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Assessment of the in vitro anti-biofilm effect of micafungin in candidaemic patients: which concentration of micafungin is necessary to eradicate *Candida* biofilms?

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Objectives. Our study aims were to assess the in vitro effect of micafungin against formed *Candida* biofilms by measuring the MIC's and sessile MIC's (SMIC) of micafugin achieving the highest fungal damage. We also assessed the necessary concentration of micafungin to inhibit sessile cells' re-growth after the experiment.

Methods. For the sake of the study, we collected 37 *Candida* strains (*C. albicans*, 29; *C. parapsilosis*, 8) representing 37 episodes of candidemia which demonstrated to be biofilm-producers by the crystal violet binding assay (OD≥0.5 was used to define biofilm production). We evaluated the activity of micafungin against planktonic and sessile forms of the 37 clinical isolates. Planktonic MICs were determined using broth microdilution method (EUCAST). Sessile MICs (SMICs) were determined with biofilms formed in 96-well, flat-bottomed microtiter plates incubated 24h at 37ºC. Then, serial twofold of micafungin dilutions ranging from 16 to 0.015 µg/mL were studied (24h incubation at 37ºC). To determine which concentration of micafungin was necessary to inhibit sessile cells' re-growth, we performed the same experiment followed a by a 24h incubation (37ºC) of the wells with 100 µL of YPD.

To assess the SMIC, we measured each well's absorbance at 492 nm using XTT to determine the lowest concentration associated with a 50% reduction in absorbance compared with the level for the control well (percent fungal damage = [1-X/C] x 100, where X is the absorbance of experimental wells and C is the absorbance of control wells).

To assess the necessary concentration of micafungin to inhibit sessile cells' re-growth, we measured each well's absorbance at 492 nm to calculate the percent growth inhibition ([1-X/C] x 100, where X is the absorbance of experimental wells and C is the absorbance of control wells). A calibration curve was performed to establish a correlation between the absorbance and the number of cells.

Results. All micafungin MICs for planktonic *C. albicans* isolates were <=0.015 µg/mL whereas the MICs for *C. parapsilosis* ranged from 0.015 µg/mL to 2 µg/mL. Overall, median SMIC of micafungin was 4 times greater than the median MIC against planktonic cells (0.015 µg/mL vs. 0.25 µg/mL). All strains showed >80% of growth inhibition after 24h of incubation with micafungin at concentrations >= 2 µg/mL. The percentage of re-growth inhibition was >50% for *C. albicans* and *C. parapsilosis*, at the following micafungin concentrations, respectively: 0.25 µg/mL, and 1 µg/mL (Figure).

Conclusion. Our study shows that micafungin was active against *C. albicans* strains both in planktonic and sessile forms and moderately active against *C. parapsilosis* sessile cells. Concentrations of micafungin above 2 µg/mL are enough to inactivate the *Candida* sessile cells' re-growth within the biofilm.

Figure