Journal of Antimicrobial Chemotherapy (1997) **40**, 441–444

JAC

Therapeutic effects of omoconazole nitrate on experimental tinea pedis, an intractable dermatophytosis, in guinea-pigs

Toshio Itoyama[a]*, Katsuhisa Uchida[a], Hideyo Yamaguchi[a,b] and Shigeru Fujita[c]

[a]Teikyo University Institute of Medical Mycology, 359 Otsuka, Hachioji-shi, Tokyo 192-03; [b]Department of Microbiology and Immunology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo 173; [c]Department of Dermatology, Tachikawa General Hospital, 3-2-11 Kanda, Nagaoka, Niigata 940, Japan

The therapeutic efficacy of omoconazole nitrate was investigated in an experimental tinea pedis model produced by topical inoculation with *Trichophyton mentagrophytes* in guinea-pigs, which is pathologically similar to naturally infected tinea pedis in humans.Treatment with omoconazole nitrate cream was started on week 2 postinfection and continued for 3 or 4 weeks. Once-a-day application of 1% omoconazole nitrate to the site of infection exhibited an excellent therapeutic efficacy, and was superior to 1% bifonazole cream in culture result. This result suggests that omoconazole nitrate has a potential usefulness for the treatment of tinea pedis in humans.

Materials and methods

Female Hartley strain guinea-pigs, 300–350 g in body weight, purchased from Sankyo Laboratory Service Co., were used. Animals were maintained in an air-conditioned room at 23 ± 2ºC and were allowed access to food and water *ad libitum*.

A zoophilic isolate, *Trichophyton mentagrophytes* TIMM 2789 (SM110), stored in the Research Centre, was used to infect guinea-pigs. Against this strain, the MIC value of omoconazole nitrate was 1.25 mg/L and that of bifonazole was 2.5 mg/L on Sabouraud dextrose agar (SDA) when incubated at 27ºC for 4 days. Conidia were harvested from cultures grown on K agar slants (0.2% Bacto-peptone, 0.1% glucose, 0.1% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$ and 2% agar)[1] at 27ºC for 3 weeks. The conidial fluid suspended with sterile saline supplemented with 0.05% Tween 80 was finally adjusted to 1×10^8 conidia/mL for use as the inoculum.

The procedures for the infection of guinea-pigs' dermatophytes were the same as reported previously.[2,3] Plantar parts of the hind feet were the sites of inoculation of the fungus. The locus was cleaned with a cotton swab dipped with 80% v/v ethanol. Adhesive tape (12 mm × 24 mm; Band Aid, Johnson & Johnson Co., Ltd, Japan) was dampened with 0.1 mL of the conidial suspension (1×10^8 conidia/mL), fixed on to the plantar part of the foot by covering with adhesive elastic tape (Elastopore; Nichiban Co., Ltd, Japan), and allowed to remain for 7 days to develop local infection.

The topical preparations of omoconazole nitrate and placebo preparation (vehicle) were provided by Hisamitsu Pharmaceutical Co., Japan, and a commercially available preparation of 1% bifonazole (Mycospor) was used as the reference drug. Once-a-day topical application of preparations was commenced on week 2 postinfection and continued for three or four consecutive weeks. Cream preparations in the daily dose of 0.5 g were applied uniformly, using sterilized spatulas, to the entire infected foot.

Two days after the last treatment, all animals were killed using ether anaesthesia and the skin of the infected sites was excised and cut into ten small blocks. All skin blocks were implanted on to SDA plates containing cycloheximide 500 mg/L, mezlocillin 50 mg/L and sisomicin 50 mg/L and incubated at 27ºC for 10 days. The skin blocks yielding fungal growth were regarded as culture-positive, and the infected site with more than one culture-positive skin block was considered fungus-positive. In addition, the intensity of infection was given a score of 10+ to zero according to the number of culture-positive skin blocks amongst the ten skin blocks studied.

Statistical analyses for the average intensity of infection were done by the Mann–Whitney *U* test, respectively.[4] Values of $P < 0.05$ were considered significant.

*Corresponding author. Present address: Hisamitsu Pharmaceutical Co., Inc., 1-25-11 Kannondai,Tsukuba, Ibaraki 305, Japan. Tel: +81-298-37-0701; Fax: +81-298-37-0685.

