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INTRODUCTION

?–103 is a novel topical triazole having a methylenepiperizine / at the C–3 position (Figure 1). In abstract No.792, we reported P–103 has a broad antifungal spectrum against pathogenic fungi ng dermatophytes, *Aspergillus* spp., and yeasts. The antifungal / of KP–103 was less affected by hair and serum and KP–103 ed excellent fungicidal activity against dermatophytes grown on rny layer, suggesting that KP–103 is retained at a high activity n the horny layer.

his study, we examined the therapeutic efficacy and prophylactic of KP–103 on the experimental dermatomycosis model in guinea nd the relapse after the treatment in comparison with those of ference drugs (Figure 2), clotrimazole (CTZ), neticonazole (NCZ), nazole (LCZ) and butenafine (BTF).

MATERIALS AND METHODS

ls.

ale Hartley strain guinea pigs (300–500 g) were divided into four to groups of 10 animals each.

isms.

nentagrophytes KD–04

throspores of *T. mentagrophytes* were harvested and the um was prepared by the method of Fujita *et al.* [1]. A suspension µl, 10^6 conidia) of *T. mentagrophytes* was applied onto a rane filter placed on a brain heart infusion agar plate and grown days at 30°C under 17% CO_2.

?albicans KC–36

. albicans was grown for 2 days at 35°C on a Sabouraud se agar slant.

ions.

a corporis

kin infection was induced by the method of Sakai *et al.* [2]. Hair plucked by hand from an area (2.5 x 2.5 cm) on the back of a pigs and the skin was lightly abraded with sandpaper. Each was inoculated with a suspension of *T. mentagrophytes* spores (0.1 ml, 10^6).

a pedis and Interdigital *Tinea pedis*

fection was induced by a slight modification of the method of and Matsuyama [3]. For *Tinea pedis*, a paper disk (diameter, 13 was wetted with a suspension of *T. mentagrophytes* arthrospores ml, 10^7) and applied onto the plantar of the hind feet. For igital *Tinea pedis*, two paper disks (4 x 8 mm) were moisten with spension of arthrospores (10^8/ml) and applied onto the region en the toes of the hind feet. Each disk was fixed with a form pad dhesive elastic tape and was removed day–7 after postinfection.

n candidiasis

ifection was induced by a slight modification of the method of ashi *et al.* [4]. Prednisolone was subcutaneously administered twice day before and after the inoculation) at a dose of 30 mg/kg of weight each time. Hair was plucked by hand from an area(2.5 by m) on the back of guinea pigs. Each locus was inoculated with a nsion of yeast cells of *C. albicans* (0.1 ml, 10^7 cells).

ungal agents.

P–103 was formulated in polyethylene glycol 400–ethanol (75:25; l). One percent solutions of neticonazole, lanoconazole, and nazole were purchased from SS Pharmaceutical Co., Tumura Co., Bayer Yakuhin Co, respectively. One percent butenafine solution he product of our company.

ment.

ea corporis and skin candidiasis

ach guinea pig was topically treated with 0.2 ml of the drug on once a day. The treatment on *T. corporis* was started on day–tinfection and continued for 10 consecutive days. The treatment of diasis was started on day–2 postinfection and continued for 3 ecutive days.

ea pedis and interdigital *tinea pedis*

ach foot of guinea pigs was treated with 0.1 ml of the drug on once a day. The treatment was started on day–10 postinfection continued for consecutive 10 days.

uation.

rmatophytosis

Two days after the last treatment, all animals were sacrificed, and *T. corporis*), twelve (*T. pedis*), and fifteen (interdigital *T. pedis*) skin s were obtained from treated sites. Each block was implanted onto bouraud glucose agar plate containing 1000 µg of cycloheximide ml, 50 µg of chloramphenicol per ml, 100 µg of gentamicin per ml, 50 µg of 5–fluorocytosine and were grown at 30° for 10 days. The nent was assessed as complete cure if no fungal growth was ted in all skin blocks of an animal or foot.

