EFFECT OF HIV ASPARTIC PEPTIDASES INHIBITORS ON CELLULAR GROWTH AND ENZYMACTIC ACTIVITIES PRODUCED BY *Fonsecaea pedrosoi*

[1]Paiva-Pereira, E., [1]Sousa, I.S.,[1]Granato, M.Q., [2]Alviano, C.S., [2]Santos, A.L.S., [1]Kneipp, L.F.

[1]Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos, IOC, Fiocruz, RJ, Brazil; [2]Departamento de Microbiologia Geral, Instituto de Microbiologia, CCS, UFRJ.
E-mail: lucimar@ioc.fiocruz.br

Fonsecaea pedrosoi is the main etiological agent of chromoblastomycosis, a subcutaneous mycoses extremely difficult to treat. Our group identified the presence of aspartic peptidase in *F. pedrosoi* and demonstrated the involvement of this enzyme in the cellular growth, morphogenesis and fungus-host cell interaction. The aim of this study was to evaluate the effect of different HIV aspartic peptidase inhibitors (HIV-PIs, second generation) on the fungal growth and enzymatic activities of *F. pedrosoi*. To determine the minimum inhibitory concentration of HIV-PIs, the broth microdilution assay was performed according to the M38-A2 protocol recommended by CLSI. The results demonstrated that up to the maximum concentration tested (400 μM) of HIV-PIs (atazanavir, darunavir and lopinavir) the fungal growth was not affected (MIC_{100} >400 μM). Thus, other experiment to evaluate the effect of inhibitors on fungal proliferation was carried out using the spread plate assay for determining the colony forming unit (CFU). Among the inhibitors tested, lopinavir was the only one capable of inhibiting around 60% and 40% the fungal growth, at 400 and 200 μM, respectively. Furthermore, the effect of HIV-PIs on the activities of two enzymes (aspartic peptidase and ecto-phosphatase), involved with *F. pedrosoi* pathogenesis, was also investigated. Using fluorogenic peptide substrate, we showed that lopinavir was able to inhibit 70% of the aspartic peptidase secreted by *F. pedrosoi*. In contrast, our results showed that lopinavir (400 μM) did not affect ecto-phosphatase activity. Experiments to evaluate the ability of conidia to adhere to polystyrene (abiotic substrate) and differentiate into hyphae were also performed. After adhesion (24 h), microscopic inspection of the polystyrene surface revealed the presence of germinated conidia and germ tube-like structures. In addition, after conidia interaction with polystyrene for 72 h, it was possible to observe the formation of intertwined mycelia monolayer adhered to the substrate. After that, three distinct parameters were analyzed, such as biomass by the incorporation of crystal violet dye, metabolic activity by the reduction of XTT and extracellular matrix by the absorption of safranin. Preliminary results showed that viable cells of *F. pedrosoi* were able to produce biomass and extracellular matrix. Additional experiments are being carried out to investigate the effect of HIV-PIs on the formation of this multicellular structure produced by *F. pedrosoi.*

Keywords: HIV aspartic peptidases inhibitors, *Fonsecaea pedrosoi*, cellular growth, peptidase activity.

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