In Vitro Antifungal Activity of KP-103, a Novel Triazole Derivative, and Its Therapeutic Efficacy against Experimental Plantar Tinea Pedis and Cutaneous Candidiasis in Guinea Pigs

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The in vitro activity of KP-103, a novel triazole derivative, against pathogenic fungi that cause dermatomycoses and its therapeutic efficacy against plantar tinea pedis and cutaneous candidiasis in guinea pigs were investigated. MICs were determined by a broth microdilution method with morpholinepropanesulfonic acid-buffered RPMI 1640 medium for Candida species and with Sabouraud dextrose broth for dermatophytes and by an agar dilution method with medium C for Malassezia furfur. KP-103 was the most active of all the drugs tested against Candida albicans (geometric mean [GM] MIC, 0.002 μg/ml), other Candida species including Candida parapsilosis and Candida glabrata (GM MICS, 0.0039 to 0.0442 μg/ml), and M. furfur (GM MIC, 0.025 μg/ml). KP-103 (1% solution) was highly effective as a treatment for guinea pigs with cutaneous candidiasis and achieved mycological eradication in 8 of the 10 infected animals, whereas none of the imidazoles tested (1% solutions) was effective in even reducing the levels of the infecting fungi. KP-103 was as active as clotrimazole and nitrofurazone but was less active than lanoconazole and butenafine against Trichophyton rubrum (MIC at which 80% of isolates are inhibited [MIC80], 0.125 μg/ml) and Trichophyton mentagrophytes (MIC80, 0.25 μg/ml). However, KP-103 (1% solution) exerted therapeutic efficacy superior to that of nitrofurazone and comparable to those of lanoconazole and butenafine, yielding negative cultures for all samples from guinea pigs with plantar tinea pedis tested. This suggests that KP-103 has better pharmacokinetic properties in skin tissue than the reference drugs. Because the in vitro activity of KP-103, unlike those of the reference drugs, against T. mentagrophytes was not affected by hair as a keratinic substance, its excellent therapeutic efficacy seems to be attributable to good retention of its antifungal activity in skin tissue, in addition to its potency.

The imidazole antimycotics clotrimazole (12), miconazole (35), econazole (11), oxiconazole (28), and bifonazole (13) have been widely used for topical treatment of dermatomycoses. In recent years, an imidazole (lanconazole) (20) and three classes of antifungal compounds, a benzylamine (butenafine) (17), an allylamine (terbinafine) (27), and a morpholine (amorolfine) (32), have been successfully developed and introduced into clinical use. These newer drugs are more highly active than the former imidazoles against dermatophytes in vitro (17, 20, 27, 32) and in vivo in guinea pig models of dermatophytosis (2, 3, 4, 5, 25, 26, 29, 38).

Dermatophytes represented by Trichophyton mentagrophytes and Trichophyton rubrum parasitize the keratinized tissues of the horny layer of the epidermis, hair, and nails because they utilize keratin for their growth. Therefore, when antifungal agents are topically applied to the skin, therapeutic efficacy depends not only on their antifungal activities but also on their pharmacokinetic properties in skin tissue (31, 34). Several antifungal drugs such as lanoconazole (22), terbinafine (34), and butenafine (5) have been demonstrated to accumulate well in the horny layer when they are applied topically. However, almost all of these drugs appear to be inactivated in the horny layer because it was shown by in vitro experiments that they are strongly bound to keratin, with a resultant loss of the activity (1, 21; Y. Tatsumi, M. Yokoo, T. Arika, H. Ogura, K. Nagai, T. Naito, and H. Yamaguchi, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F79, p. 113, 1996.). It therefore appears likely that the persistence of an antifungal agent in an active form in the horny layer is a prerequisite for the exertion of sufficient therapeutic efficacy and that such a pharmacokinetic property is held by those drugs that have a low affinity for keratin.

In the course of screening for new antidermatophytic triazole compounds with a low affinity for keratin, (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methyleneepiperidino)-1-(1H-1,2,4-triazol-1-yl)-2-butanol, whose development code is KP-103 (Fig. 1), was selected (24). KP-103 has potent in vitro activity against dermatophytes, as well as against Candida and Malassezia species (Tatsumi et al., 36th ICAAC, abstr. 113), all of which are pathogens that are major causes of dermatomycoses. This compound is being developed as the first topical triazole antymycotic for the treatment of dermatomycoses.