n candidiasis

Two days after the last treatment, all animals were sacrificed. The was excised from the treated site and homogenized in saline ining 0.05% tween 80. A 100 µl portion of the homogenate and 0–fold serial dilutions were mixed with 10 ml of Candida GS Agar (Eiken Chemical Co., Japan), which contains 4.5 mg of yeast–extract per ml, 10 mg of peptone per ml, 20 mg of glucose per ml, 0.5 mg ofnitrofuran derivatives per ml, and 15 mg of agar per ml, and grown at 35°C. Colonies grown in the agar plate were counted. The treatment was assessed as complete cure if no fungal growth was detected in skin homoganate of an animal.

Prophylactic effect.

A hairless area (3 by 3 cm) was produced on the back of guinea pigs. On the following day, the skin was treated with 0.2 ml of the drug solution. At 24, 48, 72, or 96 h after drug application, a suspension of arthrospores (0.1 ml, 10^6) was rubbed onto the pretreated area of the skin. On 24 h after application of drug, the unabsorbed drug was wiped off. On day–17 postinfection, culture studies were done as described above.

Relapse after treatment.

T. corporis and interdigital *T. pedis* models of guinea pigs were treated with 1% drug solutions in the same schedule as described above. Guinea pigs were sacrificed on day–9 (*T. corporis*) and day–30 (interdigital *T. pedis*) after the last treatment and culture studies were done. Adhesive elastic tape was applied onto the infected area to prevent reinfection after treatment.

Figure 2. Chemical structures of reference drugs

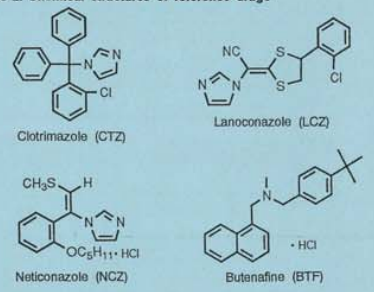

Table 1. Therapeutic efficacy of KP–103 in the guinea pigs *Tinea corporis* model

Treatment	No. of animals with negative cultures / total no. of animals	Cure rate (%)
0.25% KP–103 solution	1/10	10
0.5% KP–103 solution	9/10	90
1% KP–103 solution	10/10	100
2% KP–103 solution	10/10	100
None	0/10	0

The solutions were applied once a day for 10 days.

Table 2. Therapeutic efficacy of KP–103 and the reference drugs in the guinea pigs *Tinea corporis* model

Treatment	No. of animals with negative cultures / total no. of animals	Cure rate (%)
1% KP–103 solution	10/10	100
1% NCZ solution	1/10	10
1% LCZ solution	9/10	90
1% BTF solution	10/10	100
None	0/10	0

The solutions were applied once a day for 10 days.

Table 3. Therapeutic efficacy of KP–103 in the guinea pigs *Tinea pedis* model

Treatment	No. of feet with negative cultures / total no. of feet	Cure rate (%)
0.25% KP–103 solution	5/20	25
0.5% KP–103 solution	5/20	25
1% KP–103 solution	19/20	95
2% KP–103 solution	20/20	100
None	0/20	0

The solutions were applied once a day for 10 days.

Table 4. Therapeutic efficacy of KP–103 and the reference drugs in the guinea pigs *Tinea pedis* model

Treatment	No. of feet with negative cultures / total no. of feet	Cure rate (%)
1% KP–103 solution	20/20	100
1% NCZ solution	6/20	30
1% LCZ solution	20/20	100
1% BTF solution	20/20	100
None	0/20	0

The solutions were applied once a day for 10 days.

Table 5. Therapeutic efficacy of KP–103 and the reference drugs in the guinea pigs interdigital *Tinea pedis* model

Treatment	No. of feet with negative cultures / total no. of feet	Cure rate (%)
0.25% KP–103 solution	9/20	45
0.5% KP–103 solution	15/20	75
1% KP–103 solution	20/20	100
1% NCZ solution	8/20	40
1% LCZ solution	20/20	100
1% BTF solution	20/20	100
None	0/20	0

The solutions were applied once a day for 10 days.

