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Antibiofilm and antifungal effects of a nitro-chalcone against mixed and monospecies biofilms of *Candida albicans* and *Streptococcus mutans*, and its toxicity in *Galleria mellonella*

Gabriela F.Bombarda*; Pedro L.Rosalen; Irlan A. Freires; Eder R. Paganini; Fernanda A.S.Emeri; Luis O.Regasini; Janaina C.O.Sardi

Abstract

Biofilms are organized microbial communities formed from an ecological succession. Biofilm formation functions as a mechanism of virulence and favors the development of diseases, including oral diseases such as dental caries and periodontal disease, in which the microorganisms *Streptococcus mutans* and *Candida albicans* are closely related. Previous studies have shown that interactions between *S. mutans* and *C. albicans* are associated with the pathogenesis of early childhood caries (ECC). Therefore, there is a great interest in finding new prototypes for antimicrobial drugs, mainly for the development of structural analogues of chalcones, which constitute one of the largest classes of natural products belonging to the flavonoid family (Tristão et al, 2012) and are considered strategic molecules for this purpose.

Key words:

Biofilms, toxicity, nitro-chalcone

Introduction

Biofilms are associated with many infectious diseases in humans, including those occurring in the mouth. *S. mutans* can efficiently cope with environmental stresses, which contributes to its ability to establish biofilms, to persist in the host, and to compete with other oral bacteria, particularly when conditions are conducive to initiation and progression of dental caries. Interestingly, results from several clinical studies reveal that *C. albicans* is often detected in high numbers with *S. mutans* in biofilms from children with early childhood caries. In biofilms formed *in vitro*, the presence of *C. albicans* dramatically modifies the physical environment by increasing biomass and EPS production, thereby enhancing biofilm accumulation and stability. Biofilms mixed are considered resistant to conventional therapies and this fact has stimulated the search for alternative antimicrobial agents that can prevent biofilms formation and the onset of associated diseases. Therefore, the objective of this project was to evaluate the effect of the nitro-chalcone E08 on monospecies and mixed, mature and forming, biofilms of *C. albicans* and *S. mutans*, through total quantification of biomass, cell viability (XTT and plating), Microscopy Scanning Electron (SEM), and finally determine its *in vivo* toxicity using the *Galleria mellonella* invertebrate model.

Results and Discussion

Cell viability results of biofilms treated with NC-E08, in addition to studies of their *in vivo* toxicity using the *Galleria mellonella* invertebrate model, demonstrated that NC-E08 was able to inhibit planktonic cell growth of *S. mutans* and *C. albicans* with MIC values between 15.75 and 0.97 µg/mL, respectively. In forming biofilms, concentrations of 1xMIC and 10x MIC of NC-E08 reduced by 23% and totally inhibited *C. albicans*, respectively. *S. mutans* biofilms were reduced by 13% when treated with 1x MIC and 42.5% when using 10x MIC. In mature mixed biofilms, there was a reduction in the amount of both *C. albicans* (21% at 1x MIC and total inhibition at 10xMIC), and of *S. mutans* (18% at 1x MIC

and 30.5% at 10xMIC) biofilms. In mature biofilms, concentrations of 1x MIC and 10x MIC of NC-E08 reduced *C. albicans* by 20% and 60%, respectively. *S. mutans* was reduced by 29% when treated with 1x MIC and 38% when treated at 10xMIC. In relation to the mixed mature biofilm of *C. albicans* and *S. mutans*, there was a reduction in both *C. albicans* (23% at 1xMIC and 41% at 10x MIC) and *S. mutans* (23% at 1xMIC and 46% at 10xMIC). In order to verify acute toxicity *in vivo*, tests were performed in *G. mellonella*. The results demonstrated that at its effective antibiofilm concentration (10Xmic) NC-E08 presented low toxicity in the systemic model (viability of about 80% of the larvae), as it can be seen in figure 1.

Figure 1- *In vivo* systemic toxicity of the compound NC-E8 in *G. mellonella* larvae. The compound did not show acute toxic effects at antibiofilm concentrations (corresponding to the 696.2mM concentration). $p = 0.27$ (Log-Rank Test).

Conclusions

Nitro-Chalcone (NC-E08) demonstrated excellent antifungal, antibacterial and antibiofilm activity on *Candida albicans* and *Streptococcus mutans*, as well as low toxicity *in vivo*.

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¹ Tristão TC, Campos-Buzzi F, Corrêa R, Cruz RC, Cechinel Filho V, Bella Cruz A. Antimicrobial and cytotoxicity potential of acetamido, amino and nitrochalcones. *Arzneimittelforschung*. DOI: 10.19146/pibic-2017-78037