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Efficacy of Terbinafine in Guinea Pigs Experimentally Infected with *Microsporum gypseum* Isolated from Naturally Infected Dog
II. Clinical and Mycological Efficacy

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자연감염된 개에서 분리한 *Microsporum gypseum*을 인공감염시킨 기니픽에서 Terbinafine의 효과
II. 임상적 및 진균학적 효과

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요 약 : 알비노 기니픽의 피부에 *Microsporum gypseum*을 접종한 후 체중 kg당 20, 40 및 80 mg의 terbinafine을 접종 당일부터 9일간 연속 투여하였다. 대조군의 피부는 인설과 가피를 동반한 현저한 염증이 발생되었다. 이와 대조적으로 terbinafine을 경구투여한 처치군에서는 피부 증상이 현저하게 차도를 보였다. 진균학적인 평가를 위해 모근침범시험을 실시하였다. 체중 kg당 20~80 mg의 terbinafine을 경구투여한 처치군은 진균학적으로 97~100%의 치료효과를 나타냈으며, 대조군에서는 치료효과가 전혀 없었다.

Key words : terbinafine, clinical efficacy, mycological efficacy, hair root invasion test, guinea pig

Introduction

Many different kinds of antifungal agents have been used in treating animal fungal diseases.

In veterinary practice, griseofulvin has been used in dogs to treat dermatophyte infections of skin, hair and claws. Griseofulvin arrives at the stratum corneum from skin surface after being secreted into eccrine sweat in human[10,53].But eccrine sweat glands are only found in the footpads of dogs[34]. Moreover, eccrine sweating is shown only on the footpads of excited or agitated dogs[54]. Although apocrine sweat glands are distributed throughout all haired skin in dogs[36], apocrine sweating is nearly not seen[54].

Ketoconazole has been also used widely in veterinary medicine. Ketoconazole reacts to the skin by its concentrating in the sweat[21]. Ketoconazole inhibits the fungal ergosterol synthesis via binding to cytochrome P-450[3]. The cytochrome-binding is not restricted to fungi, and the majority of mammalian cytochrome P-450-dependent enzymes from the adrenal, liver and testis can be affected by ketoconazole[41]. This induces hepatitis and inhibition of steroid synthesis in the testis and adrenal cortex[8,24,29,35,40,51].

In addition, itraconazole represents new compound with greater efficacy and lower mammalian toxicity. This drug has only recently been introduced to veterinary medicine. Itraconazole is excreted in sebum[5]. This drug is lipophilic and this accounts for its accumulation in skin and hair.

A new antifungal, terbinafine[30] is currently under evaluation in human. *In vitro* study has shown terbinafine to be more effective than other currently available antifungal agents against dermatophytes[7]. It has a broad activity against dermatophytes, yeasts, moulds and biphasic fungi[6,39,52].

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Since terbinafine is lipophilic[25], it is present with high concentrations in the sebum[12,13,27,57]. Terbinafine is delivered by rapid diffusion through basal layers of skin to stratum corneum[28] where it binds to the lipophilic keratinocytes[56,57]. In human, short-term therapy with terbinafine may be effective in the treatment of several dermatomycoses due to strong binding of terbinafine to stratum corneum for a long time after completion of medication[13]. In dogs, sebaceous glands are distributed throughout all haired skin and the sebum produced by the sebaceous glands, spreads over the surface of the stratum corneum and hair shafts[34].

Griseofulvin, ketoconazole and itraconazole are fungistatic in action[2,19,33] and this mode of action requires prolonged treatment of dermatophytes, but terbinafine is fungicidal[42,45]. The fungicidal action of terbinafine produces very high cure rate in trichophytosis[4,14,22,23,26,46-48,53,58] and dermatophyte onychomycosis [11,16-18,20,49,59] when it is given orally for short period in human.

In the present experiment, the efficacy of orally administered terbinafine was evaluated in guinea pigs experimentally infected with *M. gypseum*.

Materials and Methods

Experimental animals

Albino guinea pigs, weighing 250 to 350 g were used. Eight animals were randomly assigned to each high-dose (80 mg/kg/B.W.) group and medium-dose (40 mg/kg/B.W.) group, nine animals to low-dose (20 mg/kg/B.W.) group and seven animals were used as control. The animals were housed individually in cages through the test. The animals were allowed free acess to pelleted feed and water. They were applied to the study after a 7-day period of adaptation.

Inoculation and treatment

The preparation of fungal inoculum and the method of inoculation were same as described in experiment on the biological cycle of *M. gypseum*.

Terbinafine (Lamisil® , Sandoz Forschungsinstitut, Vienna) was used as a test drug. For oral treatment the compound was suspended in 2% methyl cellulose with 0.5% Tween 80, and was administered orally.

