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EFFICACY OF TERBINAFINE AND ITRACONAZOLE ON A EXPERIMENTAL MODEL OF SYSTEMIC SPOROTRICHOSIS

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ABSTRACT

Itraconazole is currently considered the drug of choice to treat the diverse clinical presentation of sporotrichosis. On the other hand terbinafine by virtue of its excellent *in vitro* activity is under comparative evaluation for its therapeutic potential for a wide range of fungal infections. In this study, our aim was to determine the *in vivo* efficacy of terbinafine and itraconazole on a experimental model of systemic sporotrichosis. 120 rats Wistar received an injection of $2x10^3$ *S. schenckii* cells by via the lateral tail vein. After 3 days the animals were treated with terbinafine (250mg/kg) and itraconazole (100 mg/kg) and their respective diluents. In our model, terbinafine and itraconazole were effective in reducing the number of clinical lesions and positive organ cultures. There was statistical difference between the groups treated with the antifungals in relation to the control groups ($p<0,05$) concerning the clinical alterations, anatomic-pathological findings and in the positive organ cultures of the agent, being that the treated animals resulted in the absence and/or reduction of all the evaluated parameters. As for the treatments, terbinafine showed similar or higher activity that itraconazole in the evaluation of the testicle alteration ($p=0,0004$), as well as in the positive organ cultures of microorganism from the organ ($p=0,0142$). With these results it is possible to conclude that the antifungals studied are effective in the treatment of experimental systemic sporotrichosis.

Key words: itraconazole, terbinafine, sporotrichosis, antifungal.

INTRODUCTION

Sporothrix schenckii, the etiological agent of sporotrichosis, is a dimorphic fungus widely distributed in nature, presenting a saprophytic mycelial form on plant debris and soil. The traumatic inoculation of conidia and hyphae of *S. schenckii* leads to a subcutaneous mycosis and in the infected tissue the fungus differentiates to the yeast form and may spread to other tissues. The systemic form of sporotrichosis may evolve from an initial cutaneous lesion or be associated with inhalation of conidia. Recently, more severe clinical forms of this disease have been associated with immunocompromised patients, such as human immunodeficiency virus (HIV)-infected patients, suggesting that *S. schenckii* is an emerging opportunistic pathogen (13,14).

Itraconazole is generally used for the treatment of lynphocutaneous infections, while amphotericin B is indicated for severe infections. Although these drugs are generally effective, the long duration of therapy and the toxicity make it necessary to explore new alternatives for the treatment of severe infections (1,2,10,16). Promising *in vitro* results with terbinafine for both the fixed and lymphocutaneous forms of sporotrichosis have been compared and confirmed clinically. The antifungal activities of these compounds are based on the inhibition of fungal ergosterol biosynthesis at the point of squalene epoxidation, leading to the intracellular accumulation of

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squalene, which causes the rapid death of fungi. It has demonstrated activity against most superficial fungal infections, including onychomycosis and dermatomycosis, and systemic fungal infections, such as histoplasmosis, *Pneumocystis carinii* infection, and aspergillosis (3,4,9,17,18,19,20). In this study, our aim was to determine the *in vivo* efficacy of terbinafine and itraconazole on a experimental model of systemic sporotrichosis.

MATERIALS AND METHODS

Sporothrix schenckii

Two *Sporothrix schenckii* isolates were obtained from clinical cases of feline and canine cutaneous sporotrichosis, being these stored in the fungal collection of the Mycology lab from the Federal University of Pelotas (UFPel). For the fungal inoculum it was utilized the agent in its filamentous form, cultured on 4% Sabourad's dextrose agar in plates at 25ºC for 10 days. Mycelium was removed using a blade and washed twice in PBS. It was filtered twice in a double layer of sterile gauze and centrifuged at 1500rpm for 15 minutes. The inoculum was patterned in $2X10^3$ cells of *S. schenckii*/mL, according to the MacFarland´s scale with the subsequent culture in Sabouraud´s dextrose agar medium with addition of cloranphenicol and cicloheximide for the Colony Forming Units (CFUs) counting.

