ANTIFUNGAL ACTIVITY OF PYRIDINIUM COMPOUNDS IN COMBINATION WITH FLUCONAZOLE AGAINST *CANDIDA ALBICANS* PLANKTONIC CELLS AND BIOFILMS

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Candida albicans (*C. albicans*) is a dimorphic pathogenic fungus which causes life-threatening candidemia and disseminated candidiasis [1]. Pathogenic fungi can form biofilms on medical catheters, implanted devices, heart valves, dentures and others, hereby protecting themselves with self-produced extracellular matrix and biofilm cells become resistant to antifungals [2]. *C. albicans* formed biofilms can develop highly resistance to a first-choice drug fluconazole, which is used to treat *C. albicans* infections [3]. Therefore, combined antifungal treatment is considered as a relevant tool.

Aim. To evaluate the synergistic antifungal effects of pyridinium compounds (IB-254, IB-358 and IB-385) in combination with fluconazole against *C. albicans* biofilms and planktonic cells.

Material and methods. The susceptibility of *C. albicans* standard strain ATCC® 10231™ and clinical strain 11017, isolated from patients' ascitic fluid was tested against combinations of fluconazole and pyridinium compounds. For planktonic cells studies the overnight culture was transferred to 96-well polystyrene plates with modified RPMI-1640 medium, supplemented with 2% glucose and L-glutamine, without sodium bicarbonate, and buffered with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) to pH 7.0 in the presence of different concentrations of fluconazole (0,002 – 1 µg/ml) and constant concentration of pyridinium compounds (4 µg/ml of IB-254 and IB-358, 2 µg/ml of IB-385). The cells were incubated for 24 h without shaking at 37 ˚C. To assess the growth, ODs of planktonic yeast suspensions were measured spectrophotometrically at 612 nm using the TECAN GENios Pro™ plate reader.

For better adherence biofilms of *C. albicans* were developed in the medium without glucose in 96-well flat bottom polystyrene plates for 90 min at 37 ˚C, washed twice with PBS. Biofilms were grown for 24 h in the presence of tested compounds and their combinations. Metabolic activity of biofilm cells was determined using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay.

Results. In case of fluconazole, 1 µg/ml efficiently inhibited the growth of planktonic cells of both *C. albicans* strains, while biofilms were more resistant. Fluconazole at concentration of 2048 µg/ml was not able to completely inhibit biofilm formation. Pyridinium compounds, at concentrations of 4 µg/ml for IB-254 and IB-358, 2 µg/ml for IB-385, had no significant fungicidal effect on the tested *C. albicans* cells. Whereas, in combination with a low concentration of fluconazole (4 µg/ml) pyridinium compounds demonstrated significant synergism against *C. albicans* biofilms and planktonic cells of standard and clinical strains.

Conclusions. Our study demonstrated that combination of pyridinium compounds with fluconazole is effective against *C. albicans*. Pyridinium compounds could be promising candidates in the development of new antifungal drugs.

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