Revista Brasileira de Biociências
Brazilian Journal of Biosciences

ISSN 1980-4849 (on-line) / 1679-2343 (print)

SHORT COMMUNICATION

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

Valeriano Antonio Corbellini[1]*, Maria Lúcia Scroferneker[2,3], Mariana Carissimi[4], Luciana Maria Konrad[1] and Cheila Denise Ottonelli Stopiglia[3,5]

Received: June 16 2010 Received after revision: August 4 2010 Accepted: August 5 2010
Available online at http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/1622

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 jeanselmei* 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, Engelhardt *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 ºC.

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 1μg/mL, were made in Sabouraud dextrose broth (Oxoid Limited, Hampshire, UK).

β-oestradiol (Sigma Chemical Co., St. Louis, USA), progesterone and testosterone (Merck, Darmstad, 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 5×10^6 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).

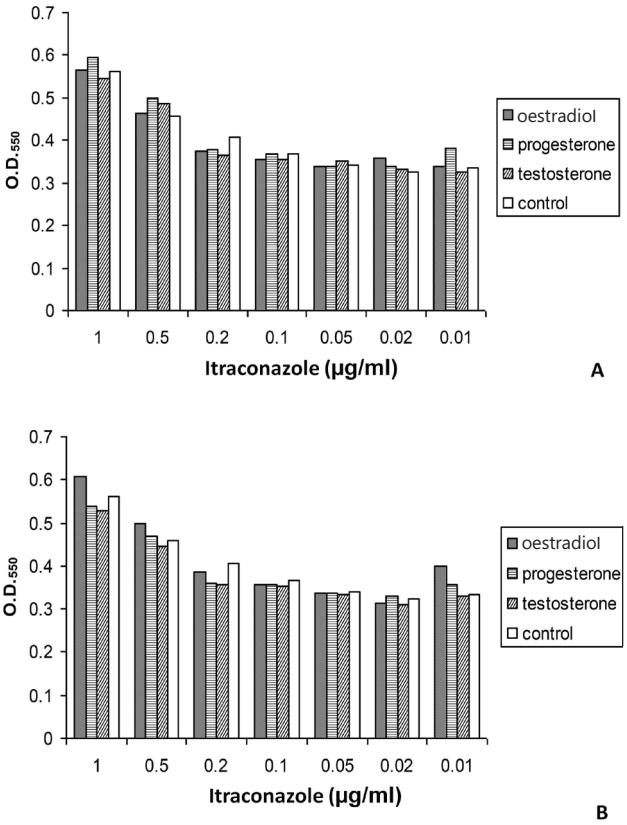

Figure 1. Mean optical densities of *Fonsecaea pedrosoi* treated with itraconazole combined with β-oestradiol, progesterone and testosterone at physiological (A) and pharmacological (B) concentrations.

According to Queiroz-Telles *et al.* (1997), analysis

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.

Source	Itraconazole (μg/ml)						
	1.0	0.5	0.2	0.1	0.05	0.02	0.01
H[a] (df=2)	10.6	0.7	6.4	5.4	1.1	0.6	2.9
HC[b] (df=2)	8.3	3.0	50.2	24.0	4.9	3.7	2.4
H x HC (df=4)	18.3	2.5	3.8	3.4	3.8	0.6	2.7

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.

ACKNOWLEDGMENTS

We thank Jansen Pharmaceutica for supplying the itraconazole, and FAPERGS and CAPES for financial support.

REFERENCES

BEAGLEY, K.W. & GOCKEL, C.M. 2003. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. *FEMS Immunology and Medical Microbiology*, *38*: 13-22.

BONIFAZ, A., CARRASCO-GERARD, E. & SAÚL, A. 2001. Chromoblastomycosis: clinical and mycologic experience of 51 cases. *Mycoses*, *44*: 1-7.

BRASCH, J. & GOTTKEHASKAMP, D. 1992. The effect of selected human steroid hormones upon the growth of dermatophytes with different adaptation to man. *Mycopathologia, 120*: 87-92.

CHENG, G., YEATER, K.M. & HOYER, L.L. 2006. Cellular and molecular biology of *Candida albicans* estrogen response. *Eukaryotic Cell*, *5*: 180-191.

CLEMONS, K.V., SCHÄR, G., STOVER, E.P., FELDMAN, D. & STEVENS, D.A. 1988. Dermatophyte-hormone relationships: characterization of progesterone-binding specificity and growth inhibition in the genera *Trichophyton* and *Microsporum*. *Journal of Clinical Microbiology*, *26*: 2110-2115.

