TITLE: ANTIFUNGAL ACTIVITY OF MILTEFOSINE AGAINST *Cryptococcus neoformans* AND *Cryptococcus gattii*.

AUTHORS: SPADARI, C.C [1]; ISHIDA, K. [1]

INSTITUTION: 1. DEPARTAMENTO DE MICROBIOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SP (AV. PROF. LINEU PRESTES 1374, LAB. 150, CEP 05508-900, SÃO PAULO - SP, BRAZIL)

ABSTRACT

Cryptococcosis is a fungal disease caused by two species complex: *Cryptococcus neoformans* and *Cryptococcus gattii*. Amphotericin B (AMB) is the cornerstone of treatment for severe cryptococcal infection, but its use has been limited due to high hepatotoxicity and nephrotoxicity. Thus, the search for new therapeutic options is necessary for the treatment of this mycosis. Miltefosine (hexadecylphosphocholine) (MFS) has shown a broad spectrum of antifungal activity, including the genus Cryptococcus. The aim of this study is to evaluate the activity of MFS on isolates of *C. neoformans* and *C. gattii*. Initially the broth microdilution assay was performed to determine the minimum inhibitory concentration (MIC) of the MFS following the M27-A3 document (CLSI, 2008) on standard strains (*C. neoformans* CAP59, *C. neoformans* H99, *C. gattii* ATCC 56990) and 10 clinical isolates. Subsequent assays were performed with the standard strains to evaluate the post-antifungal effect (PAFE) of MFS, effects on cell viability and membrane permeability, drug interaction with exogenous ergosterol and the effect of MFS on yeast morphology. MFS exhibited MIC values of 0.5 to 4 μg/mL and fungicidal activity for all strains tested. The PAFE found for MFS varied in the different isolates and it increased with higher concentrations of MFS: 8.15 h on *C. neoformans* CAP59 at MIC value, 2.71 h for MIC and 6.73 h for 4x MIC on *C. neoformans* H99, and *C. gattii* ATCC 56990 presenting 2.75 h for 4x MIC. Yeasts treated with MIC of MFS for 24 h presented no damage to the plasma membrane and to cell viability, but at higher concentrations (up to 4x MIC) extravasation of DNA and proteins was observed, as well as loss of cell viability. The presence of exogenous ergosterol increased the MIC values of MFS, as result similar to that obtained with AMB, suggesting a possible molecular interaction of MFS with ergosterol. Changes in cell morphology were also observed after MFS treatment: capsule thickness reduction, apparent decreased cell wall, cytoplasmic membrane irregularity and mitochondrial swelling. These findings suggest that MFS could be a potential option for cryptococcosis treatment and additional investigations should be pursued to understand the action mechanism of the drug in *Cryptococcus* spp. cells.

Keywords: Cryptococcosis; miltefosina; antifungal
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