Título: ANTIFUNGAL ACTIVITY OF MILTEFOSINE AGAINST *Paracoccidioides brasiliensis* AND *Paracoccidioides lutzii* ISOLATES

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Resumo:
Paracoccidioidomycosis (PCM) is a systemic and endemic mycosis in Latin America caused by the dimorphic fungus *Paracoccidioides* spp. The treatment of PCM is based in the administration of itraconazole (ITZ), amphotericin B (AMB) or combination of sulfamethoxazole (SMX) and trimethoprim (TMP); however, it is long and can last from six months to two years and relapses may occur. Thus, the search for new therapeutic options is necessary for the treatment of this mycosis. Miltefosine (MLT), an analogue of alkylphospholipids, has displayed a good antifungal activity against different species of yeast and filamentous fungi. The aim of this study is to evaluate the activity of MLT on *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii* isolates. The minimum inhibitory concentrations (MIC) of MLT and standard antifungal drugs (ITZ, AMB, SMX, and TMP) were determined by broth microdilution assay. Firstly, yeasts from *Paracoccidioides* sp. (*P. brasiliensis* - Pb18, Pb339, Pb8334, Pb113 Pbcao and *P. lutzii* - Pb01, Ed01 Pb1578, Pb66) were cultivated in the McVeigh and Morton (MVM) medium at 37 º under orbital agitation for 7-10 days. After incubation, yeast suspensions were standardized to obtain 1-5 x 10^5 cfu/ml and the antifungal drugs were serially diluted (1:2) in MVM medium into the flat-bottomed microdilution plates (96 wells). A volume of 100 µl of yeast suspension was added to each well to obtain final concentration 0.5-2.5 10^5 cfu/ml and the antifungal drugs concentrations as follow: 0.03 - 16 µg/ml for MLT, AMB, and ITZ; 0.12-64 µg/ml for SMX and TMP. The microplates were incubated at 37ºC in the humid and dark chamber and the MIC values were determined visually after 14 days of incubation. Among antifungal drugs evaluated ITZ was more effective to inhibit the yeast growth of *P. lutzii* and *P. brasiliensis* (MICs ≤ 0.12 µg/ml). MLT presented inhibitory activity on the yeasts in the concentrations from 0.12 to 1 µg/ml; similar effect to the AMB (0.25 – 1 µg/ml) and SMX/TMP combination (0.12-8 µg/ml for SMX and 0.02-1.6 µg/ml for TMP). SMX alone showed antifungal action quite varied (MIC values range from 0.25 to 16 µg/ml); and TMP alone presented the lowest inhibitory effect on both *Paracoccidioides* species (32 - >64 µg/ml). In conclusion, MLT presented antifungal activity against isolates of *Paracoccidioides* sp. and more studies should be conducted for possible use in the treatment of the PCM.

Palavras-chaves: *Paracoccidioides*, Miltefosine, Antifungal
Agência de Fomento: CAPES, CNPq, FAPESP