The isolates of feline and canine *S. schenckii* utilized for the inoculum were identified by micromorphological characteristics and by the demonstration of typical dimorphism. They were maintained in brain heart infusion (BHI) solid medium at controlled temperature of 4ºC, with replication onto new medium at 6-month intervals.

Antifungal agents

Terbinafine and itraconazole were tested for their efficacy in this murine model. Terbinafine was suspended in 1% Tween 20 and 5% DMSO in distilled water. Itraconazole was dissolved in distilled water to obtain the use concentration.

Murine model of systemic sporotrichosis

A total of 120 rats Wistar, 8 weeked, weighing 300 g were used in the experiment, and maintained in water and food ad libitum. They were housed at room temperature in four experimental groups for each isolate of *S. schenckii* studied (feline and canine), being them: group treated with terbinafine with 40 animals (20 inoculated with the feline isolate and 20 with the canine isolate) to which it was administered 250mg/Kg of terbinafine dissolved in a solution containing 1% of Tween 20 and 5% of DMSO; group treated with itraconazole with 40 animals (20 inoculated with the feline isolate and 20 with the canine isolate), to which it was administered 100mg/kg of itraconazole dissolved in sterile distilled water; and the diluent terbinafine group with 20 animals (10 inoculated with the feline isolate and 10 with the canine isolate) and diluent itraconazole group with 20 animals (10 inoculated with the feline isolate and 10 with the canine isolate) to which it was administered the respective diluent of each drug.

Each rat received an injection of $2x10^3$ *S. schenckii* cells in 0.1 ml of PBS via lateral tail vein. Treatments with drugs or diluents were begun 3 days after inoculation with *S. schenckii* and were done daily for 30 days. Terbinafine and itraconazole or diluent were administered by gavage of 1,0 ml. All mice were observed daily for appearance, ataxia, and mortality during treatment. At the end of the experiment, all surviving mice were weighed, sacrificed, and autopsied. Organ cultures of testicle, liver, and spleen were made in triplicate on Sabouraud's dextrose with cloramphenicol and cicloheximide at 25ºC and 37ºC during 10 days.

Statistical analysis

The statistical analysis to evaluate the treatment´s and the microbiologic evolution was done using Epi Info 6.0, and qui-square test, according to Mantel-Henzel, with degree of significance of 95%.

RESULTS AND DISCUSSION

Sporotrichosis remains a difficult infection to treat despite the use of iodides for the lymphocutaneous form and amphotericin B for the extracutaneous form. The potential *in vivo* therapeutic value of terbinafine has been confirmed, up to the present, only for cases of cutaneous and lymphocutaneous sporotrichosis (6,8), although the use of elevated doses of the drug have been suggested (above 500 mg day) to ensure clinical efficacy (3). Efficacy has not been observed with the use of terbinafine for the treatment of murine systemic sporotrichosis.

The anatomic-pathologic alterations derived from experimental systemic sporotrichosis were characterized by ulcerated and exsudative lesions on the tail, testicles and limbs. The statistic analyses resulted in significant differences ($p<0,05$) between the groups treated with the antifungals in relation to the groups treated with diluents which showed a higher number of animals affected (Fig. 1).

The anatomic-pathological alterations when present, were characterized by nodular and whitish lesions on the spleen, liver, testicles, and subcutaneous tissue in a localized and/or disseminated manner, being later more frequent among the animals from the diluent groups, differing statiscally ($p<0,05$) from the groups treated with the antifungals. Concerning the terbinafine and itraconazole treatments, there was statistic difference ($p=0,0004$) only in the testicular lesions, resulting in a smaller frequency of animals from the group treated with terbinafine with alteration in relation to the group treated with itraconazole. (Table 1).

