

Therapeutic Efficacy of Lanconazole, a New Imidazole Antimycotic Agent, for Experimental Cutaneous Candidiasis in Guinea Pigs

YOSHIMI NIWANO,^{1*} AKIRA SEO,² KAZUO KANAI,¹ HIROSHI HAMAGUCHI,¹
KATSUHISA UCHIDA,³ AND HIDEYO YAMAGUCHI³

Pharmaceutical Research Institute, Nihon Nohyaku Co., Ltd., Kawachinagano, Osaka 586,¹ Chemical Research Center, Nihon Nohyaku Co., Ltd., 5-1-34 Tsukuda, Nishiyodogawa-ku, Osaka 555,² and Research Center for Medical Mycology, Teikyo University, 359 Otsuka, Hachioji, Tokyo 192-03,³ Japan

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The therapeutic efficacy of 1% cream and 1% solution of lanconazole, a new imidazole antimycotic agent, in the model of cutaneous candidiasis in prednisolone-treated guinea pigs was evaluated in comparison with that of comparable formulations of bifonazole. Each preparation was topically applied once a day for 3 consecutive days, starting on the fifth day postinfection, and quantitative culture study was conducted on the ninth day postinfection. Both formulations of lanconazole were much more highly effective in terms of eradication of fungi than the bifonazole formulations.

Lanconazole, (\pm)-(E-[4-(2-chlorophenyl)-1,3-dithiolan-2-ylidene]-1-imidazolyl)acetonitrile (the structure is shown in Fig. 1), is a new imidazole antimycotic agent. Clinical trials with 1% cream and solution preparations of the agent recently completed in Japan demonstrated its therapeutic effectiveness for various dermatomycoses, including tinea pedis, tinea corporis, and cutaneous candidiasis (7). Preclinically, lanconazole exhibited excellent therapeutic efficacy in models of tinea corporis and tinea pedis in guinea pigs (4, 6). Preclinical evaluation for cutaneous candidiasis, however, has not yet been done, since no appropriate animal model of this dermatomycosis has been available.

Recently, Maebashi et al. (3) successfully induced *Candida* infection in prednisolone-treated guinea pigs topically inoculated with *Candida albicans* TIMM 2640 cells on the skin of their back region. Using this guinea pig model of cutaneous candidiasis, we performed preclinical evaluation of the in vivo activity of the clinical preparations of lanconazole.

Male Hartley guinea pigs (Japan SLC Inc., Shizuoka, Japan) weighing 500 to 700 g were used for the infection study. The animals were maintained in an air-conditioned room at $23 \pm 1^\circ\text{C}$ and were allowed access to feed and water ad libitum. *C. albicans* TIMM 2640 was maintained on Sabouraud's glucose agar slants. For in vivo studies, cultures were grown aerobically in yeast carbon base broth containing 0.2% (wt/vol) bovine serum albumin at 37°C . After 4 days of incubation, the cells were harvested and adjusted to 2×10^8 cells per ml in the same medium for use as the inoculum.

Lanconazole was synthesized by Sumika Fine Chemical Co., Ltd. (Osaka, Japan), and 1% cream and 1% solution of the agent were formulated by Tsumura & Co. (Tokyo, Japan). One percent cream and 1% solution of bifonazole (Mycospor; Bayer Yakuin Ltd., Osaka, Japan) were used as reference agents.

The procedures for infection were essentially identical to those described by Maebashi et al. (3). Guinea pigs were clipped on their backs, and an area with a 2-cm diameter within

the clipped zone was allocated for infection (two loci per animal). Each locus was inoculated with a fungal suspension in a volume of 0.1 ml (2×10^7 cells per locus). Prednisolone (Mitaka Pharmaceutical Co., Ltd., Tokyo, Japan) was subcutaneously administered four times (2 days and immediately before and 2 and 4 days after the inoculation) at a dose of 30 mg/kg of body weight each time. A placebo or active formulations (0.2 g of cream and 0.2 ml of solution per locus) were topically applied once a day for 3 consecutive days, starting on the fifth day postinfection. On the ninth day postinfection, all of the animals were sacrificed and the skin of the infected area was excised and homogenized in sterile physiological saline. A portion of the homogenate was cultured on a peptone yeast-extract glucose agar (2% Bacto Peptone, 1% yeast extract, 2% glucose, 1.5% agar) plate for 2 days at 37°C . Colonies grown on the agar plate were counted to calculate the number of CFU per locus.

