The effect of human serum on the efficacy of traditional antifungals against *Candida albicans* and *Candida parapsilosis* biofilms

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Objectives: To extend our knowledge about the *in vitro* anti-biofilm activity of the most frequently used traditional systemic antifungals (fluconazole, amphotericin B, caspofungin, micafungin), their activity was compared in RPMI-1640 and RPMI-1640 plus 50% human serum against *Candida albicans* and *C. parapsilosis* biofilms to mimic better the *in vivo* conditions.

Material and methods: *C. albicans* or *C. parapsilosis* cell suspension of 1x10⁶ cells/mL was prepared in RPMI-1640 to biofilm formation. From prepared standardized suspensions, aliquot of 200 µl were added into wells of pre-sterilized, polystyrene, flat-bottom 96-well microtitre plates and incubated statically at 37° C for 24 hours.

The preformed biofilms were washed three times with sterile physiological saline in order to remove non-adherent fungal cells. Afterwards, MIC determination was performed in RPMI-1640 with and without 50% human serum for fluconazole, amphotericin B, caspofungin and micafungin using XTT-assay. In case of *C. albicans*, the examined final concentrations of MIC determinations ranged from 0.015 mg/l to 32 mg/l for each tested antifungal agent. For *C. parapsilosis*, the tested concentrations ranged from 0.015 to 32 mg/l and from 0.015 to 512 mg/l for amphotericin B and for fluconazole as well as echinocandins, respectively. MICs of sessile cells was defined as the lowest drug concentration resulting at least 50% fungal damage compared to growth control cells for all antifungals. Susceptibility pattern of biofilms against antifungals was evaluated using metabolic activity based fungal damage assay. Activity was calculated using the following formula: fungal damage (%) = [1-(absorbance of experimental cells_(492 nm)/absorbance of control wells_(492 nm))] x 100. All isolates were examined in three independent experiments for each tested antifungal drug. The ratio of viable and dead cells was measured in fluorescent microscopic analysis using a LIVE/DEAD® BacLight™ viability kit.

Results

<i>Candida albicans</i> (n=10)	MICs of planktonic cells (mg/l)		MICs of planktonic cells in RPMI-1640 with 50% human serum (mg/l)		MICs of biofilms in RPMI-1640 (mg/l)		MICs of biofilms in RPMI-1640 with 50% human serum (mg/l)	
	Range	Median	Range	Median	Range	Median	Range	Median
FLU	0.06-0.5	0.25	0.25-0.5	0.5	0.25-64	32	0.015-0.25	0.03
AMB	0.5-1	1	2-4	4	0.12-1	0.25	0.25-2	0.5
CAS	0.06-0.25	0.125	0.25-0.5	0.5	0.03- >32 ¹	0.25	0.25-2	0.5
MICA	0.015-0.03	0.015	0.5-1	1	0.03- >32 ¹	0.03	0.06-32	2
<i>Candida parapsilosis</i> (n=10)								
FLU	0.125-0.25	0.188	0.5-1	1	512->512 ²	>512	0.015-0.06	0.03
AMB	0.25-0.5	0.5	1-4	2	0.5-2	1	0.015-0.06	0.015
CAS	1	1	4-8	8	128->512 ²	384	0.015-0.25	0.015
MICA	0.25-1	1	4-8	8	128->512 ²	256	0.015-0.25	0.015

Table 1. Minimum inhibitory concentrations (MICs) of fluconazole (FLU), amphotericin B (AMB), caspofungin (CAS), micafungin (MICA) against planktonic cells and biofilms formed by *Candida albicans* and *Candida parapsilosis* clinical isolates in the presence and absence of 50% human serum
¹ MIC is offscale at >32 mg/l, 64 mg/l (one dilution higher than the highest tested concentration) was used for median calculation
² MIC is offscale at >512 mg/l, 1024 mg/l (one dilution higher than the highest tested concentration) was used for median calculation

Figure 1. LIVE/DEAD fluorescence imaging of *C. albicans* (1544) (a, b) and *C. parapsilosis* (26977) (c, d) representative isolates in the presence (b, d) or absence (a, c) of 50% human serum. Live cells (green) and non-viable cells (red) were stained with Syto9 and propidium iodide, respectively. All images show typical fields of view. Scale bars represent 10 µm.

Table 3. Fungal damage of biofilms in RPMI-1640 with and without 50% human serum against *Candida albicans* (a, c, e and g) and *C. parapsilosis* (b, d, f and h) clinical isolates caused by fluconazole (a and b), amphotericin B (c and d), caspofungin (e and f) and micafungin (g and h). Each bar represents the mean of the percent fungal damage ± SEM (standard error of mean) of ten clinical isolates. Asterisks show significant differences between fungal damage observed in RPMI-1640 and in RPMI-1640 with 50% human serum (* p<0.05, ** p<0.01). The dotted lines show the border of 50% fungal damage compared to the untreated controls. Black bars and grey bars indicate for fungal damage in RPMI-1640 and in RPMI-1640 with 50% human serum, respectively.

Conclusions

- 1. In serum, the traditionally biofilm-active antifungals (echinocandins, amphotericin B) showed decreased activity against *C. albicans* biofilms due to the high rate of protein binding.
- 2. *C. parapsilosis* showed higher susceptibility against the tested antifungals due to the reduced biofilm mass in RPMI-1640 with serum and the observed fungicidal effect exerted by serum.
- 3. Fluconazole showed high antifungal activity in the presence of 50% serum both against *C. albicans* and *C. parapsilosis* biofilms.