INFLUENCE OF ASSOCIATING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS WITH ANTIFUNGAL COMPOUNDS ON VIABILITY OF SOME CANDIDA STRAINS

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ABSTRACT

Objective. The main goal of our study was to determine the effect that association of nonsteroidal anti-inflammatory drugs (NSAIDs) with antifungal drugs on viability of Candida (C.) albicans and C. krusei species.
Materials and methods. Six yeast strains were isolated from pharyngeal and vaginal secretions (C. albicans and C. krusei). Tests (diffusion and serial microdilutions methods) were carried out in the presence of some NSAIDs by using different concentrations (0.25mg/ml and 1mg/ml). Antifungal drugs were used in lower concentrations than the MIC (1µg/ml).
Results. Cell viability of C. krusei strain was of 82% for NSAIDs used and association of these with ketoconazole led to decrease of cell viability of C. krusei strain to 60% as compared to the cells treated with antifungal drugs. In the presence of NSAIDs, cell viability of C. albicans strains, decreased between 6 to 18% for diclofenac and ibuprofen. In the case of simultaneous use of the two classes of drugs, there was observed an increase of antimicrobial activity, especially for diclofenac association, cell viability was reduced up to 60% and respectively 70% as compared to the cells treated only with ketoconazole and fluconazole.
Conclusion. We observed a synergistic action of certain NSAIDs with two antifungal drugs used on some Candida species.

Keywords: Candida, Cell viability, NSAID

INTRODUCTION

The most common systemic fungal infection is candidiasis, which accounts for well over half of these invasive mycoses. A single species, *Candida albicans* (*C. albicans*) causes the majority of these infections. *Candida albicans,* which also causes oropharyngeal thrush and vaginitis, is a normally a commensal of the human gastrointestinal tract, in which it lives without adverse effects on the host. Candidemia and invasive candidiasis are frequently associated with high morbidity and high mortality rates. Several antifungal drugs, such as fluconazole, ketoconazole, nystatin, amphotericin B and 5-fluorocytosine can interfere with virulence factors. The prophylactic and curative treatments with antifungal drugs can cause the appearance of *Candida* resistant-strains. Nonsteroidal anti-inflammatory drugs (sodium diclofenac, aspirin, ibuprofen) are inhibitors of the cyclooxygenase isoenzymes (COX-1 and COX-2). These drugs specifically block the biosynthesis of mammalian prostaglandins by inhibiting one or both of COX isoenzymes. Diclofenac has recently been discovered to inhibit microbial biofilms. A biofilm is a population of cells growing on a surface and enclosed in an exo polysaccharides matrix. Biofilms confer resistance on micro-organisms to antibiotic treatment. The development of resistance by microorganisms to antimicrobial drugs has been one of the greatest prob-

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lems hampering antimicrobial therapy. Bacterial biofilms show enormous levels of antibiotic resistance, which is a general feature of all biofilms.

The main goal of our study was to determine the effect that association of nonsteroidal anti-inflammatory drugs (NSAIDs) with antifungal drugs on viability of *Candida (C.) albicans* and *C. krusei* species.

MATERIALS AND METHODS

Yeasts isolates

Six strains of *Candida* species (C1, C2, C3, C4, C6, C8) isolated from the Pantelimon Hospital patients with pharyngeal and vaginal candidiasis were used (*C. albicans* and *C. krusei*). Clinical isolates have been identified by the urease and yeast API 20 C AUX tests (bioMerieux, France). Yeasts were stored at -70°C on peptone glucose (YPG) medium (yeast extract 5 g/l, peptone 10 g/l, glucose 20 g/l) supplemented with 20% glycerol.

Antifungal susceptibility test was performed using a fungitest kit, according to manufacturer's recommendation (Bio-Rad's KIT, USA).

