Short Communication

EXPERIMENTAL DERMATOPHYTOSIS ON THE OUTER EAR OF GUINEA PIGS: A MODEL THAT MIMICS NATURAL INFECTION

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Abstract

In the present study a new model of experimental dermatophytosis was tested. The aim was to achieve maximum similarity to a natural infection through a procedure that is easy to carry out. The experimental infection was induced by a single inoculation of 0.5 mL of a suspension with hyphal fragments of *T. mentagrophytes* var. *mentagrophytes* on the outer ear of guinea pigs. The first signs of infection were observe on the seventh day after inoculation. The experiment demonstrated that this method, besides being effective, causes less stress and the clinical aspects the lesions here were similar to natural lesions.

Key-words: Experimental. Dermatophytosis. *T. mentagrophytes*. Infection. Lesions.

Introduction

Experimental infection is a valuable method of studying the pathogenesis of fungal infections, to evaluate the prophylactic efficacy of antifungal therapeutic drugs and to study the immunology of dermatophytosis [1-3]. Dermatophytes are a group of highly specialized fungi that infect keratinized tissues, such as the hair, nail and stratum corneum of humans and other animals. The dermatophytes include three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. The species that most commonly infect animals are *Microsporum canis*, *Trichopyton mentagrophytes* and *Microsporum gypseum* [4-6]. The use of guinea pigs as animal models for

dermatophytosis is based on the predisposition of this species to skin fungal infections with clinical features comparable to those seen in humans [2].

When establishing an animal model it is crucial to find a method that ensures a high infection rate. Various methods have previously been used to render the skin more susceptible to infection, such as scarification, abrasion with a scalpel and inoculate fixed to the flank by bandaging for approximately 24hs [10]. In these studies, the experimental infections are commonly induced in the posterior dorsal region, cheek pouch, hind thigh and other skin areas [1,7-9], causing suffering and stress to the animals.

The idea for the present study began with our observation of an outbreak of dermatopytosis caused by *T. mentagrophytes* var. *mentagrophytes* in a colony of guinea pigs, where the main site affected was the outer ear. Since our work entails *in vitro* and *in vivo* evaluation of the antifungal activity of natural products, we felt that induced infection at this site could serve as a new model that more nearly mimics natural infection and is easy to carry out while at the same time reducing the animals' suffering.

Materials and Methods

Animals

Twelve guinea pigs, of both sexes, were used in the study. The animals, weighing approximately 600g, were kept in controlled rooms (temperature: 23±2° C, relative humidity: 50±10%, frequent ventilation and 12h light cycle). All protocols that included animals were approved by the research ethics committee of State University of Ceará, Fortaleza, Brazil. The animals were used as recommended by the guide for the care and use of laboratory animals from the National Academy Press (USA; 1996), which is in line with the principles for animal use in Brazil.

Fungal strain

In the present study, *T. mentagrophytes* var. *mentagrophytes* strain (CEMM 1-4-085) was obtained from the fungal collection of the Specialized Medical Mycology Center – CEMM (Federal University of Ceará, Brazil), where it was maintained in saline (0.9% NaCl), at 28° C. At the time of the analysis, an aliquot of each suspension was taken and inoculated in potato dextrose agar (Difco, Detroit, USA), and then incubated at 28° C for six days. The identification of *T. mentagrophytes* was based on phenotypic features, such as a description of the macro and micromorphology. Skin perforation and

vitamin requirement tests were considered, as well as the production of the enzyme urease [6].

Inoculation

The stock inocula were prepared on day 10, grown on potato dextrose agar (Difco, Detroit, USA), at 28° C. The suspensions with hyphal fragments of *T mentagrophytes* var. *mentagrophytes* were transferred to a sterile tube and adjusted by turbidimetry to obtain an inoculum of approximately 10^6 cfu/ml. The optical densities of the suspensions were spectrophotometrically determined at 530 nm. The suspension (0.5 ml) was gently inoculated on the ear flap with a swab, a single time. The guinea pigs were divided into three groups of four animals each: two groups treated on both the right and left ears and a negative control that was not inoculated with the fungal suspension. One of the groups infected with *T. mentagrophytes* var. *mentagrophytes* was then treated with cetoconazol. The fungal infection in each animal was confirmed by hair cultures and clinical evaluation of infected skin lesions.

