TITLE: SUSCEPTIBILITY OF *ASPERGILLUS* SPP. CLINICAL ISOLATES TO MILTEFOSINE IN ALGINATE NANOCARRIER

AUTHORS: BARRETO, T. L.[1]; TRINDADE, M.R.S.[2]; MELO, A.S.A.[2]; LOPES, L.B.[3]; ISHIDA, K.[1]

INSTITUTIONS: [1]LABORATÓRIO DE QUIMIOTERAPIA ANTIFÚNGICA, DEPARTAMENTO DE MICROBIOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO, SP; [2]LABORATÓRIO ESPECIAL DE MICOLOGIA, DEPARTAMENTO DE MEDICINA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO, SP; [3]LABORATÓRIO DE NANOFÁRMACOS E SISTEMAS DE LIBERAÇÃO, DEPARTAMENTO DE FARMACOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO, SP

ABSTRACT:

Aspergillus spp. are responsible for causing opportunistic diseases in immunocompromised patients leading to high mortality rates. Despite the effectiveness of treatment with triazole agents, the number of resistant isolates has increased in recent years due to continued use in the clinic and agriculture. These problems make it important to search for alternatives and treatment strategies. Miltefosine (MFS), an akylphosphocholine compound, is licensed for the treatment of leishmaniasis and breast cancer in several countries. In addition, previous studies have shown that MFS presents a broad spectrum of action on pathogenic fungi and fungicidal effect, but it has high toxicity. Our research group has been working on the development of a formulation of miltefosine in alginate nanocarriers for its sustained release, toxicity reduction and maintenance of antifungal activity *in vitro* and *in vivo* against *Candida* and *Cryptococcus*. Thus, the objective of this work is to evaluate the *in vitro* antifungal efficacy of miltefosine free in solution (MFS) or in alginate nanocarriers (MFS.Alg) against *A. fumigatus* and *A. flavus* clinical isolates. The minimum inhibitory concentration (MIC), minimum effective concentration (MEC) and minimum fungicidal concentration (MFC) of MFS or MFS.Alg on 21 strains of *Aspergillus* spp. were determined by the broth microdilution method as described in the document M38-A2 (CLSI, 2008) using amphotericin B (AMB) and voriconazol (VCZ) as a controls. The MIC values of AMB ranged from 0.06 to 0.5 μg/mL on *A. fumigatus* isolates, from 0.1 to 0.5 μg/mL for VCZ, from 0.5 to 2 μg/mL for MFS and from 18.7 to 600 μg/mL for MFS.Alg. The morphological alterations of the hyphae were also verified to determine the MEC values of 0.06 to 0.25 μg/mL for AMB, 0.5 to 2 μg/mL for VCZ, 0.5 to 2 for MFS and 4.7 to 37.5 μg/mL for MFS.Alg. *A. flavus* exhibited a more tolerant profile to the compounds tested when compared to *A. fumigatus*. The MIC was 0.12 to 1 μg/mL for AMB, 0.06 to 0.25 μg/mL for VCZ, 2 to 16 μg/mL for MFS, and 300 to 600 μg/mL for MFS.alg. MEC values ranged from 0.12 to 2 μg/mL for AMB, 0.5 to 2 μg/mL for VCZ, 0.5 to 16 μg/mL for MFS, and 18.7 to 300 μg/mL for MFS.alg. Furthermore, the fungicidal effect was observed for all compounds tested including MFS. Thus, miltefosine free or encapsulated in alginate nanocarriers presented antifungal activity against all clinical isolates of *Aspergillus* tested and displays a great therapeutic potential for fungal diseases.

Keywords: fungal disease, release system, nanocarrier

Development Agency: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (#2015/07993-0 and #2018/03708-8).