Table 6. Therapeutic efficacy of KP–103 and the reference drugs in the guinea pigs skin candidiasis model

Treatment	No. of animals with negative cultures / total no. of animals	Cure rate (%)
0.25% KP–103 solution	1/10	10
1% KP–103 solution	8/10	80
Vehicle	0/10	0
1% CTZ solution	0/10	0
1% NCZ solution	0/10	0
1% LCZ solution	0/10	0
None	0/10	0

The solutions were applied once a day for 3 days.

Table 7. Prophylactic effect of KP–103 and the reference drgs in the guinea pigs *Tinea corporis* model

Pretreatment	Time before inoculation (h)	No. of animals with negative cultures / total no. of animals	Prophylaxis rate (%)
1% KP–103 solution	24	9/10	90
1% KP–103 solution	48	9/10	90
1% KP–103 solution	72	6/10	60
1% KP–103 solution	96	5/10	50
1% NCZ solution	48	0/10	0
1% LCZ solution	48	0/10	0
1% BTF solution	48	9/10	90
None		0/10	0

The solutions were applied once before inoculation.

Table 8. Relapse rate of infection in the guinea pigs treated with KP–103 and the reference drugs for *Tinea corporis*

Treatment	No. of animals with positive cultures / total no. of animals	Relapse rate (%)
1% KP–103 solution	3/10	30
1% LCZ solution	8/10	80
1% BTF solution	3/10	30
None	10/10	100

The solutions were applied once a day for 10 days.
Culture studies were performed 9 days after last treatment.

Table 9. Relapse rate of infection in the guinea pigs treated with KP–103 and the reference drugs for interdigital *Tinea pedis*

Treatment	No. of feet with positive cultures / total no. of feet	Relapse rate (%)
1% KP–103 solution	4/20	20
1% NCZ solution	14/20	70
1% LCZ solution	11/20	55
1% BTF solution	4/20	20
None	20/20	100

The solutions were applied once a day for 10 days.
Culture studies were performed 30 days after last treatment.

RESULTS

1) KP–103 solutions (0.5, 1, and 2%) were highly effective in the treatment of *T. corporis* in the guinea pigs by once daily application for 10 days. The treatment with a 1% KP–103 solution resulted in complete eradication of fungi from the infected site in all animals and the efficacy was higher than that of a 1% NCZ solution and equal to that of 1% solutions of LCZ and BTF (Tables 1 and 2).

2) KP–103 solutions (1 and 2%) were highly effective in the treatment of *T. pedis* in the guinea pigs by once daily application 10 days. The treatment with a 1% KP–103 solution resulted in complete eradication of fungi from the infected site in all animals and the efficacy was higher than that of a 1% NCZ solution and equal to that of 1% solutions of LCZ and BTF (Tables 3 and 4).

3) KP–103 solutions (0.5 and 1%) were highly effective in the treatment with iterdigital *T. pedis* in the guinea pigs. The treatment with a 1% KP–103 solution resulted in complete eradication of fungi from the infected site in all animals and the efficacy was higher than that of a 1% NCZ solution and equal to that of 1% solutions of LCZ and BTF (Table 5).

4) A 1% KP–103 solution was highly effective in the treatment of skin candidiasis in the guinea pigs by once daily application for 3 days, while 1% solutions of all reference drugs were less effective. The treatment with 1% KP–103 solution resulted in complete eradication of fungi from the infected site in 80% of the animals (Table 6).

5) When 1% KP–103 solution was applied on the back of the guinea pig 48 h before inoculation, KP–103 had a prophylactic effect in 90% of the animals. However, a 1% solution of NCZ or LCZ had no such prophylactic effect (Table 7).

6) Relapse of infection occurred in 80% of the guinea pigs treated with a 1% LCZ solution for *T. corporis*, but in only 30% of those treated with a 1% solution of KP–103 or BTF (Table 8).