DE HOOG, G.S., QUEIROZ-TELLES, F., HAASE, G., FERNANDEZ-ZEPPENFELDT, G., ANGELIS, D.A., GERRITS, A.H.G., MATOS, T., PELTROCHE-LLACSAHUANGA, H., PIZZIRANI-KLEINER, A.A., RAINER, J., RICHARD-YEGRES, N., VICENTE, V. & YEGRES, F. 2000. Black Fungi: clinical and pathogenic approaches. *Medical Mycology, 38*: 243-250.

DRUTZ, D.J., HUPPERT, M., SUN, S.H. & MCGUIRE, W.L. 1981. Human sex hormones stimulate the growth and maturation of *Coccidioides immitis*. *Infection and Immunity*, *32*: 897-907.

ENGELHARDT, D., WEBER, M.M., MIKSCH, T., ABEDINPOUR, F. & JASPERS, C. 1991. The influence of ketoconazole on human adrenal steroidogenesis: incubation studies with tissue slices. *Clinical Endocrinology, 35*: 163-168.

HAMZA, S.H., MERCADO, P.J., SKELTON, H.G. & SMITH, K.J. 2002. An unusual dematiaceous fungal infection of the skin caused by *Fonsecaea pedrosoi*: a case report and review of the literature. *Journal of Cutaneous Pathology*, *30*: 340-343.

HERNÁNDEZ-HERNÁNDEZ, F., DE BIEVRE, C., CAMCHO-ARROIO, I., CERBON, M.A., DUPONT, B. & LOPEZ-MARTINEZ, R. 1995. Sex hormone effects on *Phialophora verrucosa in vitro* and characterization of progesterone receptors. *Journal of Medical Veterinary Mycology*, *33*: 235-239.

KINSMAN, O.S. & COLLARD, A.E. 1986. Hormonal factors in vaginal candidiasis in rats. *Infection and Immunity*, *53*: 498-504.

LACAZ, C.S., PORTO, E., MARTINS, J.E.C., HEINS-VACCARI, E.M. & MELO, N.T. 2002. *Tratado de Micologia Médica*. São Paulo: Sarvier. p. 441-461.

LOOSE, D.S., STOVER, E.P., RESTREPO, A., STEVENS, D.A. & FELDMAN, D. 1983. Estradiol binds to a receptor-like cytosol binding protein and initiates a biological response in *Paracoccidioides brasiliensis*. *Proceedings of the National Academy of Science*, *80*: 7659-7663.

MINOTTO, R., BERNARDI, C.D., MALLMANN, L.F., EDELWEISS, M.I. & SCROFERNEKER, M.L. 2001. Chromoblastomycosis: a review of 100 cases in the state of Rio Grande do Sul, Brasil. *Journal of the American Academy of Dermatology*, *44*: 585-592.

NCCLS. 2002. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: approved standard. NCCLS Document M38-A. *NCCLS, 18*: 1–29.

POWELL, B.L., DRUTZ, D.J., HUPPERT, M. & SUN, S.H. 1983. Relationship of progesterone- and estradiol-binding. *Infection and Immunity*, *40*: 478-485.

POWELL, B.L. & DRUTZ, D,J. 1984. Identification of a high-affinity binder for estradiol and a low-affinity binder for testosterone in *Coccidioides immitis*. *Infection and Immunity*, *45*: 784-786.

QUEIROZ-TELLES, F., PURIM, K.S., BOGUSZEWSKI, C.L., AFONSO, F.C.S. & GRAF, H. 1997. Adrenal response to corticotrophin and testosterone during long-term therapy with itraconazole in patients with chromoblastomycosis. *Journal of Antimicrobial Chemotherapy*, *40*: 899-902.

RESTREPO, A., SALAZAR, M.E., CANO, L.E., STOVER, E.P., FELDMAN, D. & STEVENS, D.A. 1984. Estrogens inhibit mycelium-to-yeast transformation in the fungus *Paracoccidioides brasiliensis*: implications for resistance of females to paracoccidioidomycosis. *Infection and Immunity, 46*: 346-353.

RIPPON, J.W. 1982. *Medical mycology: the pathogenic fungi and the pathogenic actinomycetes.* Philadelphia: The W. B. Saunders Co. p. 277-302.

SAAG, M.S. & DISMUKES, W.E. 1988. Azole antifungal agents: emphasis on new triazoles. *Antimicrobial Agentsand Chemotherapy, 32*: 1-8.

SCHÄR, G., STOVER, E.P., CLEMONS, K.V., FELDMAN, D. & STEVENS, D.A. 1986. Progesterone binding and inhibition of growth in *Trichophyton mentagrophytes*. *Infection and Immunology, 52*: 763-767.

SILVA, J.P., SOUZA, W. & ROZENTAL, S. 1999. Chromoblastomycosis: a retrospective study of 325 cases on amazonic region (Brazil). *Mycopathologia*, *143*: 171-175.