The antimicrobial activity of some anti-inflammatory drugs (diclofenac, ibuprofen) was determined using the diffusion and serial microdilutions methods (CLSI) (1,2). The disks with known concentrations of anti-inflammatory drugs were used to evaluate the antifungal activity of NSAID on the specified strains. Disks of 6 mm diameter sterile paper were impregnated with 5 µl of anti-inflammatory drugs (1).

Disk diffusion test

The antimicrobial effects of the NSAID were investigated by the disk diffusion method (1,2). The yeasts strains were cultured overnight at 37°C in grown in *Sabouraud Dextrose Agar* **(**SDA). The cultures were centrifuged and the pellet was resuspended in phosphate buffer saline (PBS; pH 7.2). The suspension obtained had a value of 0.5 McFarland. The yeast suspension was spread on the surface of the plates containing SDA agar broth. The antifungal disk and disk impregnated with aspirin, sodium diclofenac, ibuprofen (5µl) of 10mg/ml stock solution were placed on the plates. After 24h incubation of the plates at 37°C, the diameters of inhibition area were measured in mm for the tested yeast strains compared to the antifungal control disk (2).

Microdilution method was performed in 96 wells polystyrene plates. A volume of cell suspension (10^3 cells/ml) in RPMI 1640 supplied with 0.165M morpholino propanesulfonic acid (MOPS) and 0.3 g/l L-glutamine (200 µl) were incubated for 48h at 37^0C in presence of 1µg/ml antifungal drugs and different concentration of NSAIDs (12.5-50 µg/ml) without shaking. Cell growth was estimated by determining the absorbance to 660 nm using microtiter plate reader.

RESULTS

Most of the *Candida albicans* strains were susceptible to antifungal drugs which were in used (fluconazole and ketoconazole). The *C. krusei* strain was resistant to the azole compounds.

The antimicrobial activity of some anti-inflammatory drugs (diclofenac, ibuprofen) was determined using the diffusion and serial microdilutions methods (CLSI). Results obtained using disk diffusion method shown that sodium diclofenac and ibuprofen in association with fluconazole and ketoconazole induced growth inhibition of *C. albicans* strains (Figure 1 and 2). This combination of nonsteroidal antiinflamatory drugs and azoles had no effect on *C. krusei* strain. Aspirin has no effect in combination with azole compounds.

Combination of non-steroidal antiinflamatory drugs (diclofenac and ibuprofen) with fluconazole or ketoconazole presented a synergic effect against *C. albicans* strains cell viability, inducing a decrease of cellular viability with 20-30% (Figure 3 and Figure 4). Cell viability of *C. krusei* strain was of 82% for NSAIDs used and association of these with ketoconazole led to decrease of cell viability of *C. krusei* strain to 60% as compared to the cells treated with antifungal drugs. In the presence of NSAIDs, cell viability of *C. albicans* strains, decreased between 6 to 18% for diclofenac and ibuprofen. In the case of simultaneous use of the two classes of drugs, there was observed an increase of antimicrobial activity, especially for diclofenac association, cell viability was reduced up to 60% and respectively 70% as compared to the cells treated only with ketoconazole and fluconazole.

DISCUSSIONS

The physiological and immune condition of the host and the yeasts adaptation in surviving in many anatomical sites are important factors in the transition from commensally to disease-causing yeasts. Many putative virulence factors can contribute to the yeasts invasiveness and pathogenicity, such as their ability, conversion of unicellular yeasts into filamentous forms and expression of extracellular enzymes. Several antifungal drugs, such as flucon-

FIGURE 1. *Influence of diclofenac, aspirin and ibuprofen on C. albicans C4 strain azole susceptibility by disk diffusion after 48h*

FIGURE 2. *Influence of diclofenac, aspirin and ibuprofen on C. albicans C2 strain azole susceptibility by disk diffusion after 48h*

