Effect of steroid hormones on the in vitro antifungal activity of itraconazole on *Fonsecaea pedrosoi*

Valeriano Antonio Corbellini1,2, Maria Lúcia Scroferneker2,3, Mariana Carissimi4, Luciana Maria Konrad4 and Cheila Denise Ottonelli Stopiglia1,3

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ABSTRACT: (Effect of steroid hormones on the *in vitro* antifungal activity of itraconazole on *Fonsecaea pedrosoi*). The *in vitro* antifungal activity of itraconazole, combined with oestradiol, testosterone or progesterone, on the growth of *Fonsecaea pedrosoi* was evaluated. *Fonsecaea pedrosoi* was cultured in Sabouraud supplemented with dimethyl sulphoxide dilutions in β-oestradiol (0.04 μg/mL and 0.04 mg/mL), progesterone (0.06 μg/mL and 0.06 mg/mL) or testosterone (1.2 μg/mL and 1.2 mg/mL) combined with 0.01 to 1 μg/mL of itraconazole. The sex steroid hormones did not modify the growth pattern of *F. pedrosoi* when they were in the presence itraconazole. The absence of synergistic or antagonistic activity of oestradiol, progesterone, testosterone and itraconazole on the growth of *F. pedrosoi* observed in this work does not rule out *in vivo* factors related to the development of the disease and to the success of an antifungal treatment.

Key words: chromoblastomycosis, antifungal, oestradiol, progesterone, testosterone.

RESUMO: (Efeito de hormônios esteróides na atividade antifúngica *in vitro* de itraconazol em *Fonsecaea pedrosoi*). A atividade antifúngica *in vitro* de itraconazol, combinada com estradiol, testosterona ou progesterona no crescimento de *Fonsecaea pedrosoi* foi avaliada. *F. pedrosoi* foi cultivado em Sabouraud suplementado com β-estradiol (0.04 μg/mL e 0.04 mg/mL), progesterona (0.06 μg/mL e 0.06 mg/mL) ou testosterona (1,2 μg/mL e 1,2 mg/mL) combinados com itraconazol diluído em dimetilsulfóxido na faixa de concentração entre 0,01 e 1 μg/mL. Os hormônios esteróides sexuais não modificaram a inibição de *F. pedrosoi* pelo itraconazol. A ausência da atividade de sinergismo ou antagonismo de estradiol, progesterona, testosterona e itraconazol no crescimento de *F. pedrosoi* observada neste trabalho não exclui fatores *in vivo* relacionados ao desenvolvimento da doença e para o sucesso de um tratamento antifúngico.

Palavras-chave: cromoblastomicose, antifúngico, estradiol, progesterona, testosterona.

INTRODUCTION

Chromoblastomycosis is a subcutaneous fungal disease caused by dematiaceous fungi, which is characteristic of tropical and subtropical regions (Hamza et al. 2002, Lacaz et al. 2002). Infection begins with the traumatic implantation of conidia or hyphal fragments into subcutaneous tissues, producing initial lesions consisting of papules or nodules that become verrucous (De Hoog et al. 2000). In Brazil, the most frequent etiologic agents are *Fonsecaea pedrosoi*, *Phialophora verrucosa* and *Cladosporium carrionii* (Silva et al. 1999, Minotto et al. 2001). More rarely, *Fonsecaea compacta*, *Exophiala jeaneselmei* and *Rhinocladiella aquaspersa* are the cause of the infection (Rippon 1982). Hernández-Hernández et al. (1995) found that both testosterone and progesterone were able to diminish the *in vitro* growth of *Phialophora verrucosa*. Sex steroid hormones exert inhibitory or stimulatory *in vitro* effects on the growth and maturation of some fungi (Drutz et al. 1981, Loose et al. 1983, Powell et al. 1983, Powell & Drutz 1984, Kinsman & Collard 1986). However, there are no data on the participation of sex hormones in the successful itraconazole treatment of chromoblastomycosis, caused by *Fonsecaea pedrosoi*, because triazoles are capable of down-regulating adrenal and gonadal androgen biosynthesis (Saag & Dismukes 1988, Englarhardt et al. 1991). The aim of this work was to evaluate and compare the antifungal activity of itraconazole combined with oestradiol, testosterone or progesterone on the *in vitro* growth of *Fonsecaea pedrosoi*.

MATERIALS AND METHODS

The ATCC 46428 strain of *Fonsecaea pedrosoi* was obtained from the Laboratory of Pathogenic Fungi of the Department of Microbiology of the Institute of Basic Health Sciences of the Universidade Federal do Rio Grande do Sul, and stored on Sabouraud dextrose agar (Oxoid Limited, Hampshire, UK) slants at 25 °C.

Itraconazole (Jansen Pharmaceutica, Beerse, Belgium) was obtained from the manufacturer as reagent grade powder. The stock solution of 10 mg/mL was prepared

1. Department of Physics and Chemistry, Universidade de Santa Cruz do Sul. CEP 96815-900, Santa Cruz do Sul, RS, Brazil.
2. Department of Microbiology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul. CEP 90050-170, Porto Alegre, RS, Brazil.
3. Graduate Program in Medicine, Medical Sciences, Universidade Federal do Rio Grande do Sul. CEP 90035-003, Porto Alegre, RS, Brazil.
4. Local Department of Environmental Protection, City Hall of Caxias do Sul. Caxias do Sul, RS, Brazil.
5. CAPES Researcher.