The treatment was given once daily on 9 consecutive days, starting at the day of inoculation. Infected animals were treated with 20 (low dose), 40 (medium dose), and 80 (high dose) mg of terbinafine per kilogram of body weight, respectively. The animals of untreated group were used as control.

Evaluation

The efficacy of the treatment was evaluated both clinically and mycologically.

On 10th day postinfection, the clinical assessment of local changes of the infected skin area was scored from 0 to 4, as follows.

Score 0: no findings.
Score 1: few slightly erythematous places on the skin or a small number of papular erythema.
Score 2: well-defined redness with scaling or moderate erythema spreading over the entire infected loci with scaling.
Score 3: large areas of marked redness, scaling, swelling or partly intense erythema with signs of swelling and scaling
Score 4: same as the control or lesions severely erythematous with extensive and intense crusting spreading over the exposed area, firmly swollen.

The petcentage efficacy was calculated by the following formula:

$$\% \text{ efficacy} = 100 - \frac{T \times 100}{K}$$

Where T is the mean clinical score in the treatment group and K is the same for the control group.

Hair root invasion test[37] was used for mycological evaluation. On 10th day postinfection, the mycotic focus of each animal was divided into quadrants and four hair samples (one sample per quadrant with about 10 hairs per sample) were removed with sterile forceps. Dermatophyte test medium plates similarly divided into quadrants, were inoculated with the hair samples from the corresponding quadrants of the

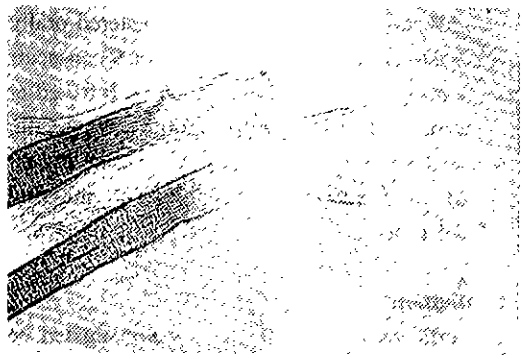

Fig 1. Mycological finding of negative cultures in hair root invasion test. These are normal hair roots of guinea pig.

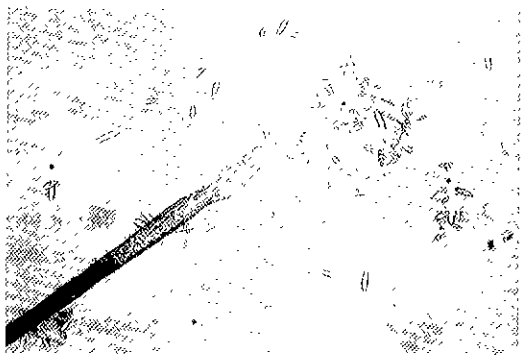

Fig 2. Mycological finding of positive cultures in hair root invasion test. Broadly spindle-shaped macroconidia with four to six septa can be seen.

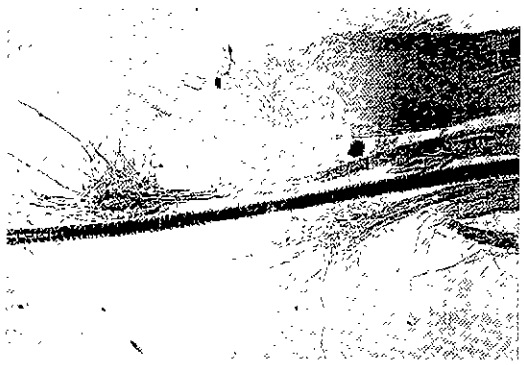

Fig 3. Mycological finding of positive cultures in hair root invasion test. Fungal hyphae radiating from the hair root can be seen.

skin. These plates were incubated at 30°C for 7 days and were then examined undermicroscope for fungal growth at the hair root. When fungal growth is positive in a quadrant, the corresponding hair sample was considered positive mycologically (Fig 1~4).

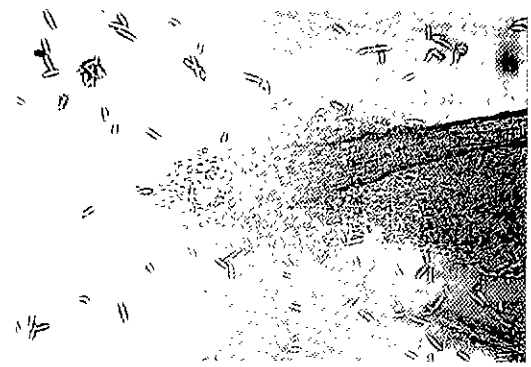

Fig 4. Mycological finding of positive cultures in hair root invasion test. Fungal hyphae radiating from the hair root can be seen.

