Anti-biofilm Activity of the synthetic peptide hLF 1-11 against different *Candida* species in Lumen Catheters

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Introduction

Candida species are human opportunistic fungal pathogen responsible for superficial as well as systemic infections, mainly in immunocompromised individuals. The ability to grow as a biofilm embedded community resistant to most antifungals is a common trait shared by several clinically relevant species. Among these, *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*, represent common causative agent of nosocomial infections associated with medical prosthetic devices. The high mortality rate associated to catheter related infections points to the pressing need for new antifungal drugs, which are able to eradicate biofilm-associated mycoses. The present study aimed at analyzing the inhibitory activity of the synthetic N-terminal lactoferrin-derived peptide (hLF 1-11) against biofilms produced by clinical isolates of different *Candida* species characterized for their biofilm forming ability and different fluconazole susceptibility in an *in vitro* model of catheter-associated biofilm production.

Antibiofilm activity in a catheter models

First, Catheters and *Candida* strains were incubated in four-fold diluted RPMI or in a 10% glucose solution, in absence or presence of two or three different concentrations of hLF 1-11, for 24 h at 37°C. After incubation cells were removed by vortex and sonication, diluted in PBS and plated on SDA. Activity of hLF 1-11 on mature biofilm (24 hours) produced by CP7 on the catheter lumen following incubation at 37° for 24 h. Data are expressed as means of three independent experiments $\pm$ SEM. A = inhibition of biofilm formation; B = activity on mature biofilm

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

- A more pronounced anti-biofilm effect (up to 3.5-log reduction) was observed in 10% glucose solution, mimicking the environment associated to parenteral nutrition, on both early and preformed *C. parapsilosis* and *C. albicans* biofilms.
- hLF 1-11 showed a less pronounced inhibitory activity on *C. tropicalis* and *C. glabrata* biofilms, while no activity could be detected on mature biofilm formed by these two species..

Imaging of *in Vitro* Formed Biofilms by CLSM

Confocal laser scanning microscopy (CLSM) was used to evaluate the presence of biofilm formed in a catheter model of *C. parapsilosis* colonization, following hLF 1-11 treatment for 24 h at 37°C. Untreated biofilm grown in the catheter lumen for 24 h at 37°C served as positive control. CLSM imaging confirmed the peptide activity on biofilm formation depicting low number of viable fungal cells on the peptide-treated catheter lumen compared to untreated control.

Conclusions

The overall findings candidate hLF 1-11 as a promising agent to prevent biofilm formation by all the *Candida* species tested and to treat *Candida albicans* and *Candida parapsilosis* mature biofilms grown on PVC catheters.

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