Statistical analysis for the number of colonies in the in vivo study was done by Dunnett's multiple comparison test. *P* values of <0.05 were considered significant.

To test the in vitro susceptibility of *C. albicans* TIMM 2640 to lanconazole, as well as to bifonazole (Sigma Chemical Co., St. Louis, Mo.), the fungal suspension was prepared in Sabouraud's glucose broth, with the cell count adjusted to 10^4 cells per ml. In test tubes, 9.9 ml of the fungal suspension was mixed with 0.1 ml of each testing agent dissolved in dimethyl sulfoxide. All of the tubes were incubated at 37°C with shaking, and the inhibitory activity of each agent against fungal growth was monitored for up to 96 h by reading the optical density at 650 nm.

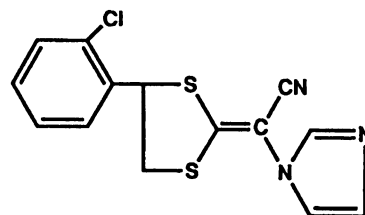


FIG. 1. Chemical structure of lanconazole.

* Corresponding author. Mailing address: Pharmaceutical Research Institute, Nihon Nohyaku Co., Ltd., 326-2 Oyamada-cho, Kawachinagano, Osaka 586, Japan. Phone: (0721)56-9000. Fax: (0721)55-1622.

TABLE 1. Effects of topically applied lanconazole and bifonazole on experimental cutaneous candidiasis in prednisolone-treated guinea pigs

Treatment	CFU/infected locus ^a
None (control).....	12,130 ± 2,308
Cream vehicle ^b	8,070 ± 1,493
1% LCZ ^c cream.....	4,470 ± 921 A
1% BFZ ^d cream.....	10,620 ± 2,770
Solvent ^e	7,060 ± 1,294
1% LCZ solution.....	2,220 ± 409 ABC
1% BFZ solution.....	9,070 ± 2,097

^a Each value is the mean ± the standard error of the mean. The total number of loci for each group was 10. Significant differences from the untreated control, the comparable solvent, and the comparable formulations of bifonazole are shown as $P < 0.01$ (A), $P < 0.01$ (B), and $P < 0.01$ (C), respectively.

^b Cream vehicle of 1% lanconazole cream.

^c LCZ, lanconazole.

^d BFZ, bifonazole.

^e Solvent of 1% lanconazole solution.

The results of the in vivo studies performed with the cutaneous-candidiasis model are shown in Table 1. Both of the placebo-treated groups of animals showed the slight tendency to have lower colony counts than did the untreated control group of animals. The cream vehicle contained 0.14% (wt/wt) methylparaben and 0.06% (wt/wt) propylparaben, both of which are known to have antimicrobial activity (1, 2, 8), and the solvent contained 48% (vol/vol) ethanol. Therefore, the placebo effects may be attributable to these constituents. The colony counts of the group of animals treated with 1% cream of lanconazole were significantly lower than those of the untreated control group of animals and tended to be lower

than those of the comparable placebo-treated group of animals. Similarly, significantly lower colony counts were obtained with the group of animals treated with 1% solution of lanconazole than were obtained with the untreated control and the comparable placebo-treated groups of animals. Although the colony counts of both of the bifonazole-treated groups of animals appeared to be lower than those of the untreated control group, there was no significant difference between the two groups.

