Could liposomal amphotericin B (L-AMB) lock solutions be useful to inhibit Candida spp. biofilms on silicone biomaterials?

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Received 1 September 2011; returned 7 October 2011; revised 13 October 2011; accepted 16 October 2011

Objectives: Candida infections associated with catheters remain difficult to manage. Antifungal lock strategies could be a therapeutic option when the device is difficult to remove or in combination with systemic treatment to increase efficacy. This study deals with the antibiofilm potential of liposomal amphotericin B (L-AMB) used as a lock solution to inhibit Candida albicans, Candida glabrata and Candida parapsilosis biofilms in vitro.

Methods: Biofilms aged 12 h and 5 days were formed on silicone catheters. L-AMB (200 or 1000 mg/L) was added to biofilms and catheters were incubated for 4, 12 or 24 h at 37°C. L-AMB was then removed by washing. The metabolic activity of yeasts was assessed by the XTT method up to 48 h after the end of the locks to evaluate the persistence of the antibiofilm activity. Controls without antifungal were used as references to calculate the inhibition percentages induced by L-AMB lock solutions.

Results: L-AMB (200 and 1000 mg/L) inhibited, for up to 48 h, C. albicans and C. glabrata biofilms by >70%, regardless of the lock duration. The activity of L-AMB (200 mg/L) against C. parapsilosis mature biofilms was lower and less sustained, especially for 4 h locks.

Conclusions: L-AMB (1000 mg/L) lock solutions strongly inhibited Candida spp. in young and mature biofilms for up to 48 h after the end of the lock. However, overall eradication of the biofilm was not obtained using 1000 mg/L L-AMB as a single lock. These results suggest the usefulness of systemic treatment combined with an L-AMB lock to control Candida spp. biofilms associated with catheters.

Keywords: catheter, Candida albicans, Candida glabrata, Candida parapsilosis

Introduction

The lock technique can enable the maintenance of catheters in patients; however, the usefulness of antifungal lock therapy has not yet been well defined.

Echinocandins and amphotericin B (AMB) lipid formulations are known for their antibiofilm activity.1,2 We recently showed that micafungin (~100- and 500-fold MIC) used in vitro as lock solutions displayed sustained efficacy against both young and mature Candida albicans and Candida glabrata biofilms. However, micafungin never managed to eradicate the biofilm, suggesting the need to combine a micafungin lock with systemic therapy.3 The value of liposomal AMB (L-AMB) lock solutions has been poorly investigated up to now. Moreover, authors often studied lock durations of ≥1 day, which are not always compatible with clinical practice.3,4 In addition, the culture media for biofilm formation were different, depending on the study, making the comparison of experimental results difficult. Moreover, Martins et al.6 recently showed that the composition of the extracellular matrix in C. albicans biofilms varied, depending on the culture media. In any case, the available data suggest a variability in the efficacy of L-AMB lock solutions as a function of dose, duration and Candida species.3,5

The aim of the present study was to evaluate the in vitro antibiofilm activity of L-AMB solutions, used as short-term (4 or 12 h) or long-term (24 h) locks, against three major species of Candida (C. albicans, C. glabrata and C. parapsilosis). Furthermore, the experimental conditions are similar to those recently used to investigate the activities of three echinocandin and azole molecules against C. albicans and C. glabrata biofilms.5

Materials and methods

Six clinical strains isolated from infected catheters were used.3 The MIC values were determined using Etest strips in accordance with the manufacturer’s instructions (AB BIODISK, Solna, Sweden), and were ≤0.094 mg/L for C. albicans strains and ≤0.25 mg/L for C. glabrata and C. parapsilosis strains. The activity was evaluated for up to 48 h after drug removal. Lock durations were chosen in light of those recommended...
by Andris et al. The investigated concentrations of L-AMB were 200 and 1000 mg/L.

Yeasts and catheter sections were prepared as previously described. A 4000 mg/L stock solution of L-AMB (AmBisome, Gilead Sciences) was produced in sterile water: 50 mg of the drug was suspended in 12 mL of water, as recommended. L-AMB final solutions at 200 and 1000 mg/L were produced in Yeast Nitrogen Base (YNB) medium supplemented with 30 mM glucose (YNB-Glc) and added to 12 h or 5 day preformed biofilms for 4, 12 or 24 h at 37 °C. All silicone sections were then incubated with YNB-Glc, without drug, at 37 °C for 24 or 48 h. The antibiofilm effects were monitored using a previously described metabolic assay based on the reduction of a tetrazolium salt (XTT). All experiments were performed twice, with eight replicates. Analysis of variance and Scheffe’s test were applied to determine statistical differences between the groups (P < 0.001).

To facilitate the analysis of the results, the inhibition percentages were calculated as follows: inhibition (%) = 100 × [1 – (A530 lock-treated strain/ A530 untreated strain)], where A530 is the absorbance at 450 nm. Decreases were calculated as the mean inhibition for each Candida species.

