

## NOTES

### Effects of Butenafine Hydrochloride, a New Benzylamine Derivative, on Experimental Tinea Pedis in Guinea Pigs

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**Butenafine is a new antifungal benzylamine. The efficacy of butenafine was investigated in an experimental tinea pedis model in guinea pigs, which is pathologically similar to natural infections in humans. Butenafine (0.1 ml) in 0.2 to 1.0% solutions was applied to the site of infection. Treatment was started on day 10 postinfection and was continued for 20 days. Butenafine applied once daily exhibited excellent dose-related therapeutic efficacy. The efficacy of butenafine was significantly superior to those of tolnaftate, clotrimazole, and bifonazole.**

*N*-4-*tert*-Butylbenzyl-*N*-methyl-1-naphthalenemethylamine hydrochloride (butenafine) has a broad spectrum of antifungal activity and is particularly active in vitro against dermatophytes, aspergilli, dimorphic fungi, and dematiaceous fungi (T. Maeda, T. Arika, K. Amemiya, and K. Sasaki, Chem. Pharm. Bull., in press). Butenafine showed excellent therapeutic efficacy against the conventional dermatophytosis caused by *Trichophyton mentagrophytes* or *Microsporum canis* in guinea pigs. This efficacy might be attributable to its fungicidal activity and prolonged cutaneous retention (1).

Dermatophytic infections produced in the dorsal skin of guinea pigs has been widely used as an experimental model for evaluating the antidermatophytic activity of topical antifungal agents. However, this dermatophytosis model is not necessarily satisfactory in that the course of infection is not as prolonged as it is in human dermatophytosis, such as tinea pedis.

Recently, Fujita and Matsuyama (2) have developed a new model of tinea pedis by inoculating *T. mentagrophytes* into the planta of guinea pigs. The infection lasts for more than 6 months without spontaneous healing and histopathologically and symptomatically mimics human infections.

In the present study, we investigated the therapeutic efficacy of topical butenafine using this experimental model.

(These data were presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, 4 to 7 October 1987, New York [T. Arika, M. Yokoo, T. Maeda, K. Amemiya, and K. Sasaki, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 980, 1987].)

Male Hartley strain guinea pigs (weight, 450 to 600 g) were divided into groups of 8 to 10 animals.

A clinical isolate of *T. mentagrophytes*, KD-04, was kindly supplied by H. Takahashi, Teikyo University School of Medicine, Tokyo, Japan. *T. mentagrophytes* was grown on potato sucrose agar at 27°C for 3 weeks. The culture was flooded with sterile saline containing 0.1% Tween 80, and the

fungal growth was scraped off with a loop. The fungal suspension was then pipetted into a test tube. After shaking, the suspension was filtered through gauze. Then, it was adjusted to yield a final conidial concentration of  $2 \times 10^7$  cells per ml after enumeration with a hemacytometer.

Skin infection was induced by a slight modification of the method by Fujita and Matsuyama (2). In brief, the site of inoculation was the planta of the hind paw of guinea pigs. A paper disk (diameter, 13 mm; AA disk; Whatman) was wetted with 50  $\mu$ l ( $10^6$  cells) of the conidial suspension, applied onto the planta with a form pad (Reston Self-adhering Form Pads No. 1560; 3M Co.), and fixed with an adhesive elastic tape (Elastopore, Nichiban, Japan). The disk was removed on day 7 postinfection. This infection did not heal spontaneously over a 6-month period.

Butenafine (lot 840317), naftifine (lot 831011), and clotrimazole (lot 840614) were synthesized in our laboratories. Tolnaftate (lot 8509) was purchased from Sagami Kasei Kogyo, Co., Ltd., Machida, Japan. Butenafine, naftifine, and clotrimazole were dissolved in polyethylene glycol 400-ethanol (75:25; vol/vol); and tolnaftate was dissolved in polyethylene glycol 400-acetone (75:25; vol/vol) (3). Bifonazole cream (lot B019) and clotrimazole cream (lot B189) were purchased from Bayer Yakuhin Ltd., Osaka, Japan. Each guinea pig was treated with a 0.1-ml volume of the test compound as a solution or cream. The treatment with butenafine and reference drugs was started on day 10 postinfection (3 days after disk removal).

On day 2 after the last treatment, all animals were sacrificed under anesthesia, and the infected sites were washed with a soap solution and thoroughly rinsed. Twelve skin sections were made from all parts of the infected planta. Each section was implanted onto a Sabouraud glucose agar plate containing 500  $\mu$ g of cycloheximide per ml, 50  $\mu$ g of kanamycin per ml, and 5  $\mu$ g of gentamicin per ml and cultured at 27°C for 10 days. The treatment was assessed as effective if no growth was detected.

The percentage of negative cultures was tested by the  $\chi^2$  test. *P* values of less than 0.05 were regarded as significant.

