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Efficacy of Terbinafine in Guinea Pigs Experimentally Infected with *Microsporum gypseum* Isolated from Naturally Infected Dog

1. The Biological Cycle of *M. gypseum* in Experimentally Infected Guinea Pigs

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자연감염된 개에서 분리한 *Microsporum gypseum*을 인공감염시킨 기니픽에서 Terbinafine의 효과

1. 인공감염된 기니픽에 있어서 *Microsporum gypseum*의 생물학적 주기

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요 약 : *Microsporum gypseum*을 인공감염시킨 기니픽에서 terbinafine의 치료효과 실험을 위한 전단계 실험으로서 인공감염시킨 기니픽에서 *M. gypseum*의 생물학적 주기를 조사하였는데, 이것은 인공감염 후 효과적인 치료와 평가의 시기를 결정하기 위해 필요하였다. *M. gypseum*으로 인공감염된 기니픽의 생물학적 주기는 급성기 이후에 자연치유되는 것이 특징이었다. 중식기는 감염 후 5일부터 11일까지 지속되었다. 따라서 다음 번의 치료효과 실험에서 인공감염 당일부터 9일간 계속 terbinafine을 경구투여하고, 마지막 투여 다음 날 진균학적 및 임상적 평가를 하기로 결정하였다.

Key words : Microsporum gypseum, terbinafine, guinea pig

Introduction

The dermatophytosis caused by species of *Microsporum, Trichophyton*, or *Epidermophyton* is an infection of the keratinized tissues, nail, hair and stratum corneum. The dermatophytes are unique fungi that can invade and maintain themselves in keratinized tissues. These fungi share the ability to utilize keratin as a nutrient substrate[7]. Three fungi, *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton mentagrophytes*, cause almost all dermatophyte infections in dogs.

Many different kinds of antifungal agents have been used in treating fungal diseases in dogs, but they showed limited efficacy and different side effects in dogs. A new antifungal, terbinafine[5] is currently under evaluation in human. *In vitro* study has shown that terbinafine is more effective than other currently available antifungal agents against dermatophytes in human[3]. It has a broad activity against dermatophytes, yeasts, moulds and biphasic fungi[2,10-12].

The goal of the studies is to evaluate the efficacy of terbinafine in guinea pigs experimentally infected with *M. gypseum* isolated from naturally infected dog. Guinea pig was used as a model animal of canine dermatophytosis because histologic structures of its skin are similar to those of dog. Each sebaceous gland has an associated hair follicle whereas sweat glands are apparently absent in guinea pigs as in dogs[1]. Moreover, for experimental dermatophytosis, the animal must be susceptible to dermatophyte infection and the infection must have quantifiable clinical features and moderate duration (2~3 weeks). Guinea pigs are suitable to fulfill these requirements[4].

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In the first experiment the biological cycle of *M gypseum* in experimentally infected guinea pigs was examined to determine the effective time for treatment and its evaluation.

Materials and Methods

Experimental animals

Ten albino guinea pigs, weighing 250 to 350 g were used to this study. Four were used for daily skin biopsy. The animals were housed individually in cages through the test. The animals were allowed free access to pelleted feed and water. They were applied to the study after 7-day period of adaptation.

Test organism

Microsporum gypseum used in this study was recently obtained from the lesion of a dog with active infection. The hair samples from dogs suspected to have dermatophytosis were sent to our laboratory from local animal hospitals by mail. The hair samples were cultured in dermatophyte test medium (DTM) consisted of Sabouraud dextrose agar (SDA) supplemented with chloramphenicol, cycloheximide, and phenol red. The fungal strains turned DTM into red color within 10 days were considered as dermatophytes. The isolated dermatophytes were recultured in SDA and morphology of fungi in cultures were examined by 'PHOL' stain[8]. Thirteen strains of dermatophytes were isolated from 40 hair samples. Three of thirteen strains were identified as *Microsporum gypseum*. The culture which grows most quickly among the three strains was used as an organism for the study.

Preparation of inoculum

The test organism was cultivated on SDA slants at 30°C for 7 days. The colonies were flooded with sterile saline containing 0.1% Tween 80 and scraped off with the aid of a wire loop under sterile condition. The suspension was finely dispersed with glass homogenizer. The preparation was composed of numerous macroconidia and a few hyphal fragments. The macroconidia were counted with hemocytometer and were diluted to a conidial concentration of 10^7 per ml.

Inoculaltion

One day prior to inoculation the hair was shorn from backs of guinea pigs with electric clippers. To make a hairless square in backs of guinea pigs, adhesive tapes were applied and then were taken off forcefully. This procedure was repeated 5 to 6 times to depilate completely and to abrade the upper horny layer of skin in backs.

On the following day, the skin was shaved. An open glass cylinder (2 cm high and 3.5 cm in diameter) was laid on the widest part of the back (lumber region). The inoculum (0.1 ml containing 10^6 spores) was pipetted onto the surface of the skin and abraded into the portion of skin encircled by the cylinder with a roughened glass pestle. Care was taken to maintain the spores within the prescribed circular area.

Clinical sign

Infected skin region of all guinea pigs except the guinea pigs used for skin biopsy were observed clinically.

Skin biopsy

Infection was confirmed histopathologically through skin biopsy. Biopsied skins were fixed in 10% neutral buffered formalin for 24 hours and routinely processed and embedded in paraffin in an automated processor. Sections were cut at 4 μm thick and stained with periodic acid-Schiff.

