Title: EXPRESSION OF SECRETED ASPARTYL PROTEASE GENE (SAP4) IN *Candida albicans* EXPOSED TO SUBINHIBITORY CONCENTRATIONS OF FLUCONAZOL AND AMPHOTERICIN B BEFORE AND AFTER PHAGOCYTOSIS BY MACROPHAGES

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Abstract:

Fungal infections have become a major public health problem especially in hospital settings. *Candida* spp. are considered the main pathogen in invasive fungal infections. The production of secreted aspartic proteinases (Sap) by *Candida* spp. can be related to the increase in the number of infections and drug resistance. The production of proteinases is encoded by a family of 10 genes known as SAP1-10. The expression of SAP4-6 is associated to production of Sap4-6 enzymes and hyphae formation which can contribute to the invasion of host tissues and destruction of macrophages in infectious processes.This study evaluates the SAP4 gene expression in *C. albicans* ATCC 10231 before and after the process of phagocytosis by macrophages. *C. albicans* was grown in Sabouraud Dextrose Agar for 24 h at 37ºC and after in yeast carbon base more 0,2% bovine serum albumin in the absence or presence of subinibitory concentrations of Fluconazole and Amphotericin B. The human monocytic leukemia cell line (THP1) was grown in RPMI-1640 Medium supplemented with 10% fetal bovine serum, penicillin (100U/ml) and streptomycin (100µg/ml) for 10 days. For the induction of cell differentiation, 10^6 cells were seeded in RPMI-1640 medium supplemented and phorbol 12-myristate 13-acetate (PMA) 200 nM for 48 h. The samples: 5 x 10^6 *C. albicans*, 10^6 macrophages, and 5 x 10^6 *C. albicans* in the presence of 10^6 macrophages. All samples were incubated in culture test plates with 6 flat-botton wells for 1 h at 37 °C and 5% CO_2. Total RNA was extracted from the samples using TRIzol® reagent, after RNA purification with DNaseI it was converted to cDNA, and then, the relative quantification of the SAP4 gene was performed by Real-Time Polymerase Chain Reaction (PCR) using ACT1 gene as normalizing endogenous gene. The results indicate that *C. albicans* grown in the presence of macrophages and 1/4 IC50 of fluconazole expressed 1,59 fold less SAP4 compared to *C. albicans* grown on 1/4 IC50 fluconazole and absence of macrophages. *Candida albicans* grown in the presence of macrophages and 1/4 IC90 amphotericin expressed 4,47 fold less SAP4 compared to *C. albicans* grown on 1/4 IC90 amphotericin. Understanding the gene expression of this enzyme in fungal pathogenesis may aid in research and development of new drugs for treating candidiasis, thus contributing to reducing the incidence of morbidity and mortality associated with fungal infections.

Keywords: *Candida albicans*, secreted aspartic proteinases, virulence factors, macrophages, gene expression.

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