In the first experiment, once daily treatment with 0.2, 0.5, and 1.0% solutions of butenafine was started on day 10

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TABLE 1. Therapeutic efficacy of butenafine solution in guinea pig tinea pedis by once daily application for 20 days

Treatment	No. (%) of skin sections with negative cultures (n = 240)	No. of feet with negative cultures (n = 20)
0.2% Butenafine	152 (63.3) <sup>a</sup>	0
0.5% Butenafine	179 (74.6) <sup>b</sup>	4
1.0% Butenafine	219 (91.3) <sup>c</sup>	10 <sup>d</sup>
Placebo	18 (7.5)	0
None	12 (5.0)	0

<sup>a</sup>  $P < 0.001$  versus placebo control.

<sup>b</sup>  $P < 0.05$  versus the 0.2% butenafine treatment group.

<sup>c</sup>  $P < 0.001$  versus the 0.5% butenafine treatment group.

<sup>d</sup>  $P < 0.01$  versus placebo control.

postinfection and was continued for 20 days. The results of the culture studies performed with tissue specimens excised from the planta on day 31 postinfection are given in Table 1. A dose-related therapeutic efficacy of butenafine was observed, with mycological eradication being noted in 91.8% of skin sections treated with a 1% solution of butenafine.

In the second experiment, the therapeutic efficacy of a 1% solution of butenafine was investigated in comparison with and compared with the efficacies of a 1% solution of naftifine, a 2% solution of tolnaftate, and a 1% solution of clotrimazole. The efficacy of butenafine was significantly superior to those of tolnaftate and clotrimazole (Table 2). Naftifine exhibited potent activity that was almost the same as that of butenafine. In another study with 1% creams of butenafine, clotrimazole, and bifonazole, butenafine and clotrimazole exhibited activities that were as potent as their solutions, with the efficacy of butenafine cream being superior to those of bifonazole and clotrimazole creams.

As documented in our previous report (1), 0.01 to 1.0% solutions of butenafine show excellent therapeutic efficacy when applied topically for 10 days in the conventional dermatophytosis models produced on the backs of guinea pigs. Compared with those conventional models, a longer duration of treatment was required to attain complete mycological cure in the tinea pedis model used in the present study. Because there is a distinct difference in the thickness of the horny layer between the dorsal skin and the planta in guinea pigs, the efficacies of antifungal agents may be associated with the thickness of this layer. It is well known from clinical studies that tinea pedis usually responds to antifungal chemotherapy to a lesser extent than does tinea

TABLE 2. Therapeutic efficacies of butenafine, naftifine, tolnaftate, clotrimazole, and bifonazole in guinea pig tinea pedis by once daily application for 20 days

Treatment	No. of skin sections with negative cultures/total no. of skin sections from infected sites (%)	No. of feet with negative cultures/total no.
1% Butenafine solution	214/240 (89.2)	9/20 <sup>a</sup>
1% Naftifine solution	209/240 (87.1)	6/20 <sup>b</sup>
2% Tolnaftate solution	153/240 (63.8) <sup>c</sup>	4/20
1% Clotrimazole solution	95/240 (39.6) <sup>c</sup>	0/20
None	40/240 (16.7) <sup>c</sup>	0/20
1% Butenafine cream	170/192 (88.5)	9/16 <sup>a</sup>
1% Bifonazole cream	60/192 (31.3) <sup>c</sup>	0/16
1% Clotrimazole cream	52/192 (27.1) <sup>c</sup>	0/16
None	18/192 (9.4) <sup>c</sup>	0/16

<sup>a</sup>  $P < 0.01$  versus the no treatment group.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.001$  versus butenafine treatment group.

corporis or tinea cruris (4). From this viewpoint, the experimental tinea pedis in guinea pigs seems more appropriate for prediction of the activity of antifungal agents against tinea pedis in humans.

Together with our previous results (1), results of this study indicate that topical butenafine may be promising for the treatment of all types of dermatophytosis, including tinea pedis.

#### LITERATURE CITED

1. Arika, T., M. Yokoo, T. Hase, T. Maeda, K. Amemiya, and H. Yamaguchi. 1990. Effects of butenafine hydrochloride, a new benzylamine derivative, on experimental dermatophytosis in guinea pigs. *Antimicrob. Agents Chemother.* **34**:2250-2253.
2. Fujita, S., and T. Matsuyama. 1987. Experimental tinea pedis by non-abrasive inoculation of *Trichophyton mentagrophytes* arthrospores on the plantar part of guinea pig foot. *J. Med. Vet. Mycol.* **25**:203-213.
3. Petranyi, G., A. Georgopoulos, and H. Mieth. 1981. In vivo antimycotic activity of naftifine. *Antimicrob. Agents Chemother.* **19**:390-392.
4. Takahashi, H., A. Hasegawa, O. Kaneko, A. Saito, and Y. Tanaka. 1986. Clinical studies of fungicidal agents with respect to their relationship *in vitro* and *in vivo*, p. 227-240. In K. Iwata and H. Vanden Bossche (ed.), *In vitro and in vivo evaluation of antifungal agents*. Elsevier Science Publishers, Amsterdam.