Results

The first lesions characterized by a small number of papular erythema in the infected locus (clinical score 1, Fig 1) appeared between the 5th and 7th day postinfection with *M. gypseum*. During the 6~8 days postinfection, erythematous lesions developed extensively, spreading over the entire infected locus and scaling started to be shown (clinical score 2, Fig 2). Around 8~9 days postinfection, erythema became more and more increased and were accompanied by remarkable signs of inflammatory responses of the skin, by intense scaling and by swelling (clinical score 3, Fig 3). The dermal responses continued to increase in severity reaching maximum level by 9th

Fig 1. Gross finding of dermatophyte infection in guinea pig : clinical score 1. Few slightly erythematous lesions can be seen.

Fig 2. Gross finding of dermatophyte infection in guinea pig : clinical score 2. Well-defined redness with scaling can be seen.

Fig 3. Gross finding of dermatophyte infection in guinea pig : clinical score 3. Large areas of marked redness, scaling and swelling can be seen.

Fig 4. Gross finding of dermatophyte infection in guinea pig : clinical score 4. Intense crusting can be seen.

~13th days postinfection, when thick crusting (clinical score 4, Fig 4) developed, and hemorrhagic lesions appeared 13 days postinfection. Spontaneous healing began 16 days postinfection and erythema was almost faded away by 26 days postinfection (Fig 5).

Histopathologically, numerous spherical to oval arthroconidia were formed inside of the hair follicles in guinea pigs infected with 10^6 macroconidia of *Microsporum gypseum* on 10 days postinfection (Fig 6, 7).

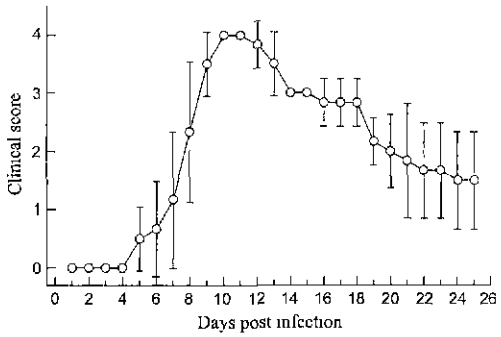

Fig 5. Changes of clinical scores in guinea pigs inoculated with *Microsporum gypseum*. Score 1: slight erythema; Score 2: erythema, scaling; Score 3: marked erythema, scaling, swelling; Score 4: crusting. Vertical bar represents standard deviation.

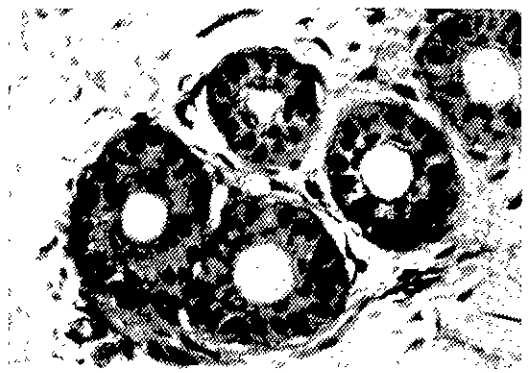

Fig 6. Histological features of normal, uninfected guinea pig skin. PAS stain. magnification×400.

Fig 7. Histological features of guinea pig infected with 10^6 macroconidia of Microsporum. PAS stain, magnification ×400. Numerous spherical to oval arthroconidia were formed inside of the hair follicles (arrow head).

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[6]. 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[9]. Despite good reliability and reproducibility of the test, several prerequisites must be met to yield satisfactory results. The hair root invasion test is only reliable if due regard is paid to the biological cycle of the infection. The experimental follicular dermatophytoses of the guinea pig show a marked tendency to spontaneous cure. Furthermore, the shedding of highly infected hair from the infected skin area after the climax of infection makes it difficult and sometimes even impossible to obtain suitable hair samples from the infected animals for the diagnostic cultivation procedure. Therefore, the treatment and subsequently the assessment of its result should be carried out and completed during the proliferation phase of the infection[9]. The course of experimental trichophytosis in guinea pigs was subdivided into several phases: an incubation phase of 2~4 days, followed by a proliferation phase lasting 3~14 days and reaching a climax 12~16 days postinoculation, followed in turn by the healing phase[9].

It is confirmed in this experiment that the biological cycle of microsporosis in guinea pigs is similar to that of trichophytosis. The proliferation phase lasted from 5 to 11 days postinoculation. Since the treatment has to be evaluated during proliferation phase, it is decided that the terbinafine is to be given once daily on 9 consecutive days starting at the day of inoculation and the efficacy is to be evaluated mycologically and clinically 1 day after the last treatment in the experiment on efficacy of terbinafine.

Conclusion

The biological cycle of *M. gypseum* in experimen-

tally infected guinea pigs was investigated to determine the effective time for treatment and its evaluation. The induced infection with *Microsporum gypseum* in guinea pigs was characterized by an acute phase followed by spontaneous healing. The proliferation phase lasted from 5 to 11 days post inoculation. Since the treatment has to be evaluated during proliferation phase, it is decided that the terbinafine is to be given once daily on 9 consecutive days starting at the day of inoculation and the efficacy is to be evaluated mycologically and clinically 1 day after the last treatment in the experiment on efficacy of terbinafine.

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