In this paper, we describe the in vitro activity of KP-103 against dermatophytes and other pathogenic fungi and the effect of the addition of serum or hair to the assay medium on its in vitro activity, along with the in vivo activity of topically applied KP-103 in guinea pig models of plantar tinea pedis and cutaneous candidiasis.

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In vitro study. (i) Antifungal agents. KP-103, neticonazole hydrochloride (neticonazole), and lanconazole were synthesized at the Central Research Laboratories, Kaken Pharmaceutical Co., Ltd., Kyoto, Japan. Butenafine hydrochloride was obtained from the factory of Kaken Pharmaceutical Co., Ltd. (Shiga, Japan). Clotrimazole was purchased from Sigma Chemical Co., St. Louis, Mo. (ii) Media. Yeast morphology agar (YMA) contained 5 g of yeast extract, 5 g of malt extract, 5 g of peptone, 10 g of glucose, and 15 g of agar per liter. Liquid RPMI 1640 medium supplemented with L-glutamine (Nissui Seiyaku, Tokyo, Japan) was added to 2% sodium bicarbonate and 0.165 M morpholinepropanesulfonic acid (MOPS) and adjusted to pH 7.0. Sabouraud dextrose broth (SDB; 2% dextrose [pH 5.6]) and Sabouraud dextrose agar (SDA) were purchased from Difco Laboratories, Detroit, Mich. Potato dextrose agar (PDA) was purchased from Nichiban, Tokyo, Japan. Glucose Trypticase yeast-extract agar (GTYA) contained 1 g of glucose, 1 g of Trypticase (BBL, Becton Dickinson and Company, Cockeysville, Md.), 0.5 g of yeast extract, and 20 g of agar per liter and was adjusted to pH 5.8 with 1 N HCl. Medium C agar was prepared by the method of Faergermann and Bernander (9).

(iii) Organisms. A total of 143 strains of 16 fungal species were used in the study. Almost all of the strains used in susceptibility tests were clinical isolates collected from over 20 different medical centers in Japan.

(iv) Preparation of inocula. Dermatophytes were grown on GTYA or SDA slants at 30°C for 11 to 14 days. The mature culture was flooded with sterile saline containing 0.05% (vol/vol) Tween 80, scraped off with a platinum loop, and homogenized in a glass homogenizer. The suspension was adjusted to a concentration of 2 × 10^6 conidia or hyphal fragments per ml by counting in a hemocytometer. Then, the suspensions were diluted 100-fold with SDB to give a final inoculum of 2 × 10^5 cells per ml. All isolates of Candida species were subcultured at 35°C for 48 h on YMA plates. Yeast cells were recovered from at least five 1-mm-diameter colonies and suspended in 5 ml of sterile saline. The suspension was mixed for 15 s with a vortex mixer, and the turbidity of each suspension was adjusted to a 0.5 McFarland standard (corresponding to 1 × 10^8 to 5 × 10^8 CFU/ml) at a wavelength of 530 nm by the method of the National Committee for Clinical Laboratory Standards (NCCLS) (18). Each suspension was diluted 1,000-fold with RPMI 1640 medium to give a final inoculum of 1 × 10^4 to 5 × 10^4 CFU/ml.

Malassezia furfur and Malassezia pachydermatis were grown on PDA slants containing 1% yeast extract, 1% peptone, and 2% olive oil and on YMA slants, respectively, at 30°C for 5 days. The yeast cells were collected and suspended in sterile saline containing 0.05% Tween 80 to prepare an inoculum of 2 × 10^5 cells per ml by counting with a hemocytometer.

(v) Antifungal susceptibility tests. Susceptibility testing of dermatophytes was performed by the following method. The fungal inocula (100 μl) were added to each well of 96-well flat-bottom microdilution plates; each well contained 100 μl of twofold serial dilutions of the drugs tested (at 2% final concentrations) in RPMI 1640 medium. All plates were incubated at 35°C for 7 days.

The antifungal susceptibility of Candida species were tested by a slight modification (39) of the broth microdilution method outlined by NCCLS (18). The yeast inocula (100 μl) were added to each well of 96-well flat-bottom microdilution plates; each well contained 100 μl of twofold serial dilutions of the drugs (at 2% final concentrations) in RPMI 1640 medium. After each plate was incubated at 35°C for 24 h, the turbidity of each well was measured at 620 nm with a microplate reader.

The antifungal susceptibilities of the Malassezia species were determined by agar dilution methods with medium C agar and PDA containing 1% yeast extract, 1% peptone, and 2% olive oil. Five microliters of each inoculum (10^6 cells) was spotted with an inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto the agar plates containing twofold serial dilutions of drugs. All plates were incubated at 30°C for 5 days.