The effects of lanconazole and bifonazole on the in vitro growth of *C. albicans* cultures are shown in Fig. 2. Lanconazole at concentrations higher than 0.1 µg/ml revealed strong inhibition of fungal growth in a dose-dependent manner, in contrast to the growth of the control culture. The inhibitory activity of bifonazole was clearly weaker than that of lanconazole at concentrations of 0.1 and 1 µg/ml.

In the present guinea pig model of cutaneous candidiasis, 1% cream and 1% solution of lanconazole showed activity that was significantly superior to that of comparable 1% formulations of bifonazole. The in vitro anti-*Candida* activity of lanconazole was also greater than that of bifonazole, particularly at relatively low concentrations, which was probably due to more potent inhibition by lanconazole of ergosterol synthesis in *C. albicans* (5). This is considered contributory to the excellent therapeutic efficacy of this new imidazole antimycotic agent in the animal model used in the present study.

The results of these preclinical studies favorably support those of clinical trials which proved that both 1% cream and 1% solution of lanconazole had excellent therapeutic efficacy in cutaneous candidiasis (7).

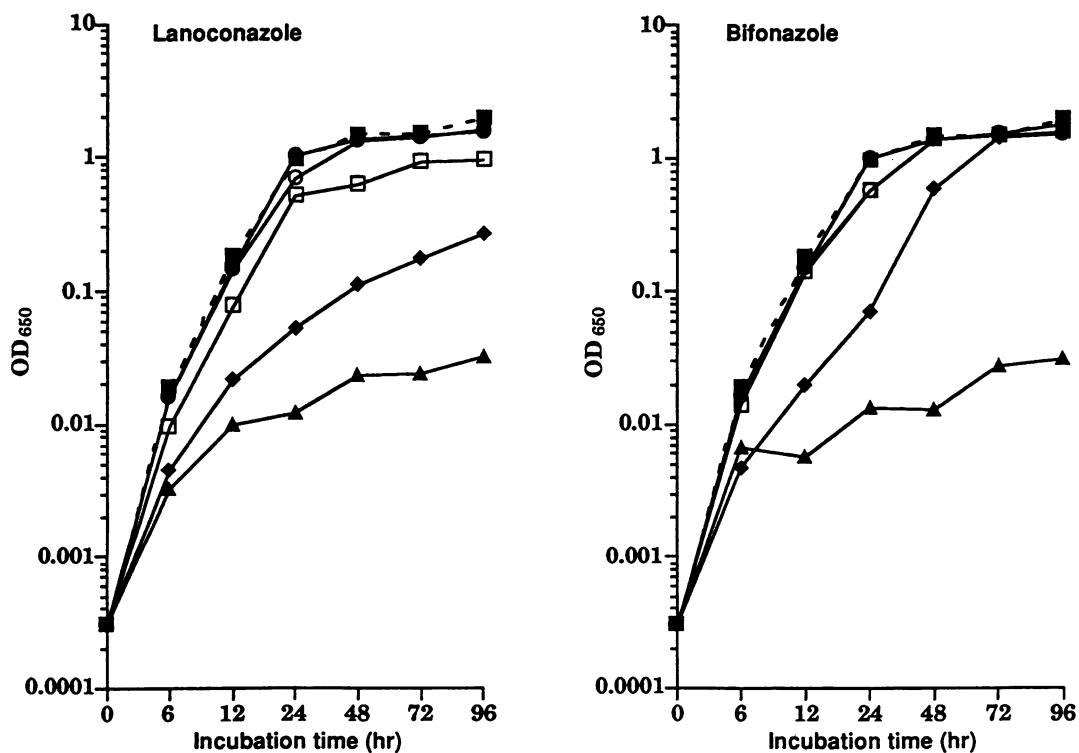


FIG. 2. Effects of lanconazole and bifonazole on the growth of *C. albicans* TIMM 2640. OD₆₅₀, optical density at 650 nm. Symbols: ■, control; ●, dimethyl sulfoxide; ○, 0.01 µg/ml; □, 0.1 µg/ml; ◆, 1 µg/ml; ▲, 10 µg/ml.

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