The obtainment of the positive organ cultures of *S. schenckii* resulted in the growth of colonies with macro and micro-

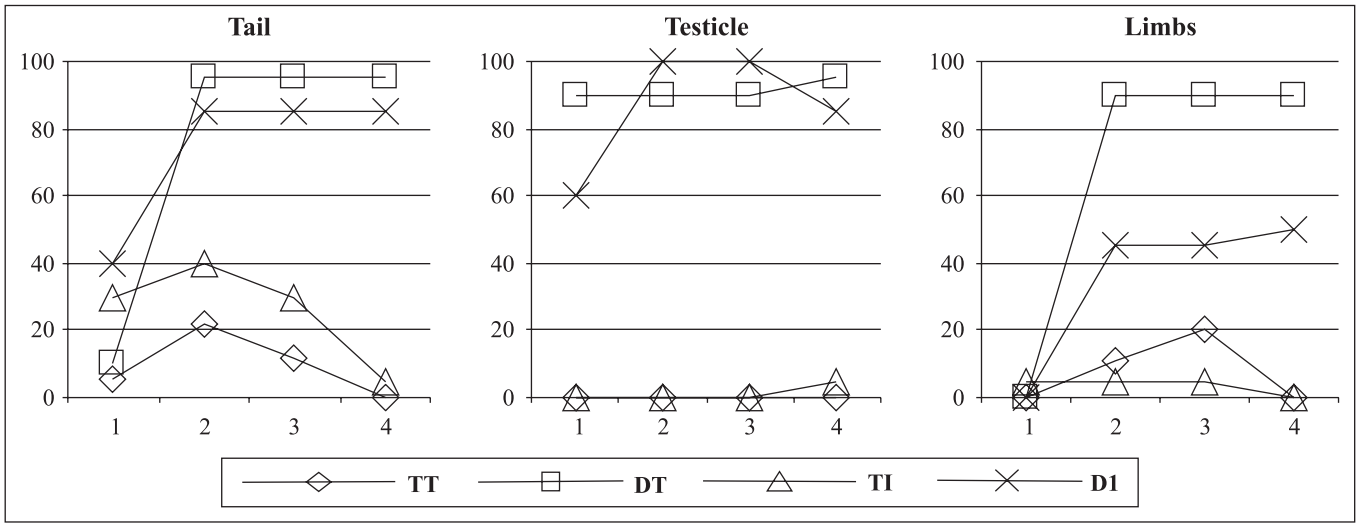

Figure 1. Percentage of tissue lesions evaluated weekly on tail, testicle, limbs of 114 rats with experimental systemic sporotrichosis treated with terbinafine (TT), itraconazole (TI) and respective diluents (DT) e (DI).

Table 1. Frequency of animals with macroscopic lesions in spleen, liver, testicle, subcutaneous nodules and disseminated lesions on the postmortem exam of 114 rats sacrificed 30 days after inoculation with *Sporothrix schenckii* according to the treatments.

	TT (%)	DT (%)	TI (%)	DI (%)
Spleen	$0^{a,A}$	100^{b}	$25^{a,A}$	95^{b}
Liver	$11,7^{a,A}$	100^{b}	$25^{a,A}$	85^{b}
Testicle	$26,4^{a,A}$	100^{b}	$67,5^{a,B}$	95^{b}
Subcutaneous nodules	$8,8^{a,A}$	100^{b}	$12,5^{a,A}$	85^{b}
Disseminated lesions	$0^{a,A}$	100^{b}	$0^{a,A}$	85^{b}

TT Treatment terbinafine; DT Diluent terbinafina; TI Treatment itraconazole; DT Diluent itraconazole; (%)= percentage; *p<0,05; ^{a,b,A,B} different exponents at the same line vary on minimum p< 0,05.

Table 2. Isolation of *S. schenckii* in spleen, liver and testicle of 114 rats after 30 days of treatment with terbinafine (TT), itraconazole (TI) and respective diluents (DT, DI).