(vi) Endpoints for determining MICs and MFCs. The MICs of all drugs tested for the yeasts were defined as the lowest drug concentrations that yielded turbidity less than or equal to that for 80% inhibition compared with the growth in control wells. The MICs for dermatophytes and Malassezia species were defined as the lowest drug concentrations that inhibited visible growth of the fungi. The minimal fungicidal concentrations (MFCs) for dermatophytes were determined by subculturing 0.01 ml from each well with no visible growth onto an SDA plate. The plates were incubated at 30°C for 7 days. Afterward, the colonies were counted, and the MFC was defined as the lowest concentration of drug which yielded a negative subculture or less than two colonies (killing of more than 98% of the fungal inoculum).

(vii) Measurement of antidermatophytic activity in the presence of serum. The MICs for eight clinical isolates of T. mentagrophytes were determined as described above by using 10% human serum-containing SDB, whose pH was adjusted to 5.6 with 1 N NaOH, and the MICs were compared with those obtained with SDB only. The MICs were defined by the same endpoint described above.

(viii) Measurement of antidermatophytic activity in medium with hair. MICs and MFCs for T. mentagrophytes KD-04 were determined by the following method. Ten microliters of twofold serial dilutions of the drugs was dispensed into a tube containing 1 ml of medium with hair, which was prepared by suspending 50 mg of small pieces of defatted human hair per ml in saline. Each tube was seeded with 10 μl (10^6 cells) of the fungal inoculum and then incubated at 30°C for 7 days. For reference, a test was also run in which SDB was used in place of medium with hair.

The MICs were defined by the same endpoint described above. The MFCs were determined by subculturing pieces of hair or 100 μl of SDB cultures from all tubes with no visible growth onto SDA plates, and the MFC was defined as the lowest concentration of drug which yielded a negative subculture.

In vivo efficacy. (i) Animals. Male Hartley strain guinea pigs (weight, 375 to 470 g) were used in the study of in vivo efficacy. The experiments were done with groups of 10 animals.

(ii) Challenge organism. A clinical isolate of T. mentagrophytes, isolate KD-04, was kindly supplied by H. Takahashi, Teikyo University School of Medicine, Tokyo, Japan. A clinical isolate of Candida albicans, isolate KC-36, was isolated from a clinical specimen in Mitsui Commemoration Hospital, Tokyo, Japan.

(iii) Preparation of inocula. For a guinea pig plantar tinea pedis model, arthroconidia of T. mentagrophytes were prepared by the method of Fujita et al. (13). The arthroconidial suspension (0.1 ml, 10^6 cells) was applied to a petri dish (diameter, 47 mm; pore size, 0.45 μm; Millipore Corp.), the filter was placed on a plate of brain heart infusion agar (Nissui Seiyaku), and the plate was cultured in the presence of 17% carbon dioxide for 7 days at 30°C. Arthroconidia were harvested and suspended in saline containing 0.05% Tween 80. The suspension was homogenized in a glass homogenizer, filtered through gauze, and adjusted to give a concentration of 10^8 per ml by counting with a hemocytometer.

For a guinea pig cutaneous candidiasis model, C. albicans KC-36 was grown on an SDA slant for 2 days at 35°C. Yeast colonies were suspended in saline containing 0.05% Tween 80 to give 10^8 cells per ml by counting with a hemocytometer.

(iv) Production of plantar tinea pedis in guinea pigs. Plantar tinea pedis was induced by a previously described method (3). 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 dampened with 100 μl (10^5 cells) of the arthroconidial suspension, applied onto the planta with a foam pad (Reston self-adhering foam pads; No. 1560; 3M Co.), and fixed with an adhesive elastic tape (Elastopore; Nichiban, Tokyo, Japan) (day 0). The disks were removed on day 7 postinfection.

