TITLE: SYNERGISTIC ACTION OF THE ANTIMICROBIAL PEPTIDE ToAP2 WHEN COMBINED TO AMPHOTERICIN B AND FLUCONAZOLE IN BIOFILMS OF *Candida albicans*

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ABSTRACT

One of the major concerns of *Candida albicans* infections is their ability to form biofilms, that are complex structures resistant to antifungal agents and less susceptible to the action of the immune system. Thus, the objective of the present study was to evaluate the synergistic activity of the antimicrobial peptide (AMP) ToAP2 when combined to the antifungal agents Amphotericin B and Fluconazole in the two phases of *C. albicans* biofilm formation. ToAP2 was chemically synthesized based on the putative AMP cDNA sequence obtained from the venom gland of scorpions. The biofilm formation was assayed incubating $1x10^6$ cells of *C. albicans* SC 5314 in 96-well plates with RPMI medium, at 37 °C. After 4 and 24 hours (adhesion phase and mature biofilm) of incubation, the cells were washed with PBS and different concentrations of ToAP2, Amphotericin B and Fluconazole were added and incubated for 24 hours. Biofilm activity was determined using the AlamarBlue reagent, with fluorescence determined on a SpectraMax® plate reader, at 585 nm. Fluorescence images of the dead cells were obtained with an inverted fluorescence microscope, using the Phloxine B dye. The results indicate that ToAP2 has activity in the biofilm formation, reducing significantly the cell mass in the concentrations of 50 and 200 μM in the phases of adhesion and mature biofilm, respectively. Amphotericin B was also effective in reducing both phases of biofilm, in concentrations of up to 1 μM, reducing the cell mass to less than 50%. Fluconazole, on the other hand, had no effect in both phases of the biofilm. The combined activity of ToAP2 and Amphotericin B showed efficacy only in the biofilm adhesion phase, at combined concentrations that when used alone had no effect (0.06 and 0.12 μM Amphotericin B and 25 μM ToAP2). Although Fluconazole alone had showed no activity on both phases of biofilm formation, when associated to ToAP2, resulted in a reduction of up to 100% of the biofilm mass in the adhesion phase. Additionally, a marked reduction in the mature biofilm was observed when subinhibitory concentrations were used reaching to reduce up to 50% in some cases. These results suggest that the peptide ToAP2 could be used as an alternative agent against *C. albicans* biofilms, both alone or in combination with antifungals, especially Fluconazole, which is currently used in the treatment of candidiasis.

Keywords: Antimicrobial peptide, Antifungals, *Candida albicans*

Development Agencies: CNPq; FAPDF; PPG em Patologia Molecular/FM, Universidade de Brasília