**In vitro** efficacies of caspofungin or micafungin catheter lock solutions on *Candida albicans* biofilm growth

Estelle Cateau*, Marie-Hélène Rodier and Christine Imbert

Université de Poitiers, UMR CNRS 6008, Equipe de Microbiologie Fondamentale et Appliquée, 6 Rue de la Milétrie, BP 199, 86034 Poitiers Cedex, France

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**Objectives**: Caspofungin and micafungin belong to the echinocandins; the mechanism of action of echinocandins is based on the inhibition of (1,3)-β-D-glucan synthase. The aim of this study was to investigate *in vitro* the optimal antifungal lock treatment against a *Candida albicans* biofilm.

**Methods**: An *in vitro* model of a *C. albicans* (ATCC 3153 or ATCC 66396) biofilm associated with 100% silicone catheters was used. The effectiveness of the antifungal treatment was assayed against biofilms aged 12 h or 5 days, after exposure to caspofungin (2 mg/L) or micafungin (5 mg/L) for 12 h. The durability of the reduction in the biofilm metabolic activity was investigated (1–3 days after echinocandin treatment). The efficacy of caspofungin and micafungin was determined by evaluating a significant decrease (*P* < 0.0001) in the metabolic activity of biofilm yeasts.

**Results**: The results showed that the tested antifungal agents used as lock solution significantly (*P* < 0.0001) reduced the metabolic activity of *C. albicans*, whatever the biofilm maturation stage (12 h or 5 days old biofilms). The reduction in the metabolic activity of biofilm yeasts was maintained, even after 48 h.

**Conclusions**: These data suggest that caspofungin (2 mg/L) and micafungin (5 mg/L) could represent good candidates for the reduction or control of fungal biofilms associated with silicone medical devices, as part of an antifungal lock. They were able to induce a significant and persistent reduction in the yeast metabolic activity of intermediate and mature biofilms, 12 h and 5 days old, respectively, when used as catheter lock solutions.

**Keywords**: echinocandins, yeasts, silicone, susceptibility

**Introduction**

Candidaemia is frequently associated with intravascular catheter-related infection that causes increased morbidity, mortality and healthcare costs. These infections are associated with the formation of a biofilm consisting of sessile fungi, a hyphal layer and an extracellular matrix. The fungal biofilm alters the susceptibility to antifungal agents, and once infected, the current clinical procedure is the removal of the catheter and the administration of systemic antifungals. Conservative strategy of the catheter in candidaemia, including lock and systemic therapies, could be adopted in clinically stable patients to avoid intravascular catheter replacement. This hypothesis proceeds from investigations carried out in the case of the evaluation of lock technique for the treatment of catheter-related bacteraemia. The antibiotic-lock technique involves the static instillation of a concentrated solution of antibiotic into the catheter lumen and allowing it to remain for an extended period, usually 12 h.

Echinocandins have been recently approved for use in the treatment of candidiasis and their mechanism of action is based on the inhibition of (1,3)-β-D-glucan synthase, which is responsible for forming glucan polymers in the cell wall. The *in vitro* anti-biofilm activity of echinocandins has been previously demonstrated. However, the potential of echinocandins used as lock therapy is very poorly documented.

This study deals with the *in vitro* anti-biofilm potential of two echinocandins, caspofungin and micafungin, used as a lock solution to manage *Candida albicans* biofilms.

**Materials and methods**

In this study, biofilms of *C. albicans* strains ATCC 66396 and 3153, 12 h and 5 days old, respectively, were formed in microtitre plates, as described previously, using sections of 100% silicone catheters (A-M Systems, Carlsborg, WA, USA). The tested concentrations of...
caspofungin and micafungin were about 100 times MIC and so were compatible with a lock therapy strategy.4 Antifungal solutions were diluted in YNB-Glc (6.7 g of YNB, 5 g of Glc and 1 L of water). Aliquots (300 μL) of 2 mg/L caspofungin (Merck Research Laboratories, Rahway, NJ, USA) or 5 mg/L micafungin (Astellas Pharma Inc., Tokyo, Japan) were then added to the biofilms, and plates were incubated for 12 h at 37 °C. For controls, biofilms were incubated for 12 h with 300 μL of YNB-Glc. All the silicone pieces were then moved into new wells of the plate and incubated with YNB-Glc without antifungal, at 37 °C for 24, 48 or 72 h, to evaluate the persistence of the anti-biofilm activity of echinocandins.

Echinocandin effects were monitored by a previously described metabolic assay based on the reduction of a tetrazolium salt (XTT).6,8 Briefly, each catheter section coated with biofilm and treated or not with antifungal was incubated for 3 h at 37 °C with XTT (300 mg/L) and menadione (0.13 mM) in 150 μL of PBS. Absorbance was then measured at 492 nm (microplate reader LP400, Sanofi Diagnostics Pasteur) and correlated with the yeast metabolic activity within the biofilm. All experiments were performed twice with eight replicates. An analysis of variance and a Scheffe’s test were conducted to determine the statistical differences between groups.

**Results and discussion**

The biofilm growth inhibition observed after such a lock treatment was represented on a bar chart showing the percentage of biofilm inhibition induced by the antifungal function of the time of incubation after the end of the treatment for each type of biofilm: intermediate phase (12 h) or maturation phase (5 days).