(v) Production of cutaneous candidiasis. Cutaneous candidiasis was induced by a slight modification of the procedure of Maebashi et al. (16). Briefly, hair was plucked by hand from an area (3 by 3 cm) on the backs of the guinea pigs to make a hairless square. On the following day, the skin was slightly abraded with sandpaper and 100 μl of inoculum (10^5 cells) was applied with a glass rod. The guinea pigs were subcutaneously administered 30 mg of prednisolone (Nacalai Tesque, Kyoto, Japan) per kg of body weight on the day before and on the day after the inoculation.
(vi) Topical treatment of plantar tinea pedis. KP-103 was dissolved in a mixture of polyethylene glycol 400–ethanol (75:25; vol/vol) at various concentrations (0.25 to 2.0%) (2, 27). One percent solutions of neticonazole hydrochloride (Atrant) and butenafine (Astat), which are commercially available and which were the products of SS Pharmaceutical Co., Ltd. (Tokyo, Japan), and Tsumura & Co. (Tokyo, Japan), respectively, were used. A 1% solution of butenafine hydrochloride (Mentax) was obtained from the factory of Kakem Pharmaceutical Co., Ltd. Each foot of the guinea pigs was topically treated with a 0.1-ml volume of the solution of the test compound. For plantar tinea pedis, the once-a-day treatment was started on day 10 postinfection and was continued for 7 or 10 consecutive days. The control group of animals was infected but did not receive drug or vehicle therapy.

(vii) Topical treatment of cutaneous candidiasis. KP-103, neticonazole, and clotrimazole were dissolved in mixture of polyethylene glycol 400–ethanol (75:25; vol/vol) (2, 27) to give concentrations of 0.25 or 1%. Each guinea pig was topically treated with 0.2 ml of the solution of the test compound once daily for 3 consecutive days starting on day 2 postinfection.

(viii) Evaluation. (a) Plantar tinea pedis. Culture studies were done to assess the efficacy of treatment. Two days after the last treatment, all animals were killed while under ether anesthesia, and each treated foot was wiped thoroughly with a cotton swab containing 70% ethanol. Twelve skin blocks were excised from each treated foot. Each skin block was implanted onto an SDA (Difco) plate, which contained 1 g of cycloheximide, 100 mg of gentamicin, 50 mg of chloramphenicol, and 50 mg of 5-flucytosine per liter. All plates were incubated at 30°C for 10 days. The skin blocks yielding fungal growth were regarded as culture positive, and a foot with more than one culture-positive skin block was considered fungus positive. The fungal burden of an infected foot was given a score of 0 to +12, according to the corresponding number of culture-positive skin blocks among the 12 skin blocks studied.

(b) Cutaneous candidiasis. Culture studies were done to assess the efficacy of treatment. Two days after the last treatment, all animals were killed while under ether anesthesia, and each treated site was wiped thoroughly with a cotton swab containing 70% ethanol. The skin was excised from each treated site, minced with scissors, and homogenized in 4 ml of saline containing 0.05% Tween 80 with a glass homogenizer. A 200-µl portion of the homogenate and 10-fold serial dilutions of the homogenate were mixed with 10 ml of Candida GS agar (Eiken Chemical Co., Tokyo, Japan), which contained 4.5 g of yeast extract, 10 g of glucose, 0.5 g of nitrofurane derivatives, and 15 g of agar per liter. All plates were incubated at 35°C for 5 days. The number of CFU in the agar plate was counted, and the logarithm of the number of CFU per infected site was calculated. An animal was considered fungus positive when more than one fungal colony was counted (assay limit, less than 20 CFU per infected site). The frequency of fungus-positive animals and the logarithm of the number of CFU in the infected sites were analyzed by Fisher’s exact test and the Kruskal-Wallis test (a Tukey-type comparison test), respectively. P values of less than 0.05 were regarded as significant.

(i) Statistical analysis. (a) Plantar tinea pedis. The frequency of fungus-positive animals and the average fungal burden were analyzed by Fisher’s exact test and the Kruskal-Wallis test (a Tukey-type comparison test), respectively. P values of less than 0.05 were regarded as significant.

(ii) Cutaneous candidiasis. The animals that were culture negative were considered to contain the assay limit of the number of organisms for numerical and statistical purposes. The frequency of fungus-positive animals and the logarithm of the number of CFU in the infected sites were analyzed by Fisher’s exact test and the Kruskal-Wallis test (a Tukey-type comparison test), respectively. P values of less than 0.05 were regarded as significant.

