

P0008

Poster Session I

News from the fungal frontier

DIRECT EFFECTS OF TACROLIMUS AND CYCLOSPORINE ON CANDIDA ALBICANS

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Objectives: Tacrolimus (FK506) and cyclosporine (CY) are calcineurin inhibitors that are widely used in transplantation setting where the risk and mortality of invasive fungal infection (IFI) is the highest. This study investigated the morphological effects of tacrolimus and CY in *Candida albicans* exposed to different concentrations of tacrolimus and CY at specific time points by using transmission electron microscopy (TEM) and atomic force microscope (AFM). The minimal inhibitory concentrations (MIC) of tacrolimus and cyclosporine were also assessed by broth microdilution method.

Methods: *C. albicans* ATCC 10231 strain maintained in Sabouraud broth, and incubated at 37°C for 24 hours in a incubator. *C. albicans* in cell suspension were treated with 7.8 mg / 100 µL cyclosporin and 0.8 mg / 100 µL tacrolimus and incubated 37°C for 24 hours again. 100 µL of concentrated cell suspensions were dropped on the glass surface and dried. Samples were stained with methylene blue and washed. After drying completely, samples were visualized by AFM analysis. AFM images were obtained, using a Nanoscope Veeco AFM. Samples were prepared in petri dishes including SDA agar supplemented with extra 6 gr agar. Drug-treated suspensions dropped in petri dishes and incubated 37°C for 24 hours. TEM analyses were performed on samples by fixed in 3 % glutaraldehyde, rinsed in buffer, post fixed in 1% osmium tetroxide in 0.1 M potassium phosphate for 2 h at 48°C, dehydrated in ethanol and embedded in Araldite. Ultra thin sections were observed under a TEM. The minimal inhibitory concentrations (MIC) of tacrolimus and CY were also assessed by broth microdilution method.

Results: The AFM examination of *C. albicans* ATCC 10231 strain exposed to tacrolimus has shown significant changes in fungal cell morphology as the reduction in fungal cell thickness to 1 µm, reduction in fungal cells to 3-5 µm compared to pre-exposure fungal cells and wrinkled morphology in the surfaces of the fungal cell. The TEM examination revealed massive cellular deformation with the exposure of tacrolimus and CY. The direct inhibitory effect of tacrolimus was demonstrated by microdilution method and the MIC at which growth of *C. albicans* was completely inhibited was found to be 1.25 µg/ml while it was 25 µg/ml for CY. Minimal fungicidal concentration (MFC) were also calculated in SDA plates after 24 h incubation. MFC of tacrolimus was 2.5 µg/ml while it was 50 µg/ml for CY

Conclusions: To our knowledge this is the first study showing the direct antifungal effects of tacrolimus and CY on *C. albicans* by demonstrating the morphological modifications and direct inhibition at low MIC values. The findings of this study might have important implications in clinical practice where *C. albicans* is prevalent and tacrolimus and CY are widely used.