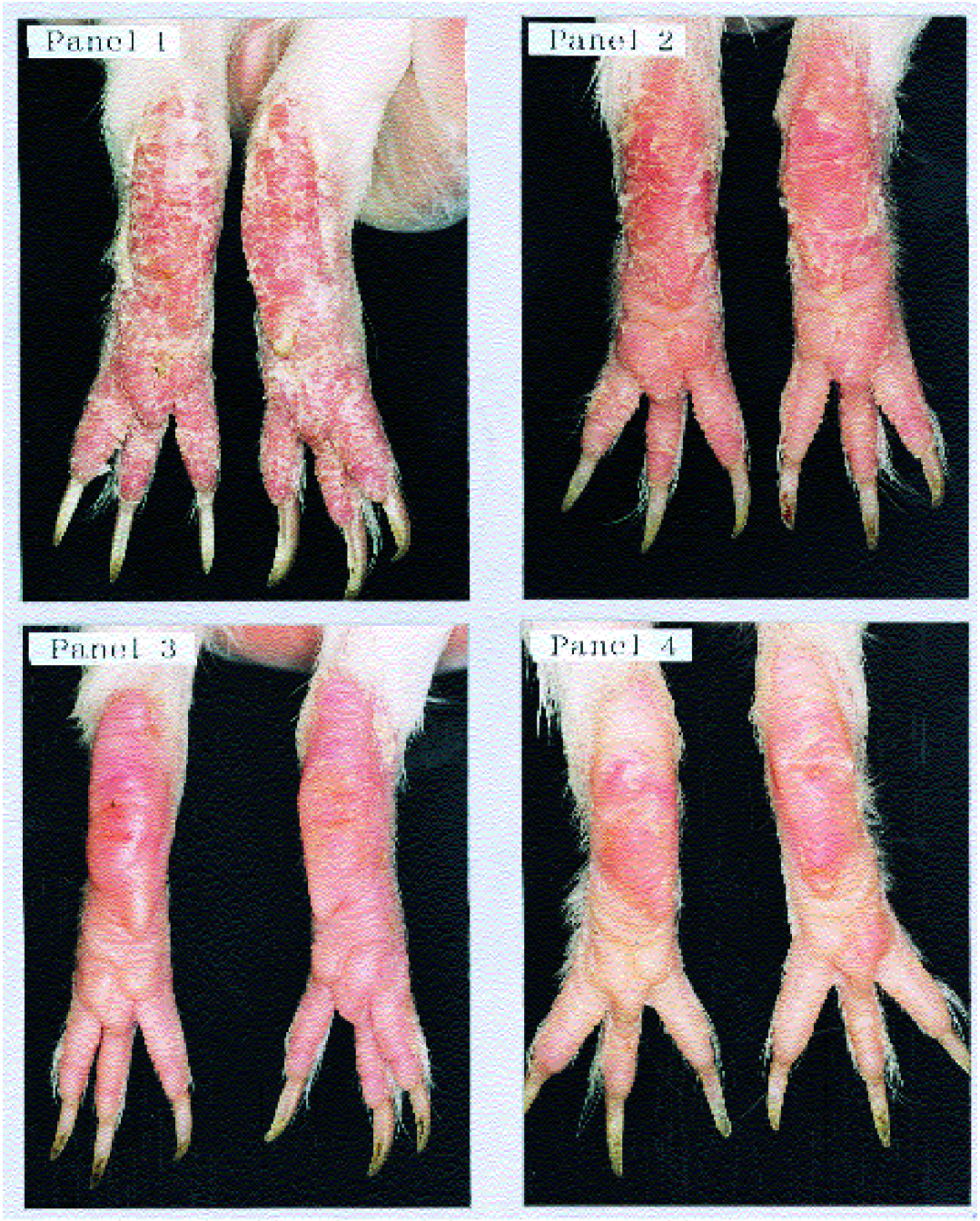

Figure. Preclinical findings of the hind leg of *T. mentagrophytes*-infected guinea-pigs as influenced by several different treatments. Panel 1, the infected feet of an animal in the untreated group 6 weeks postinfection show erythema and scale formation expanding over the whole plantar surface. Panel 2, the infected feet of an animal in the vehicle-treated group 6 weeks postinfection show skin lesions which were moister than those in the untreated group, with severe scale formation. Panels 3 and 4, the infected feet of an animal in the 1% omoconazole nitrate-treated group and the 1% bifonazole-treated group, respectively, 6 weeks postinfection show a gradual decrease in the severity of swelling, inflammation and scale formation.

Results and discussion

Dermatophytosis produced at a hairy skin site such as the back of a guinea-pig is pathologically similar to kerion celsi but quite different from tinea corporis or tinea pedis in humans.[2,3] The guinea-pig model of tinea pedis, which is persistent and similar to natural infection in humans, was successfully produced by Fujita and his colleagues, who used *T. mentagrophytes* TIMM 2789 (SM 110) as the inoculum.[2,3] This strain is a zoophilic dermatophyte characterized by deep perforation in hair and a clear proteolytic activity for bovine serum albumin.[5] It was

Table. Therapeutic efficacies of 1% omoconazole nitrate cream and 1% bifonazole cream in guinea-pig tinea pedis after 3 week and 4 week treatment

Treatment	No. of feet with negative culture (n = 10)	Average intensity of infection (n = 10)		
3 week treatment				
untreated	0	+10.0		
cream vehicle	0	+10.0	$P < 0.01$	NS
omoconazole nitrate 1.0%	0	+5.0	$P < 0.05$	
bifonazole 1.0%	0	+9.1		
4 week treatment				
untreated	0	+10.0		
cream vehicle	0	+10.0	$P < 0.01$	NS
omoconazole nitrate 1.0%	0	+1.8	$P < 0.01$	
bifonazole 1.0%	0	+8.8		