### RESULTS

In vitro activity against *T. rubrum* and *T. mentagrophytes.* The in vitro activity of KP-103 against 67 clinical isolates of *T. rubrum* and *T. mentagrophytes* was compared with those of clotrimazole, neticonazole, lanoconazole, and butenafine. Table 1 shows the MIC ranges, together with the MIC at which 50 and 80% of the strains tested were inhibited (MIC<sub>50</sub> and MIC<sub>80</sub>, respectively). KP-103 was as active as neticonazole and clotrimazole but was 8- to 16-fold less active than lanoconazole and butenafine against 39 *T. rubrum* isolates (MIC<sub>50</sub>, 0.125 µg/ml) and 28 *T. mentagrophytes* isolates (MIC<sub>50</sub>, 0.25 µg/ml). Table 1 also shows the MFC ranges and the MFCs at which 50 and 80% of the strains tested were inhibited (MFC<sub>50</sub> and MFC<sub>80</sub>, respectively). The MFC<sub>80</sub> of KP-103 for the dermatophytes was equal to or only twofold higher than its MIC<sub>80</sub>, which indicates that KP-103 has fungicidal action against the dermatophytes, like all of the reference drugs.

In vitro activity against other pathogenic fungi. The geometric mean (GM) MICs of KP-103 and the reference drugs for 76 isolates of five dermatophytic species, seven *Candida* species, and two *Malassezia* species are presented in Table 2. Against the five dermatophytes species, viz., *Trichophyton violaceum,* *Trichophyton ajelloi,* *Microsporum canis,* *Microsporum gypseum,* and *Epidermophyton floccosum,* KP-103 (GM MICs, 0.0078 to 0.0422 µg/ml) was as active as neticonazole and was more active than clotrimazole, but it was less active than lanoconazole and butenafine.

KP-103 was also highly active against all the *Candida* and *Malassezia* species tested. KP-103 showed 10-, 27-, and 55-fold greater activity than clotrimazole, neticonazole, and lanoconazole, respectively, against 44 *C. albicans* isolates (GM MIC, 0.002 µg/ml). Against the five non-*C. albicans* Candida species, viz., *Candida tropicalis,* *Candida krusei,* *Candida parapsilosis,* *Candida guilliermondii,* and *Candida utilis,* KP-103 was the most active of all the drugs tested (GM MICs, 0.0039 to 0.0442 µg/ml). KP-103 was slightly more active than neticonazole and lanoconazole against *Candida glabrata* (GM MIC, 0.0124 µg/ml) but was 18-fold more active than clotrimazole. Moreover, KP-103 showed 22- to 158-fold greater activity than the reference imidazoles against *M. furfur* (GM MIC, 0.025 µg/ml) in medium C agar. The activity of lanoconazole against *M. furfur* was little affected in the presence of 2% olive oil in PDA,

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### TABLE 1. MICs and MFCs of KP-103 and four reference drugs for *T. rubrum* and *T. mentagrophytes*

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>Compound</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range  50%</td>
<td>80%</td>
</tr>
<tr>
<td><em>T. rubrum</em> (39)</td>
<td>KP-103</td>
<td>0.0156–0.5 0.025 0.125 0.0156-1.0 0.125 0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clotrimazole</td>
<td>0.0625–1.0 0.25 0.125 0.0625-2.0 0.25 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neticonazole</td>
<td>0.0156–0.5 0.025 0.0156-1.0 0.25 0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lanoconazole</td>
<td>0.0005–0.0131 0.0039 0.0078 0.0005-0.0625 0.0078 0.0313</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Butenafine</td>
<td>0.0039–0.0156 0.0039 0.0078 0.0039-0.0156 0.0078 0.0078</td>
<td></td>
</tr>
<tr>
<td><em>T. mentagrophytes</em> (28)</td>
<td>KP-103</td>
<td>0.0625–0.5 0.25 0.25 0.125–1.0 0.25 0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clotrimazole</td>
<td>0.125–0.25 0.25 0.25 0.125–0.5 0.25 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neticonazole</td>
<td>0.0313–0.25 0.25 0.25 0.0625–1.0 0.25 0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lanoconazole</td>
<td>0.001–0.0025 0.0156 0.00313 0.0039–0.0625 0.0313 0.0313</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Butenafine</td>
<td>0.0039–0.0156 0.0078 0.0156 0.0039–0.0156 0.0078 0.0156</td>
<td></td>
</tr>
</tbody>
</table>
whereas the activities of KP-103, clotrimazole, neticonazole, and butenafine against *M. furfur* were reduced 7-, 18-, 6-, and more than 20-fold, respectively. However, KP-103 was still the most active of all drugs tested and inhibited the growth of *M. furfur* (GM MIC, 0.178 μg/ml). Overall, among the drugs tested butenafine had the lowest level of activity against the yeasts and yeast-like fungi.