Clinical evaluation

Clinical assessment of the guinea pigs was done according to a modification of the method published by Lee et al. (2007). The lesions were clinically followed-up daily, starting on day 0, until resolution was observed. Clinical evaluation of the inoculated animals was performed using a modified lesion score from 0 to 4, as follows: score 0, no visible lesion; score 1, moderate scaling; score 2, hair rarefaction and heavier scaling; score 3, hair loss and crust formation; score 4, alopecia and crusts.

Mycologic and histopathologic evaluation

Mycological evaluation. Epidermal flakes were scraped from the animals and hairs were collected manually at intervals of seven days after inoculation. The epidermal samples and hairs were seeded in tubes containing potato dextrose agar (Difco, Detroit, USA) and Sabouraud dextrose agar 2% (SGA; Difco, Detroit, USA), with chloramphenicol, and maintained in an incubator at 28° C.

Histopathological evaluation. The guinea pigs experimentally inoculated with *T. mentagrophytes* var. *mentagrophytes* were submitted to local anesthesia and skin fragments of their mycotic ears were collected with scissors and tweezers. These samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 5-m-thick sections, and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). All the samples were immediately taken to the Histology Laboratory of the Department of Pathology and Forensic Medicine of Federal University of Ceará, where they were

analyzed. The amount of fungal elements (hyphae and conidia), and degree of hyperkeratosis, acanthosis (epidermal hyperplasia), and spongiosis were evaluated semi-quantitatively by one pathologist in a blind setup. A positive histological examination was de fined as detection of fungi.