For the strain *C. albicans* 3153, for a young biofilm (12 h), the growth inhibition induced by a 12 h lock therapy and observed after a 24 h culture without antifungal was ~91% (control: \( A_{492} = 0.54 \) and caspofungin: \( A_{492} = 0.050 \)) for 2 mg/L caspofungin and ~67% (micafungin: \( A_{492} = 0.180 \)) for 5 mg/L micafungin (Figure 1a). For strain 66396, the results observed were, respectively, ~70% (control: \( A_{492} = 0.606 \) and caspofungin: \( A_{492} = 0.182 \)) for caspofungin and ~92% (micafungin: \( A_{492} = 0.050 \)) for micafungin (Figure 1b). These results showed that caspofungin and micafungin used in our experimental conditions demonstrated an inhibitory activity of ~70% on a 12 h old biofilm development, 24 h after the lock therapy, for the two studied strains. Furthermore, this anti-biofilm efficacy was maintained after the end of the lock, suggesting a persistence of the activity. To confirm and investigate this persistence, the 12 h old biofilm (strain 3153 or 66396) growth inhibition was also evaluated 48 or 72 h after the end of each echinocandin lock.

For the 3153 strain, even 48 h after the end of the lock, caspofungin and micafungin demonstrated a significant activity on the development of a 12 h old biofilm, ranging from 54% (micafungin) to 85% (caspofungin), as shown by absorbance values: control: \( A_{492} = 0.536 \); caspofungin: \( A_{492} = 0.082 \) and micafungin: \( A_{492} = 0.245 \) (Figure 1a). Whereas 72 h after the end of the lock, the inhibition observed was ~40% with caspofungin or micafungin (control: \( A_{492} = 0.517 \); caspofungin: \( A_{492} = 0.312 \) and micafungin: \( A_{492} = 0.309 \); Figure 1a).

For the 66396 strain, the anti-biofilm activity also seemed to be maintained. Our investigations have shown that 48 h after the end of the treatment, the observed growth inhibition was ~60% with caspofungin (control: \( A_{492} = 0.735 \) and caspofungin: \( A_{492} = 0.292 \)) and 93% with micafungin (micafungin: \( A_{492} = 0.054 \)). Three days after the end of the antifungal contact, the inhibition percentages were ~57% and 52%, respectively, control: \( A_{492} = 0.670 \); caspofungin: \( A_{492} = 0.286 \) and micafungin: \( A_{492} = 0.319 \) (Figure 1b).

In conclusion, these results confirmed that the anti-biofilm activity of the two echinocandins on intermediate *C. albicans* biofilms is persistent, even if 72 h post-lock, the inhibition seemed to decrease: echinocandin locks did not manage to induce more than a reduction by half of the metabolic activity of the biofilms 72 h after the end of the antifungal contact.

In the second part of this work, we investigated the efficiency of these locks on the development of a mature *C. albicans* biofilm, i.e. 5 days old. The activity persistence was tested 24 or 48 h after the end of each lock. The persistence 72 h after the treatment was not evaluated because we hypothesized that the activity decrease observed with an intermediate biofilm would have been intensified on older biofilms.

On a 5-day-old *C. albicans* 3153 biofilm, the inhibition observed 24 h after the end of the lock was ~87% for caspofungin (control: \( A_{492} = 0.608 \) and caspofungin: \( A_{492} = 0.079 \)) and 58% for micafungin (micafungin: \( A_{492} = 0.230 \)) (Figure 1a). For the 66396 strain, in the same conditions, the inhibition was 90% for caspofungin (control: \( A_{492} = 0.667 \) and caspofungin: \( A_{492} = 0.067 \)) and 89% for micafungin (micafungin: \( A_{492} = 0.075 \)) (Figure 1b). Therefore, the anti-biofilm activity described for the intermediate biofilm treated by the studied echinocandins used as lock solutions was verified on older biofilms whatever the studied strain.

The results obtained from these mature biofilms 48 h after the end of the contact corroborated the hypothesis that the activity of echinocandins used as lock solutions was persistent. For the 3153 strain, the inhibition was 88% for caspofungin (control: \( A_{492} = 0.617 \) and caspofungin: \( A_{492} = 0.073 \)) and 54% for micafungin (micafungin: \( A_{492} = 0.283 \)) (Figure 1a). For *C. albicans* 66396, the observed inhibition was 47% for caspofungin (control: \( A_{492} = 0.693 \) and caspofungin: \( A_{492} = 0.366 \)) and 91% for micafungin (micafungin: \( A_{492} = 0.059 \)) (Figure 1b).

**Conclusions**

Our results underlined the *in vitro* ability of lock solutions to reduce the metabolic activity of a *C. albicans* biofilm, whatever the echinocandin used and whatever the biofilm maturation stage, within the limits of the experimental conditions of this study. In addition, our results showed no acquisition of resistance, suggesting that echinocandins could be of interest in the management of candidiasis associated with catheters. Our results agreed with previous investigations that had already shown that caspofungin and aminocandin, another echinocandin, were capable of anti-adherent and anti-biofilm activity.6,9 These experiments were different from the presented lock methodology because the metabolic activity of the biofilm was evaluated as soon as the antimicrobial treatment was over.

In conclusion, our results showed that *in vitro*, caspofungin and micafungin managed at least to reduce by half the metabolic activity of *C. albicans* growing as a biofilm. However, all results corroborated the hypothesis that echinocandins would have a real anti-biofilm potential and could become important factors in the lock approach. The tested concentrations corresponded to the lowest concentrations that can be used as a lock strategy, if we
consider antibiotic lock therapy data. The use of higher echinocandin concentrations could be considered, but Melo et al. have recently described an in vitro paradoxical effect using supra-MIC concentrations of caspofungin, suggesting that experimental conditions have to be monitored carefully. Our study suggests that the lock therapy strategy could be extended to fungal infections.

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Transparency declarations

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References