**In vitro antidermatophytic activity in the presence of serum.** To predict the antidermatophytic activity of KP-103 in infected skin tissues, occasionally with exudates, we investigated the effect of serum or hair as a keratinic substance on the in vitro activity of KP-103. The GM MICs of KP-103 and the reference drugs for eight clinical isolates of *T. mentagrophytes* in SDB and 10% serum-supplemented SDB are shown in Table 3. The antifungal potencies of clotrimazole, neticonazole, lanoconazole, and butenafine were reduced 6-, 3-, 27-, and 6-fold, respectively, by the addition of 10% serum to SDB medium. However, the potency of KP-103 was not affected by serum.

**In vitro antidermatophytic activity in medium containing defatted hair as sole nutrient source.** Since the growth of dermatophytes in infected skin tissues is usually supported by keratin alone, we attempted to measure the antidermatophytic activities of the test compounds in assay media which mimic the nutritional environment of the human skin. For this purpose, the MICs and MFCs of KP-103 and the reference drugs for *T. mentagrophytes* KD-04 were determined in saline containing 5% human hair and were compared with the corresponding values obtained in SDB. As shown in Table 4, all the reference drugs, clotrimazole, neticonazole, lanoconazole, and butenafine, were 16- to 64-fold less active in the medium with hair than in SDB, whereas KP-103 showed similar activities in both media and its potency was little affected by keratin. Thus, KP-103 showed antifungal activity comparable to those of lanoconazole and butenafine in the medium in which hair was the sole nutrient source.

**In vivo efficacy in experimental plantar tinea pedis.** Table 5 shows the efficacy of KP-103 against plantar tinea pedis in guinea pigs in comparison with that of neticonazole, lanoconazole, or butenafine. *T. mentagrophytes* was recovered from all feet of the untreated control animals, whose feet yielded the highest average intensity of infection scores. In contrast, once-a-day treatment for 10 days with KP-103 solution led to a dose-dependent therapeutic effect, which was almost maximal at a drug concentration of 1.0%. When animals were treated with 1.0% KP-103 solution for 7 days, 16 of the 20 infected feet became culture negative, making the efficacy of KP-103 similar to those of lanoconazole and butenafine. A 10 day-treatment with a 1% solution of KP-103 achieved negative culture results for all infected feet, and the effect was significantly superior to that of neticonazole and equal to those of lanoconazole and butenafine.

**In vivo efficacy in experimental cutaneous candidiasis.** A clinical isolate of *C. albicans*, isolate KC-36, was used for production of experimental cutaneous candidiasis in guinea pigs. The MICs of KP-103, clotrimazole, neticonazole, and lanoconazole for the test strain were 0.0039, 0.0156, 0.0625, and 0.125 μg/ml, respectively, and KP-103 was 4-, 16-, and 32-fold active for all infected feet, and the effect was significantly superior to that of neticonazole and equal to those of lanoconazole and butenafine.
candidiasis in guinea pigs in comparison with those of clotrimazole, neticonazole, and lanoconazole. Viable C. albicans was recovered from the lesions of all the vehicle-treated animals, with large high average colony counts (log 3.41 ± 0.37) detected. When the animals were treated with a 1.0% solution of KP-103, 8 of the 10 infected sites became culture negative. Treatment with clotrimazole-, neticonazole-, and lanoconazole-treated groups, mycological eradication was not seen and the numbers of viable cells in the infected sites were significantly lower than the numbers in the infected sites of the vehicle-treated control group. On the other hand, for the clotrimazole-, neticonazole-, and lanoconazole-treated groups, mycological eradication was not seen and the numbers of viable cells in the infected sites were not significantly differed from the numbers in the infected sites of the vehicle-treated control group.

**DISCUSSION**

In general, imidazole antifungal agents have a relatively broad spectrum of activity against major superficial pathogens of dermatophytes and Candida and Malassezia species, but their activities against dermatophytes are lower than those of an allylamine derivative (terbinafine), a benzylamine derivative (butenafine), and a morpholine derivative (amorolfine), an exception is lanoconazole, which is known as the most potent antifungal imidazole (25, 26) against dermatophytes and which is as active as terbinfine (23) and presumably butenafine and amorolfine. The present study demonstrated that KP-103, a novel triazole compound, has a broad spectrum of activity against superficial fungal pathogens like imidazoles and that its antidermatophytic activity was almost equal to that of neticonazole and lower than those of butenafine and lanoconazole. However, when administered topically in a guinea pig model of plantar tinea pedis caused by T. mentagrophytes KD-04, KP-103 was more effective than neticonazole and was as effective as lanoconazole and butenafine, even though its MIC was similar to that of neticonazole and 32-fold higher than that of lanoconazole or butenafine for this dermatophytic strain (Table 4). These results suggest the possibility that KP-103 may be superior to all of these reference antifungal agents tested in terms of its pharmacokinetics in infected skin tissues.