The effectiveness of the treatment was evaluated by decreasing of positive hair samples in treated group, and it was expressed as percentage efficacy, and calculated by the following formula.

$$\% \text{ efficacy} = 100 - \frac{T \times 100}{K}$$

Where T is the mean number of mycologically positive hair samples from one animal in treatment group and K is the same in control group.

Results

The clinical and mycological examinations of the infected sites were carried out 1 day after the last treatment. The skin lesion of control animals developed from a sparsely occurring papular erythema to more severely inflammatory responses accompanied by intense scaling and crusting. In contrast, a marked reduction in gross lesions was noted in each dose group treated with terbinafine orally.

The clinical percent efficacy of each dose group was 92, 85 and 97, respectively and that of control group was 0 (Table 1). The clinical efficacy of terbinafine as an oral antifungal agent was significant when compared with control.

Mycologically, the percent efficacy of terbinafine in each dose group was 100, 100, and 97, respectively.

Table 1. Clinical efficacy of terbinafine in guinea pig with induced microsporosis (*M. gypseum*)

Group	Dose (mg/kg)	n	% Efficacy
Terbinafine*	20	9	92
	40	8	85
	80	8	97
Control	0	7	0

*:Oral treatment once daily on 9 consecutive days starting at the day of inoculation.

Table 2. Mycologically curative efficacy of terbinafine in guinea pig with induced microsporosis (*M. gypseum*)

Group	Dose (mg/kg)	n	% Efficacy	Cure rate
Terbinafine*	20	9	100	9/9
	40	8	100	8/8
	80	8	97	7/8
Control	0	7	0	0/7

*:Oral treatment once daily on 9 consecutive days starting at the day of inoculation.

The mycologically curative efficacy of terbinafine was significant when compared with control (Table 2).

Discussion

Experimental investigation in laboratory animals is commonly used to assess the clinical antimycotic potential of chemotherapeutic agents and has been proven to have high predictability for clinical use[31]. The hair root invasion test is a reliable semiquantitative method for the preclinical evaluation of antimycotics *in vivo* with a high degree of reproducibility. This test is based on viable dermatophytes in the mycotic lesions of guinea pigs and permits a general clinical evaluation by scoring local symptoms[37].

The present study demonstrated that terbinafine is also effective in guinea pigs infected with *M. gypseum* similar to the results which were shown in the infection with Trichophyton mentagrophytes, *Trichophyton rubrum*, and *Microsporum canis*[31,32,38].

Although both azole (for example, ketoconazole and itraconazole) and allylamine (for example, terbinafine) antifungals are potent inhibitors of fungal ergosterol synthesis, their modes of action are different. Azoles interfere with demethylation of lanosterol to ergosterol by inhibiting the cytochrome P450-dependent enzyme 14-alpa-demethylase, and this results in deficient membrane formation. Azoles are therefore primarily fungistatic Ketoconazole inhibits the fungal ergosterol systhesis via binding to cytochrome P-450. The hepatotoxicity risk associated with ketoconazole limits its use in chronic dermatophyte infection[24]. Testicular and adrenal testosterone biosynthesis is also blocked by ketoconazole[8-10,51]. In veterinary practice, ketoconazole greater than 10 mg/kg dosage may suppress adrenal cortisol production and cause drug induced hypocortisolemia[15].

However, terbinafine does not interfere with cytochrome P-450-dependent enzymes in steroid biosynthesis[1,9,35]. It inhibits the non-cytochrome P-450 enzyme squalene epoxidase, a key enzyme in ergosterol synthesis[42,45]. This leads to deficient membrane sterol production and intrafungal accumulation of squalene[43,44,50]. This is believed to be the essential mechanism by which terbinafine exerts its primary fungicidal activity, i.e. its ability to kill fungi at minimal inhibitory concentrations.

Considering the anatomic skin structure of dog is similar to that of guinea pig, clinical trial of this drug in naturally occurring dermatophytosis in dog is thought to be worthwhile.

Conclusion

The skin of Albino guinea pigs were inoculated with *M. gypseum*, and terbinafine was administered orally 20, 40 or 80 mg/kg on 9 consecutive days starting at the day of inoculation. The skin lesion of control animals showed severe inflammation accompanied by intense scaling and crusting. In contrast, a marked reduction in gross lesions was noted in each dose group treated with terbinafine orally. Hair root invasion test was used for mycological evaluation. Oral administration of 20~80 mg of terbinafine per kilogram of body weight revealed 97~100% efficacy mycologically.

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