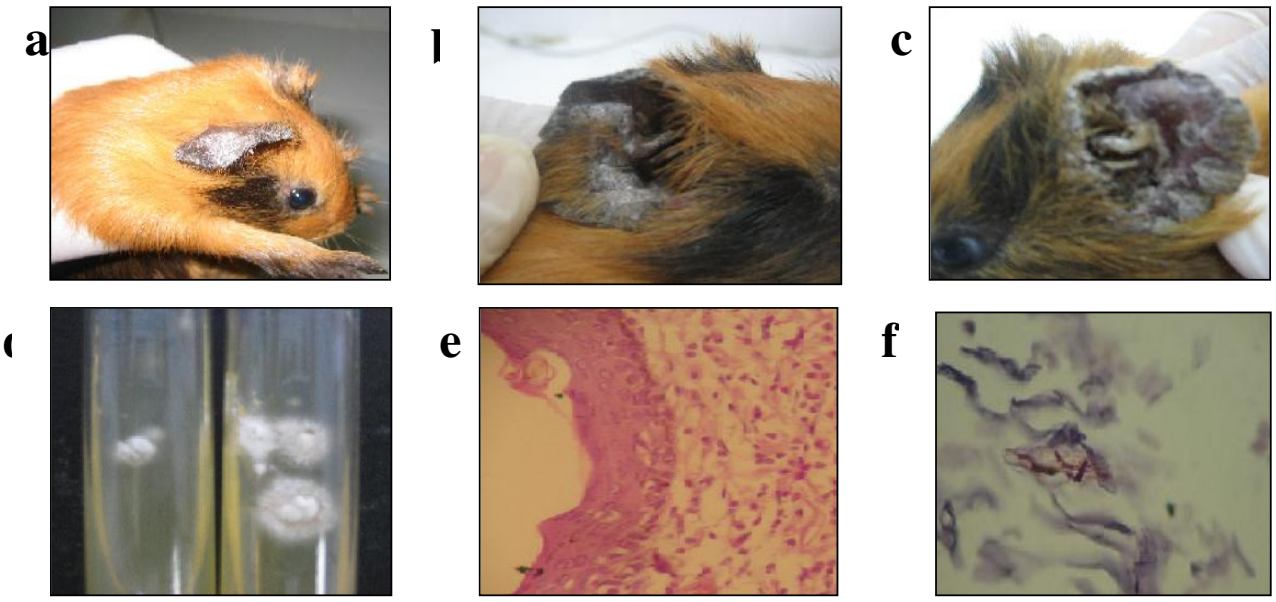
Results

The experimental infection of guinea pigs with *T. mentagrophytes* resulted in lesions in all animals that were exposed to the fungus. The first signs of infection were observed on the 7th day after inoculation in all the infected animals and were manifested in the form of moderate scaling, corresponding to lesion score1. These alterations became more evident around the 14th day, with the development of hair rarefaction and squamosis, score 2 (Fig. 1a). The lesions progressively increased in diameter, with total hair loss and crust formation, score 3, between the 18th and 21st days (Fig. 1b). Between the 22nd and 25th days, the inoculation site showed areas of alopecia and crusts, score 4 (Fig. 1c). *T. mentagrophytes* were re-isolated from hairs and epidermal flakes of all inoculated animals from day 7, 14, 21, 28 and 33 (Fig. 1d). The mycological cultures were positive in 100% of the inoculated animals.

The positive cases were related to the first week of infection. After the start of treatment with cetoconazol, on the 14th day, the mycological tests were negative. The group treated with cetoconazol had reduced lesion scores compared to the positive control during the entire experimental period.

In the histological sections stained with hematoxylin-eosin, the animals infected with *T. mentagrophytes* var. *mentagrophytes* showed moderate inflammatory infiltrate rich in lymphocytes, neutrophils and macrophages on the skin diffusely. We observed foci of granulation tissue with neovascularization, extravasation of red blood cells and collections of neutrophils (micro abscesses). The inflammatory infiltrate extended to the dermo-epidermal junction. There were also foci of parakeratosis with accumulations of neutrophils (Fig. 1e). *T. mentagrophytes* hyphae were detected in the stratum corneum in histological sections stained with PAS (Fig. 1f). The group treated with cetoconazol not had inflammatory infiltrate and *T. mentagrophytes* hyphae.

Fig. 1 - Experimental lesion produced by *T. mentagrophytes* var. *mentagrophytes* in guinea pigs after 14 days of evolution (a), after 21 days (b), after 28 days (c) and colonies (d) of *T. mentagrophytes* isolates from the guinea pigs. Histological section (e) of the skin of a guinea pig showing a moderate infiltrate rich in lymphocytes, neutrophils and macrophages, diffusely on the skin. Staining: hemosylin-eosin. Magnification: 200x. Presence of hyphal fragments (f) in the stratum corneum, visible after staining with PAS. Magnification: 400x



Discussion

In this study a new model of experimental dermatophytosis was developed and showed itself to be practical and fast, without the need to use abrasions, bandages and general anesthesia, thus causing less harm and suffering to the animals. Such procedures have been described by various authors, such as Cavalcante et al. (2002). In that study, the hair in the posterior dorsal region of the animals was removed and a skin area was submitted to scarification with a scalpel. After the inoculation of the suspension (of *Microsporum canis*), the site was covered with polyethylene film and kept in place with an elastic bandage for 24h.

In comparison with the technique described by Cavalcanti et al. (2002) and other authors [8,9], the experimental model presented in this study, using the outer ear of guinea pigs without subjecting the animals' skin to any type of aggression or invasive procedures, showed significant results, both in relation to the clinical profile and time for the infection to appear.

The first signs of infection in this work were observed on the 7th day after inoculation in 100% of the animals, manifested in the form of scaling. These findings corroborate those described by Vermout et al. (2004), who observed signs, although slight, typical of dermatophytosis on the 7th day after

inoculation in guinea pigs in evaluating immunogenicity and protective efficacy of a *Microsporum canis* metalloprotease subunit vaccine. Regarding the time for manifestation of the infection, our findings are similar to those described by Cavalcanti et al. (2002), where the first signs were detected around the 5th day after inoculation in 87.5% of the animals, through the presence of mild edema, erythema and mild shedding.

The literature, although extensive due to the many techniques of experimental infection described [1-3], does not report any study where the outer ear was the inoculation site, or techniques that do not require aggression and are less invasive for establishment of the infection. The method described here appears to cause less stress, harm and risk of death to the animals, and is quick and practical for developing experimental dermatophytosis.

The present results obtained by experimental induction of *T. mentagrophytes* var. *mentagrophytes* led us to conclude that the new model of experimental dermatophytosis in guinea pigs produces lesions similar to natural ones, is relatively simple to perform and is less traumatic for the animals.

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