*Author for correspondence. E-mail: valer@unisc.br*
in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St. Louis, USA). Serial macrodilutions were prepared as outlined in CLSI document M-38A (NCCLS 2002). Final concentrations, which ranged between 0.01 and 1 μg/mL, were made in Sabouraud dextrose broth (Oxoid Limited, Hampshire, UK).

β-oestradiol (Sigma Chemical Co., St. Louis, USA), progesterone and testosterone (Merck, Darmstadt, Germany) were prepared in the DMSO to achieve the final concentrations of 0.04 μg/mL and 0.04 mg/mL, 0.06 μg/mL and 0.06 mg/mL, and 1.2 μg/mL and 1.2 mg/mL, respectively. Each of them corresponded to normal and 1000-fold concentrations in human serum.

The mycelia were harvested from cultures grown for 14 days at 25 ºC in liquid Sabouraud dextrose broth (120 rpm). Homogenisation was performed using a tissue grinder, and the mycelia were collected, filtered with a Whatman #1 paper filter with the aid of a Büchner funnel and observed under a microscope (Olympus Inc., Center Valley, USA) to ensure that the inoculum had well-dispersed hyphal fragments. The concentration of the supernatant was determined by optical density at 550 nm (O.D. of 0.5), using an SP-2000UV ultraviolet-visible (UV) spectrophotometer (Spectrum Instruments Company, Shangai, China), corresponding to 5×10⁶ CFU/mL in Sabouraud dextrose broth. Tests were performed, in quintuplicate, by culturing the inoculum that was supplemented with each hormone concentration and combined with each itraconazole dilution. Standard solutions were obtained, in triplicate, from the supernatant cultured with only the itraconazole dilutions. The readings were made after 3 days at 25 ºC using a spectrophotometer at 550nm.

The data were analysed by two-way analysis of variance. Tukey’s test was used for each itraconazole treatment to define which mean optical density was significantly different from the other means.

RESULTS AND DISCUSSION

The study demonstrates that sex steroid hormones did not modify the in vitro inhibition of F. pedrosoi by itraconazole because they did not change fungal behaviour when compared to the controls. F values for each itraconazole concentration combined with the hormone treatments are presented in Table 1.

Data derived from studies about Coccidioides immitis (Rippon 1982), Paracoccidioides brasiliensis (Restrepo et al. 1984) and some dermatophytes (Schär et al. 1986, Clemons et al. 1988, Brasch & Gottkehaskamp 1992) indicate that the effects of mammalian steroids mediated by intracellular steroid-binding sites may be relevant to the development of fungal diseases. β-oestradiol has also been shown to cause an increase in germ tube length in Candida albicans in a dose/strain-dependent manner (Cheng et al. 2006). Hernández-Hernández et al. (1995) demonstrated that sex steroid hormones modified the normal in vitro growth of P. verrucosa, another chromoblastomycosis agent. Thus, it would be expected that the change in membrane integrity by antifungal agents could enhance inhibitory activity, leading to a better and individualised dosage of the antifungal agent. In this work, no statistical significance was observed among optical cell densities in the fungal strain tested with oestradiol, progesterone and testosterone, even at physiological and pharmacological concentrations (Fig. 1). According to Queiroz-Telles et al. (1997), analysis

![Figure 1](image-url)  
Mean optical densities of Fonsecaea pedrosoi treated with itraconazole combined with β-oestradiol, progesterone and testosterone at physiological (A) and pharmacological (B) concentrations.

Table 1. F values estimated from optical densities of Fonsecaea pedrosoi treated with itraconazole combined with β-oestradiol, progesterone and testosterone at physiological and pharmacological concentrations.

<table>
<thead>
<tr>
<th>Source</th>
<th>1.0</th>
<th>0.5</th>
<th>0.2</th>
<th>0.05</th>
<th>0.02</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺ (df=2)</td>
<td>10.6</td>
<td>0.7</td>
<td>6.4</td>
<td>5.4</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>HC⁺ (df=2)</td>
<td>8.3</td>
<td>3.0</td>
<td>50.2</td>
<td>24.0</td>
<td>4.9</td>
<td>3.7</td>
</tr>
<tr>
<td>H x HC (df=4)</td>
<td>18.3</td>
<td>2.5</td>
<td>3.8</td>
<td>3.4</td>
<td>3.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a. source of variation among the hormones tested: β-oestradiol, progesterone and testosterone, for each itraconazole concentration.  
b. source of variation among physiological concentration, pharmacological concentration and without hormone.  
df = degrees of freedom.
of testosterone levels in male patients suggested that itraconazole did not interfere with androgen synthesis. Although increased male susceptibility occurs in a variety of infectious diseases and sex hormones are known to influence immune responses (Beagley & Gockel 2003), the male:female differential ratio in chromoblastomycosis infections occurs because the disease is predominant among farmers, who are subject to multiple work-related injuries; it is likely that most of the infected women accounted for in previous studies were also farmers (Bonifaz et al. 2001). Nevertheless, the absence of synergistic or antagonistic activity of oestradiol, progesterone, testosterone and itraconazole on the in vitro growth of *F. pedrosoi* (observed in this work) does not rule out in vivo factors related to the development of the disease and to the success of an antifungal treatment.

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**REFERENCES**


