25</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>1% bifonazole cream</td>
<td>100</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td><strong>Short-term treatment study using a deactivators-supplemented medium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7 days treatment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>100</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td>1.0% luliconazole cream</td>
<td>0*</td>
<td>0#</td>
</tr>
<tr>
<td>1.0% terbinafine cream</td>
<td>75</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>1.0% bifonazole cream</td>
<td>100</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>(14 days treatment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>100</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>1.0% luliconazole cream</td>
<td>0*</td>
<td>0#</td>
</tr>
<tr>
<td>1.0% terbinafine cream</td>
<td>0*</td>
<td>0#</td>
</tr>
<tr>
<td>1.0% bifonazole cream</td>
<td>100</td>
<td>6.0 ± 0.9</td>
</tr>
</tbody>
</table>

Drug was topically applied once daily for 7 or 14 consecutive days. The culture assay was performed on 14 days after the completion of the drug treatment with or without a deactivators-supplemented medium. *p<0.05 versus comparative non-treated by Fisher’s exact probability test (n=8). #p<0.05 versus comparative non-treated by Steel’s multiple comparison rank sum test (n=8).