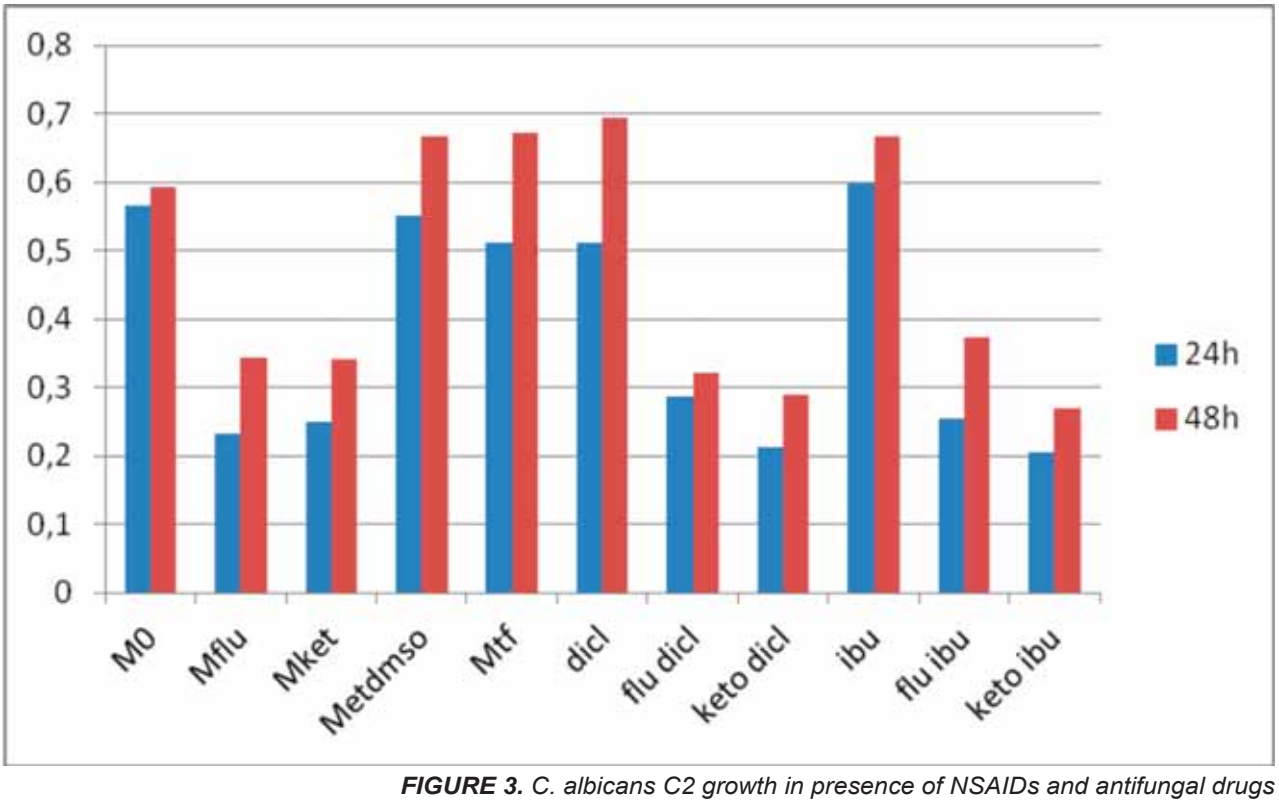

FIGURE 3. *C. albicans C2 growth in presence of NSAIDs and antifungal drugs*

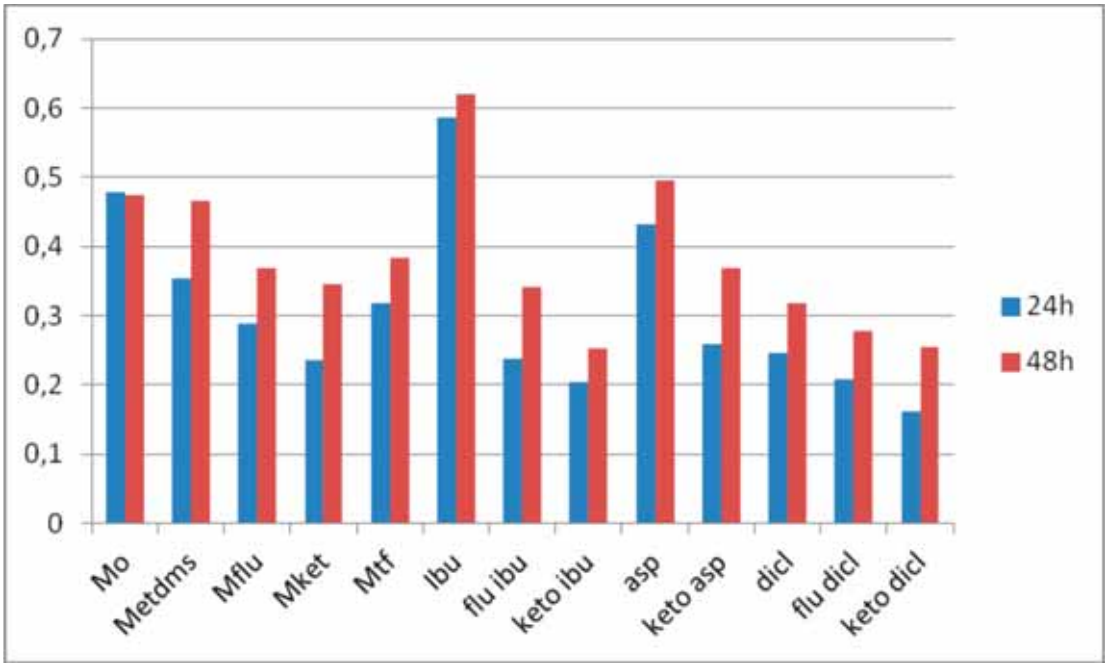

FIGURE 4. *C. albicans C4 growth in presence of NSAIDs and antifungal drugs*

azole, ketoconazole, nystatin, amphotericin B and 5-fluorocytosine can interfere with virulence factors. The prophylactic and curative treatments with antifungal drugs can cause the appearance of *Candida* resistant-strains. Antifungal drugs are used increasingly both as prophylactic and curative agents which have led to the widespread of resistant strains. This situation has prompted the search for alternative anti-Candida therapeutic agents.

Nonsteroidal anti-inflammatory drugs (sodium diclofenac, aspirin) are inhibitors of the cyclooxygenase isoenzymes (COX-1 and COX-2). These drugs specifically block the biosynthesis of mammalian prostaglandins by inhibiting one or both of COX isoenzymes. Previous authors studied the role of diclofenac in the dimorphic transition in *C. albicans*. Their results indicated the effect of diclofenac was dependent on the concentration of this compound. (3,4). Another non-steroidal anti-inflammatory drug, ibuprofen, was described as being able to reverts resistance related to efflux activity in *C. albicans* (5). Although few studies found that ibuprofen and acetaminophen has significant effects to reduce some of body disorders after bacterial infection, antibacterial action of these agents are not clear for many species of pathogenic bacteria. Ibuprofen and acetaminophen were tested for antibacterial activity against seven isolates of bacteria including gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and gram negative bacteria (*E. coli*, *Enterobacter aerogene*, *E. cloacae, Salmonella typhi* and Paracoccus yeei) (6).

Fluconazole resistant isolates revering to susceptibility after incubation with ibuprofen showed *CDR1* and *CDR2* genes overexpresion especially of the latter (7). Effect of aspirin and piroxicam of cell viability on *Candida* species was also studied (8).

CONCLUSIONS

Combination of non-steroidal antiinflamatory drugs (diclofenac and ibuprofen) with fluconazole or ketoconazole presented a synergic effect against *C. albicans* strains cell viability, inducing a decrease of cellular viability with 20-30%.

ACKNOWLEDGEMENTS

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