It has been reported that various antifungal agents developed recently, including neticonazole, lanoconazole, butenafine, amorolfine, and terbinafine, exhibit prolonged cutaneous retention times (2, 7, 25, 29, 33) and achieve concentrations far above the MICs for most dermatophytes in the horny layer when they are applied to the skin (5, 22, 34). However, it looks likely that these antifungal agents mostly exist in an inactive form in the skin tissues because several studies have demonstrated that their in vitro activities were markedly lower in the presence of keratin (1, 21, 30) or serum (6, 8, 14), probably through drug-protein interactions. The present study, in which

**TABLE 4. In vitro activities of KP-103 and four reference drugs against T. mentagrophytes KD-04 in saline with human hair in comparison with that measured in SDB**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/ml) determined in:</th>
<th>Reduced activity (ratio of B/A)</th>
<th>MFC (µg/ml) determined in:</th>
<th>Reduced activity (ratio of D/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDB (A)</td>
<td>Saline with 5% hair (B)</td>
<td></td>
<td>SDB (C)</td>
<td></td>
</tr>
<tr>
<td>KP-103</td>
<td>0.2</td>
<td>0.2</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>0.2</td>
<td>6.25</td>
<td>32</td>
<td>0.39</td>
</tr>
<tr>
<td>Neticonazole</td>
<td>0.1</td>
<td>1.56</td>
<td>16</td>
<td>0.1</td>
</tr>
<tr>
<td>Lanoconazole</td>
<td>0.006</td>
<td>0.1</td>
<td>16</td>
<td>0.025</td>
</tr>
<tr>
<td>Butenafine</td>
<td>0.006</td>
<td>0.2</td>
<td>32</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* Treatment was started on day 10 postinfection and was continued for 7 or 10 days. The culture study was done 2 days after the last treatment.  

**TABLE 6. Therapeutic efficacies of KP-103 and three reference drugs in a guinea pig model of cutaneous candidiasis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals with positive cultures/total no. of animals (%)</th>
<th>Log CFU/infected site (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10/10 (100)</td>
<td>3.66 ± 0.67</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10/10 (100)</td>
<td>3.41 ± 0.37</td>
</tr>
<tr>
<td>0.25% KP-103</td>
<td>8/10 (80)</td>
<td>2.15 ± 0.69</td>
</tr>
<tr>
<td>1.0% KP-103</td>
<td>2/10 (20)</td>
<td>1.42 ± 0.35</td>
</tr>
<tr>
<td>1.0% clotrimazole</td>
<td>10/10 (100)</td>
<td>2.90 ± 0.60</td>
</tr>
<tr>
<td>1.0% neticonazole</td>
<td>10/10 (100)</td>
<td>2.96 ± 0.55</td>
</tr>
<tr>
<td>1.0% lanoconazole</td>
<td>10/10 (100)</td>
<td>3.16 ± 0.40</td>
</tr>
</tbody>
</table>

* Treatment was started on day 2 postinfection and was continued for 3 days.  

The culture study was done 2 days after the last treatment. 

P < 0.01 versus the neticonazole-treated group.  

P < 0.05 versus the vehicle-treated group.  

P < 0.01 versus the vehicle-treated group.  

P < 0.01 versus the neticonazole- and clotrimazole-treated groups.  

P < 0.05 versus the neticonazole- and clotrimazole-treated groups.
experiments were conducted to determine the effect of keratin on the dermatophytic activity of KP-103 in comparison with those of clotrimazole, neticonazole, lanoconazole, and butenafine, showed that the activities of all the reference drugs greatly decreased in the presence of hair. This result was consistent with those reported by Niwano et al. (21) for lanoconazole and clotrimazole and by Arika et al. (1) for clotrimazole and butenafine. Uchida and Yamaguchi (34) reported that terbinafine had a high affinity for keratin and that its rate of keratin binding increased with increasing keratin concentrations in the reaction mixture. Because the concentration of keratin in the horny layer is more than 80% and is higher than the keratin concentration of 5% in the medium used in the present study, the activities of these reference drugs in the local skin tissues should be reduced to a greater extent. In contrast, the present study also demonstrated that the activity of KP-103 was not affected by hair. It is known that T. mentagrophytes depends on its keratinolytic properties for invasion and growth in skin tissues. The fungus probably produces keratinase to obtain the amino acids requisite for growth in the medium with hair. In the rich medium of SDB, in which the dermatophytes do not need keratinase, the in vitro antidermatophytic activity of KP-103 was not affected by the presence of hair (data not shown). This result suggests that the high level of activity of KP-103 in the presence of a keratin-containing substance is not due to its inhibitory effect against keratinase. This is because KP-103 has a lower affinity for keratin than the reference drugs tested, as we have reported previously (Tatsumi et al., 36th ICAAC, abstr. 113). As a result, KP-103 exhibited in vitro antidermatophytic activity comparable to those of lanoconazole and butenafine in the presence of hair.

