

P0022

Poster Session I

News from the fungal frontier

17-BETA-ESTRADIOL INCREASES RHODAMINE 6 G EFFLUX IN CANDIDA ALBICANS DETECTED BY FLOW CYTOMETRIC ANALYSIS

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Objectives: Azole efflux has been confirmed a main mechanism of azole resistance in *Candida albicans*. The function of *Candida* drug resistance protein 1 (Cdr1p) can be measured by flow cytometry using fluorescent dye Rhodamine 6G(R6G), a substrate of drug efflux transporter. Although it has been reported that 17-beta-estradiol can upregulate CDR1 expression, the influence of 17-beta-estradiol on drug efflux at the cellular level has not yet been confirmed as 17-beta-estradiol is also transported by Cdr1p. The aim of this study was to evaluate the effects of 17-beta-estradiol on the azole efflux in *Candida albicans*.

Methods: The efflux of R6G and minimal inhibitory concentrations (MICs) of two triazole agents were investigated in 2 standard strains (SC5314 and ATCC90028) and 32 clinical strains of *Candida albicans*. In all experiments, 17-beta-estradiol were added to the incubation medium to 10⁻⁵mol/l and 10⁻¹⁰mol/l . Uptake of fluorochrome was quantified after the first incubation of cells in glucose-free PBS for 60min and the residual fluorescence was quantified after the second incubation in containing 5%glucose PBS for 30 min at 525 nm by flow cytometer. Efflux was calculated as the difference of the geometric mean of the fluorescence intensity (GMF) measured after the first and second incubations. MICs of fluconazole and terconazole were measured in the absence and presence of different concentrations of 17-beta-estradiol according to the NCCLS-approved standard M27-A3.

Results: A significant difference was observed in R6G efflux between 10⁻⁵mol/l 17-beta-estradiol treatment and negative control in *Candida albicans* standard strains SC5314 (27.8±4.6 VS 23.4±4.4, n=10) and ATCC90028(35.0±5.0 VS 27.1±4.3, n=10). Moreover, 10⁻⁵mol/l 17-beta-estradiol had a greater enhancement on the R6G efflux than 10⁻¹⁰mol/l 17-beta-estradiol in SC5314 (27.8±4.6 VS 25.3±3.9, n=10) and ATCC90028(35.0±5.0 VS 29.8±4.5,n=10). However, two standard strains exposure to 10⁻¹⁰mol/l 17-beta-estradiol had no significant difference with negative control. Increased efflux had been verified in 32 clinical strains of *Candida albicans* treated with 10⁻⁵mol/l 17-beta-estradiol compared to negative control (22.2±1.5 VS 16.8±1.6). Consistent with increased efflux of R6G, the strains exposure to 10⁻⁵mol/l 17-beta-estradiol showed greater resistance to fluconazole compared to negative control in standard strains SC5314 (MIC₈₀=0.5 µg/ml VS 0.25µg/ml, n=3)and ATCC90028 (MIC₈₀=0.5 µg/ml VS 0.25µg/ml, n=3). The susceptibilities of the standard strains to the terconazole were not affected.

Conclusions:This study found 17-beta-estradiol could increase R6G efflux and promote azole resistance in *Candida albicans*. The fluctuation of 17-beta-estradiol in women's menstrual cycle should be considered as an important impact factor for antifungal therapy.