7) Relapse of infection occurred in 55% of the feet of guinea pigs treated with a 1% LCZ solution for interdigital *T. pedis*, but in only 20% of those treated with a 1% solution of KP–103 or BTF (Table 9).

REFERENCES

1) Fujita, S., T. Matsuyama, and Y. Sato. 1986. A simple and reliable culturing method for producing of arthrospores by dermatophytes. Jpn. J. Med. Mycol. 27:175–181.

2) Sakai, S., T. Kada, G. Saito, N. Muraoka, and Y. Takahashi. 1952. Studies on chemotherapy of *Trichophyton* infection. 1. Antifungal properties of halogen phenol esters. J. Sci. Res. Inst. 46:113–117.

3) 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.

4) Maebashi, K., T. Itoyama, K. Uchida, N. Suegara, and H. Yamaguchi. 1994. A novel model of cutaneous candidiasis produced in prednisolone–treated guinea pigs. J. Med. Vet. Mycol. 32:349–359.

CONCLUSIONS

A 1% KP-103 solution was highly effective in the treatment of dermatophytosis in the guinea pigs by once daily topical application, being more effective than that of a 1% NCZ solution and equal to that of 1% solutions of LCZ and BTF. A 1% KP-103 solution was also highly effective in the treatment of cutaneous candidiasis in the guinea pigs by once daily topical application, while 1% solutions of CTZ, NCZ, and LCZ were less effective.

A single topical application of 1% KP-103 within 48 h prior to challenge with *T. mentagrophytes* almost completely protected the guinea pigs from development of infection, suggesting that an effective concentration of KP-103 remains in the skin for at least 48 h after application.

The relapse rate of infection of the guinea pigs treated with a 1% KP-103 solution for interdigital *T. pedis* was lower than that of animals treated with a 1% solution of NCZ or LCZ.

In summary, the excellent therapeutic efficacy and the low relapse rate after KP-103 therapy may be attributed to the prolonged retention of active drug in the skin tissue.

Accordingly, KP-103 may be a useful topical antifungal agent for the treatment of all types of dermatophytosis and other superficial mycosis.

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Abstract No. F793

Therapeutic Efficacy of KP-103, a Novel Topical Antifungal Triazole, on Experimental Superficial Mycosis

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ABSTRACT

The therapeutic efficacy of KP-103 on dermatomycosis models in guinea pigs was compared with that of neticonazole(NCZ), lanoconazole(LCZ), butenafine(BTF), and clotrimazole(CTZ). One percent solution of each drug was topically applied once a day. Two days after last application, skin blocks or homogenates were cultured. Rate of complete cure was expressed by the percentage of animals (n=10) or feet (n=20) with negative cultures.

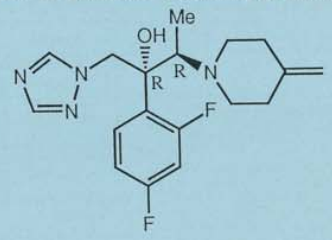
Mycosis models	Treatment duration (days)	Rate of complete cure (%)				
		KP-103	NCZ	LCZ	BTF	CTZ
Tinea corporis	10	100	10	90	100	N.D.
Tinea pedis	10	100	30	100	100	N.D.
Interdigital *tinea pedis*	10	100	40	100	100	N.D.
Skin candidiasis	3	80	0	0	N.D.[a]	0

[a] Not determined.

The efficacy of KP-103 on dermatophytosis models was superior to that of NCZ and almost equal to that of LCZ and BTF. KP-103 was effective on skin candidiasis, while the other drugs were not. To clarify the duration of retention time of the drug in skin after topical application, the prophylactic effect of KP-103 on dermatophytosis model was examined. KP-103 exerted prophylactic effect with 90% cure rate at application of 48 h before infection.

In summary, the excellent efficacies of KP-103 on dermatophytosis and skin candidiasis may be attributed to its high activity and long time retention in the horny layer.

Figure 1. Chemical structure of KP-103



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