TITLE: CASPOFUNGIN IN THE CONTROL OF INFECTIONS ASSOCIATED WITH POLYMICROBIAL BIOFILMS OF *Candida* spp. AND *Staphylococcus aureus*

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ABSTRACT:

Candida albicans and *Staphylococcus aureus* are responsible for a high number of infections impacting in the therapy especially when it involves the polymicrobial biofilms formation. Thus, the main objective of this work is to evaluate the effect of caspofungin *in vitro* and *in vivo* on *C. albicans* and *S. aureus* in planktonic or in biofilms life styles. *C. albicans* planktonic cells (SC5314 and IAL-40) were susceptible to all antifungals amphotericin B (AMB), caspofungin (CPG), Voriconazole (VRZ), Fluconazole (FLZ), except for IAL-40 characterized as FLZ- and VRZ-resistant. *S. aureus* (MSSA – ATCC 29213, MRSA – ATCC 6538 and ATCC 33591) were inhibited by CPG at 8-64 μg/mL and bactericidal activity was observed at 32-512μg/mL. Vancomycin (VCM) was active for all *S. aureus* strains (0.5-2μg/mL), but ineffective for *C. albicans*. Mono- or polymicrobial biofilms in formation or 24h pre-formed were treated with several concentrations of CPG, AMB, or VCM in RPMI 1640 medium buffered with 0.15 M MOPS for 24h at 35°C to determine the minimum concentration that inhibited 50% of the biofilm ($BMIC_{50}$) by violet crystal staining. CPG and AMB inhibited the polymicrobial biofilms formation at 16-32 μg/mL. The pre-formed polymicrobial biofilms treated with AMB were inhibited at 32-128μg/mL and those treated with CPG at 64-256μg/mL; in contrast, no inhibition was observed for VCM. All *S. aureus* biofilms in formation were inhibited by AMB (0,125μg/mL), CPG (0,125-16μg/mL) and VCM (0,125-0,25μg/mL); but pre-formed biofilms were 16-256 times more tolerant that those in formation. *C. albicans* biofilm formation was inhibited at 0.25 μg/mL for AMB and <0.125μg/mL for CPG; and on pre-formed biofilm the inhibitory effect was observed at 4μg/mL for AMB and 2 μg/mL for CPG. In *in vivo* assay, *Galleria mellonella* larvae were infected by *C. albicans* SC5314 ($5x10^7$ CFU/mL) and *S. aureus* (10^9 CFU/mL) and treated with 20 or 50mg/kg of CPG resulting in a significant ($p<0.0001$) increase in the survival rate of larvae co-infected with *C. albicans* and *S. aureus* ATCC 6538 or ATCC 33591. In contrast, CPG was unable to control mixed infection by *C. albicans* SC5314 and *S. aureus* ATCC29213. As control, CPG at both doses was able to increase the larvae survival to 95% in the monomicrobial infection with *C. albicans*. Our preliminary results indicate that caspofungin may be an alternative in the treatment of mixed infections of *C. albicans* and *S. aureus* related to biofilms.

Key words: Caspofungin; polymicrobial biofilm; *Candida albicans*; *Staphylococcus aureus*.

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