

TITLE: CLOTRIMAZOLE IS EFFECTIVE AGAINST *SPOROTHRIX BRASILIENSIS* YEASTS *IN VITRO*

AUTHORS: BORBA-SANTOS, L.P.; GAGINI, T.; ROZENTAL, S.

INSTITUTION: LABORATÓRIO DE BIOLOGIA CELULAR DE FUNGOS, INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, UFRJ, RIO DE JANEIRO, BRAZIL.

ABSTRACT:

Clotrimazole is an imidazole antifungal firstly synthesized in the late 70s that has been used in topical treatment of several fungal infections. However, clotrimazole has not been tested against *Sporothrix brasiliensis*, the species responsible for sporotrichosis outbreak in the Rio de Janeiro state. Thus, the aim of our study was to evaluate the activity of clotrimazole *in vitro* against yeasts (pathogenic form) of *S. brasiliensis*. Yeast cells of the reference isolate CBS 133021 was incubated to different concentrations of clotrimazole in RPMI 1640 medium supplemented with 2% glucose and buffered with 0.165 M MOPS (pH 7.2), for 24h or 48h, at 35°C in the dark in a humid chamber with 5% CO₂. Samples were diluted and plated on solid medium and incubated for seven days before colony forming units counting. Alterations in the intracellular neutral lipid levels and morphological structures were also evaluated by flow cytometry analysis and by transmission electron microscopy, respectively. Results were compared with those for itraconazole, the first-line antifungal therapy for sporotrichosis. The inhibitory activity of clotrimazole was stronger than that itraconazole: after 24 h, there was a 2 Log₁₀ reduction in yeast viability for clotrimazole at 0.5 µg/ml, compared with a reduction of 1 Log₁₀ for itraconazole at 4 µg/ml. After treatment of yeast cells with sub-inhibitory concentrations of clotrimazole or itraconazole, clotrimazole was able to induce accumulation of neutral lipid in *S. brasiliensis* yeasts as itraconazole, and treated yeasts displayed grooves in the cell membrane and cell wall thicker. Our results show that clotrimazole has potential as therapeutic agent against *S. brasiliensis* and could be an effective alternative for the topical treatment of sporotrichosis. However, *in vivo* studies are required to confirm the antifungal potential of clotrimazole for the treatment of sporotrichosis.

KEYWORDS: clotrimazole, *Sporothrix brasiliensis*, sporotrichosis treatment.

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