ÓRGANS	TT (%)	DT (%)	TI (%)	DI (%)
Spleen	0^{b}	80^{a}	0^{b}	80^{a}
Liver	0^{b}	100^{a}	25^{b}	80^{a}
Testicle	3^{b}	100^{a}	$22,5^{b}$	95^{a}

TT Treatment terbinafine; DT Diluent terbinafina; TI Treatment itraconazole; DT Diluent itraconazole; (%)= percentage; *p<0,05; ^{a,b}different exponents at the same line vary on minimum p< 0,05.
All rats in the groups receiving itraconazole survived, however, six of the 40 rats treated with terbinafine died during the course of the experiment, owing to gavage trauma, as determined by autopsy.

morphologic aspect characteristic of the agent. The statistic analysis revealed differences between the treated and diluent groups (p<0,05), demonstrating smaller or none positive organ cultures for the *S. schenckii* in the groups treated with the antifungals. It was evidenced a statistic difference (p=0,0142) between the groups treated with terbinafine and itraconazole, resulting in the larger number of animals with positive isolation from the testicle in the group treated with itraconazole (Table 2).

In a previous study with guinea pigs inoculated intratesticularly with *S. schenckii*, itraconazole prevented dissemination and cured 30 to 100% of animals receiving 10 to 40 mg/kg (10). The statistic analysis demonstrated that the animals treated with terbinafine and itraconazole resulted in minor and/or absence of alterations in relation to the diluent group. Although the group treated with terbinafine resulted in statistic differences in relation to the group treated with itraconazole, such as the macroscopic alterations in the testicle and positive organ cultures, observing smaller number of affected animals. Terbinafine is highly lipophilic and keratophilic, so it is extensively distributed to the adipose tissue, dermis, epidermis, and nails in humans. The apparent volume of distribution in humans is relatively high and has been reported to be in the range of 780 to 2,000 liters. The high volume of terbinafine distribution and its accumulation in peripheral tissue, as well as the low redistribution of the drug into the blood are likely to significantly influence the half-life of terbinafine. Previous studies in humans have variously reported the half-

life of terbinafine elimination in 15 h, 26 h, 290 h, and 22 days. The rapid and extensive distribution of terbinafine in tissues dominates the pharmacokinetic characteristics of this drug in the body. The slow redistribution of terbinafine from tissues is responsible for the long elimination half-life observed for this drug (5, 7, 12). Based on these pharmacokinetic characteristics, terbinafine can be expected to provide sustained protection from a relapse of fungal infections following therapy; these pharmacokinetic characteristics also provide a rationale for a potentially shorter treatment time.

RESUMO

Eficiência de terbinafina e itraconazol em um modelo experimental de esporotricose sistêmica

Itraconazol é atualmente considerado a droga de escolha para o tratamento das diferentes formas clínicas da esporotricose. Por outro lado a terbinafina devido a sua excelente atividade *in vitro* está sendo avaliada quanto ao seu potencial terapêutico frente a diversas infecções fúngicas. O objetivo deste estudo foi determinar a eficácia *in vivo* da terbinafina e itraconazol em um modelo de esporotricose experimental sistêmica. 120 ratos Wistar receberam uma injeção de $2x10^3$ células de *S. schenckii* pela veia lateral da cauda. Após 3 dias os animais foram tratados com terbinafina (250mg/kg) e itraconazol (100mg/kg) e os seus respectivos diluentes. No modelo experimental estudado, a terbinafina e itraconazol se mostraram efetivos reduzindo o número de sintomas clínicos e retroisolamento positivo para o agente. Houve diferenças estatísticas entre os grupos tratados com os antifúngicos em relação aos grupos controle ($p<0,05$) nas alterações clínicas, achados anatomopatológicos e no retroisolamento do agente, sendo que os animais tratados resultaram na ausência e/ou diminuição de todos os parâmetros avaliados. Quanto aos tratamentos a terbinafina se mostrou com atividade similar ou superior ao itraconazol quando avaliado as alterações anatomopatológicos do testículo ($p=0,0004$), assim como no retoisolamento do órgão ($p=0,0142$). Com estes resultados permite-se concluir que os antifúngicos estudados são efetivos no tratamento da esporotricose sistêmica experimental.

Palavras-chave: itraconazol, terbinafina, esporotricose, antifúngico.

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