Several dermatophytic species, such as T. mentagrophytes, Trichophyton verrucosum, and M. canis, occasionally invade the hair follicles and severe inflammatory lesions, typically kerion celsi, develop in which a serum-like exudate appears. It has been found that the in vitro activities of several imidazoles were markedly lower in the presence of serum or serum components (6, 8, 14, 36, 37). These results were supported in the present study, which demonstrated that with some imidazoles, particularly lanoconazole, higher MICs were obtained in 10% human serum-supplemented SDB than in unsupplemented SDB. However, under the same experimental conditions, the activity of KP-103 was scarcely influenced by supplementation with human serum.

Such physiochemical characteristics of KP-103, that is, that its activity is not reduced or is scarcely reduced in the presence of keratin or serum, appear to confer on the compound some advantageous pharmacokinetic properties as a topical antifungal drug for the treatment of dermatophytosis and other dermatomycoses. This is true because a larger part of the percutaneously absorbed molecule could exist in the infected skin tissues in a free and active form. These favorable physiochemical and pharmacokinetic properties of KP-103 could explain, at least in part, its excellent therapeutic efficacy in guinea pigs with experimental plantar tinea pedis with experimental plantar tinea pedis beyond what might be expected on the basis of its in vitro activity.

S-32839, a related imidazole derivative of KP-103, was also found to be highly active against dermatophytes even when the activity was measured in serum-containing medium or medium with hair (24; Tatsumi et al., 36th ICAAC, abstr. 113). This suggests that a methylenepiperidine group at the C-4 position, which is commonly shared by the two compounds, may be responsible for the favorable biological properties of KP-103.

KP-103 showed potent in vitro activity against C. albicans, as well as against non C. albicans Candida species. KP-103 was 10-, 27-, and 55-fold more active than clotrimazole, neticonazole, and lanoconazole, respectively, against clinical isolates of C. albicans. In correlation with its in vitro activity, topically administered KP-103 achieved mycological eradication in 80% of guinea pigs with experimental cutaneous candidiasis caused by C. albicans. Its potent fungicidal activity in vivo may be due to the presence of a much higher concentration of the drug in free and active form in skin tissues on the basis of its low affinity for keratin (Tatsumi et al., 36th ICAAC, abstr. 113), together with its potent antifungal activity. Under the same experimental conditions, none of the reference drugs (clotrimazole, neticonazole, and lanoconazole) was effective in even reducing the number of fungi in the infected sites. However, other researchers reported that 1% solutions of neticonazole and lanoconazole significantly reduced the number of viable fungi more than the vehicle control did in a guinea pig model of cutaneous candidiasis (15, 19). This discrepancy may be due to the different virulence of the strains used and their ability to invade the skin tissues.

Lipid-dependent M. furfur is commonly found on human skin, in particular, on the upper body, where the level of sebum excretion is highest. It is reported that the activities of many imidazole antifungal agents were reduced in the presence of some lipidic components such as unsaturated fatty acids (8, 36) and phospholipids (37). Although the activity of KP-103 against M. furfur was reduced sevenfold by 2% olive oil, it was still the most active of all the drugs tested. Therefore, KP-103 may retain a high level of activity against M. furfur in lesions with sebum excretion and some lipidic components.

In conclusion, a new triazole compound, KP-103, has been found to have potent in vitro activity against dermatophytes, as well as against Candida and Malassezia species, and this activity is not affected by keratin or serum in the assay medium. These favorable biological and physiochemical properties of KP-103 are reflected by its excellent therapeutic effect in guinea pig models of dermatophytosis and cutaneous candidiasis. On the basis of the results of in vitro and in vivo studies, KP-103 can be considered a promising topical antifungal agent candidate for the treatment of dermatophytose and other superficial mycoses caused by Candida and Malassezia species.

REFERENCES