Statistical analyses for the average intensity of infection were assessed by the Mann–Whitney U test. NS, not significant.

reported in this tinea pedis model that hyphae quickly penetrated the horny layer of an inoculated foot within 24 h, invading the whole horny layer just above the granular layer and provoking a strong inflammatory response and clinical manifestations.[5] Virtually the same guinea-pig model of tinea pedis was used for the preclinical evaluation of two recently developed antifungal drugs for topical use, butenafine hydrochloride[6] and latoconazole.[7] In these studies, the treatment was started on day 10 postinfection. However, this model was originally developed to analyse the fungal invasion and spreading process of dermatophytes in the horny layer.[2] The hyphae of *T. mentagrophytes* had not yet invaded the tip of the toe or greater parts of the heel, and it took more than 26 weeks to spread over the sole.[2] It is therefore suggested that in this animal model the outcome of treatment starting on day 10 postinfection reflects both therapeutic and preventive efficacies. This may explain why relatively high cure rates were obtained for treatment even with inactive vehicle preparations in the previous studies.[6,7]

To evaluate only the therapeutic efficacy of certain active preparations, we modified an animal model in which infection was more severe and intractable; this was done using an adhesive tape dampened with conidial suspension and fixed all over the plantar part of the foot, so that 2 weeks after inoculation, erythema had expanded over the entire infected locus and was accompanied by remarkable signs of inflammatory responses of the skin and by intense scale formation. The scale formation and swelling of the infected skin became increasingly severe 5 or 6 weeks after inoculation. All the guinea-pig feet infected with *T. mentagrophytes* showed typical symptoms, which were pathologically similar to those of naturally infected tinea pedis in humans.

Once-a-day topical treatment with 1% omoconazole nitrate cream or 1% bifonazole cream was continued for 3 or 4 weeks after initiation on week 2 postinfection. As shown in the Figure, the infected skin of the animals treated with vehicle was rendered moister than that of the infected but untreated controls without decrease in scale formation. In comparison with these two controls, the preparation of omoconazole nitrate markedly reduced the local symptoms of swelling, inflammation and scale formation after 3 or 4 weeks of once-a-day treatment. A similar improvement of local symptoms was achieved when animals were treated with bifonazole.

The results of mycological examination of the infected sites are summarized in the Table. The non- and vehicle-treated sites yielded the highest rates of intensity of *T. mentagrophytes* infection, whereas marked reduction of the intensity was noted in the animal groups treated with omoconazole nitrate cream preparation; the value of intensity of infection was +5.0 after 3 week treatment and +1.8 after 4 week treatment with omoconazole nitrate cream, whereas the comparable values obtained with bifonazole treatment were +9.1 and +8.8, respectively. The reduction rate of infection in the omoconazole nitrate-treated group of animals was statistically significantly higher than that in the non-treated, vehicle-treated or bifonazole-treated ($P < 0.01$) group.

Thus, omoconazole nitrate is viewed as a promising topical antifungal agent and potentially useful in clinical situations.

References

1. Takashio, M. (1972). Sexual reproduction of some *Arthroderma* and *Nannizzia* on diluted Sabouraud agar with or without salts. *Mykosen* **15**, 11–17.

2. Fujita, S. & Matsuyama, T. (1987). Experimental tinea pedis induced by non-abrasive inoculation of *Trichophyton mentagrophytes* arthrospores on the plantar part of a guinea pig foot. *Journal of Medical and Veterinary Mycology* **25**, 203–13.

3. Fujita, S., Matsuyama, T. & Sato, Y. (1988). [Experimental tinea pedis in guinea pig feet—scanning electron microscopic and histological study of the infection.] *Japanese Journal of Medical Mycology* **29**, 163–8.

4. Uchida, K., Matsuzaka, A. & Yamaguchi, H. (1991). [Therapeutic effect of amorolfine on experimental dermatophytosis.] *Japanese Journal of Antibiotics* **44**, 1020–31.

5. Fujita, S., Kaneko, Y., Sato, Y. & Matsuyama, T. (1989). [Invasiveness of infecting fungi and tissue response in experimental tinea pedis in guinea pigs.] *Japanese Journal of Medical Mycology* **30**, 234–40.

6. Arika, T., Yokoo, M., Maeda, T., Amemiya, K. & Yamaguchi, H. (1990). Effects of butenafine hydrochloride, a new benzylamine derivative, on experimental tinea pedis in guinea pigs. *Antimicrobial Agents and Chemotherapy* **34**, 2254–5.

7. Oka, H., Niwano, Y., Ohmi, T., Tanaka, T., Uchida, M. & Yamaguchi, H. (1992). Therapeutic efficacy of latoconazole in formulations of clinical use on experimental dermatophytosis in guinea pigs. *Arzneimittel-Forschung / Drug Research* **42**, 345–9.

Received 11 October 1996; returned 11 November 1996; revised 14 January 1997; accepted 15 April 1997