The role of antifungals agents on Candida glabrata biofilms matrix composition

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Candida glabrata was considered, for years, a relatively non-pathogenic saprophyte of the normal flora of healthy individuals and as no causative agent of serious infection in humans. However, its high mortality rate and its quick spread confirm the opposite. In fact, due to the widespread and increased use of immunosuppressive therapy together with broad-spectrum antifungal treatments, the frequency of mucosal and systemic infections caused by C. glabrata has increased significantly. Furthermore, biofilms are described as surface associated communities of microorganisms within an extracellular matrix, generally composed of carbohydrate and proteins. Biofilm formation is an important virulence factor for a number of Candida species, as it confers significant resistance to antifungal therapy by limiting the penetration of substances through the matrix and protecting cells from host immune responses. Moreover, little is known about the role of antifungals on C. glabrata biofilms. Thus, the aim of this work was to study the role of fluconazole, itraconazole and amphotericin B on 24 h pre-formed C. glabrata biofilms and specially on their matrix composition.

A total of 3 C. glabrata strains isolated from oral, urinary and vaginal tract were used, as well as a reference strain from ATCC (C. glabrata 2001). Biofilms were formed on 12-well plates on RPMI 1640, during 24h at 37ºC and 120 rpm. Then, the antifungal agents (fluconazole, amphotericin B and itraconazole) were added to the previously formed biofilms. After 48 h of action of each antifungal agent, the biofilms were evaluated in terms of total biomass by crystal violet staining and number of viable cells by colony forming units (CFUs). The role of itraconazole on biofilms of the clinical vaginal isolate (C. glabrata 534784) was also examined in terms of matrix composition. For this, biofilms were formed in 24-well plates during 24h and, after 48h of exposure to itraconazole, were scraped from the wells and the extracellular matrix was extracted by sonication. Biofilm matrix contents in proteins and carbohydrates were determined using the BCA kit and the Dubois method, respectively.

The results showed that, amphotericin B and fluconazole were able to cause a significant decreased on total biomass and CFUs of C. glabrata. However, itraconazole was not able to affect biofilms, except for the clinical vaginal isolate (C. glabrata 534784) at 256 µg/mL point concentration, which presented an increase in total biofilm biomass. Candida glabrata 534784 biofilms matrix exposed to itraconazole (256 µg/mL) presented an increase in proteins content but not in carbohydrate comparatively to the control. In summary, fluconazole and amphotericin B were able to significantly decrease the pre-formed biofilms of C. glabrata strains. Furthermore, the highest amount of total biofilm biomass of the vaginal isolate seems to be due to the increased protein content in its matrix.

Key words: Candida glabrata, Biofilms, antifungals agents; resistance

Urolithins, Metabolites Produced by Human Colonic Microflora, Act as Quorum Sensing Inhibitors of Yersinia enterocolitica Affecting its Gene Expression

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Urolithins are metabolites produced after the consumption of ellagitannins by the human colonic microflora. These hydroxydibenzopyran-6-one derivatives have beneficial effects as, cardioprotective, antioxidant and anti-inflammatory. Yersinia enterocolitica is a mammalian enteropathogen which may cause gastrointestinal syndromes after the consumption of contaminated food or through direct inoculation following a blood transfusion in humans. The three human pathogenic Yersinia spp. produce N-acyl homoserine lactone (AHL), Quorum Sensing (QS) signal molecules which are involved in the cell population density dependent regulation of virulence, secondary metabolic production, and biofilm maturation. Recent studies have associated the pathogenic activity of Y. enterocolitica to specific QS systems. Our objective was to evaluate the effect of the main microbiota-derived metabolites, urolithin-A and urolithin-B, as QS inhibitors in Y. enterocolitica and to determine whether or not these metabolites affect the expression of specific genes involved in virulence processes.

It was found that urolithin-A and urolithin-B were effective inhibiting QS of Y. enterocolitica when applied at 25 and 50 µg/mL, respectively. The quantification of AHL by LC-MS/MS showed that urolithin-A and urolithin-B (25µg/mL and 50 µg/mL) inhibited the production of N-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL) and N-hexanoyl-L-homoserine lactone (C6-HSL) in a dose-dependent manner. Urolithin A (80% reduction) showed a higher inhibition than Urolithin B (70% reduction). It was also observed that the tested urolithins affected the gene expression of Y. enterocolitica. The data obtained by Real-time PCR showed that urolithins decreased the transcriptional activity of QS regulated genes when low concentrations (25 µg/mL) were used. These findings suggest that very low concentrations (25 µg/mL) of both, urolithin-A and urolithin-B, have an antipathogenic effect against Y. enterocolitica and can be used as QS inhibitors reducing the expression of virulence related genes.

Key words: Cell-cell communication; Acyl homoserine lactones; gene expression; virulence