Results and discussion

Despite some interstrain and interspecies non-significant differences, the inhibition of 12-h-old Candida spp. biofilms always reached ≥ 75% and that activity persisted for up to 48 h post-lock (P < 0.001; Table 1). Interestingly, the highest L-AMB concentration (1000 mg/L; mean inhibition 85.9%) did not induce significantly stronger activity against young biofilms than that obtained with the lowest concentration (200 mg/L; mean inhibition 85.2%). These results need to be confirmed in clinical practice, but suggest that 4 h locks using 200 mg/L L-AMB could be sufficient to reduce an intraluminal non-mature biofilm and, thus, could be useful for the management of catheters colonized only by yeasts. Locks should be as short as possible, both to limit the risk of thrombosis and to not disturb the normal use of the catheter. Furthermore, no significant difference in antibiofilm (12 h old) activity was observed, regardless of the Candida species and the L-AMB concentration. However, we still remain cautious, as, overall, 12-h-old biofilm eradication was never reached (inhibition ≤ 93%).

The results were quite different against mature biofilms (5 days old): C. parapsilosis biofilms were less susceptible to L-AMB lock solutions compared with those formed by C. albicans and C. glabrata. Similar antibiofilm activities were observed for both C. albicans and C. glabrata, and 1000 mg/L L-AMB did not display enhanced efficacy compared with 200 mg/L L-AMB (Table 1). The mean inhibition percentages were 83.7% ± 4.6% (200 mg/L L-AMB) and 85.1% ± 2.9% (1000 mg/L L-AMB), regardless of lock and post-lock times (Table 1); lock activity persisted for up to 48 h post-lock (P < 0.001). Lastly, all the tested locks were able to inhibit > 73.5% of both young and mature biofilms made with C. albicans or C. glabrata, regardless of the study conditions (P < 0.001), suggesting the usefulness of L-AMB lock solutions in the management of catheters colonized or/and infected with these Candida species.

The lock activity of L-AMB was also investigated based on yeast viability tests (trypan blue staining and cfu counts). The results of both methods agreed with those obtained using the metabolic method (data not shown).

The activity of 200 mg/L L-AMB against mature C. parapsilosis biofilms, regardless of the lock duration, was lower than that obtained against both other Candida species (Table 1). In addition, the longer the lock, the more the activity of 200 mg/L L-AMB was sustained against mature C. parapsilosis biofilms (P < 0.001); the variability in the results also decreased with increasing lock durations (Table 1). This phenomenon, which was obvious with 200 mg/L L-AMB, was less evident with 1000 mg/L L-AMB, except for the results obtained using a 4 h lock. However, again, the variability observed decreased as the lock duration increased (Table 1).

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\text{Decrease in yeast metabolic activity (%)} = \frac{A_{530} \text{lock-treated strain}}{A_{530} \text{untreated strain}} \times 100
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\begin{array}{|c|c|c|c|c|}
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\text{Lock duration (h)} & \text{Post-lock time (h)} & \text{12-h-old biofilm} & \text{5-day-old biofilm} \\
\hline
\text{Candida albicans} & & & & \\
4 & 24 & 89.0 ± 2.8 & 86.5 ± 0.7 & 73.5 ± 17.7 & 83.5 ± 3.5 \\
4 & 48 & 84.0 ± 2.8 & 86.5 ± 0.7 & 76.0 ± 5.7 & 80.0 ± 8.5 \\
12 & 24 & 91.5 ± 3.5 & 89.0 ± 2.8 & 86.5 ± 0.7 & 84.5 ± 2.1 \\
12 & 48 & 91.0 ± 4.2 & 88.5 ± 2.1 & 87.5 ± 2.1 & 87.5 ± 2.1 \\
24 & 24 & 79.5 ± 4.9 & 84.5 ± 2.12 & 81.5 ± 0.7 & 83.5 ± 3.5 \\
24 & 48 & 86.0 ± 1.4 & 84.5 ± 2.12 & 82.0 ± 2.8 & 81.0 ± 1.4 \\
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\text{Candida glabrata} & & & & \\
4 & 24 & 75.5 ± 7.8 & 84.5 ± 4.9 & 83.0 ± 8.5 & 86.0 ± 4.2 \\
4 & 48 & 80.0 ± 7.0 & 86.5 ± 0.7 & 87.5 ± 3.5 & 87.0 ± 2.8 \\
12 & 24 & 93.0 ± 3.0 & 88.0 ± 0.7 & 89.5 ± 3.5 & 89.5 ± 0.7 \\
12 & 48 & 91.0 ± 2.8 & 88.5 ± 3.5 & 88.5 ± 4.9 & 87.0 ± 0.0 \\
24 & 24 & 77.5 ± 0.7 & 85.0 ± 1.4 & 85.0 ± 0.0 & 84.0 ± 0.0 \\
24 & 48 & 81.5 ± 0.7 & 85.5 ± 2.1 & 83.5 ± 2.1 & 88.5 ± 0.7 \\
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\text{Candida parapsilosis} & & & & \\
4 & 24 & 85.5 ± 9.2 & 83.0 ± 9.9 & 71.5 ± 20.5 & 77.5 ± 3.5 \\
4 & 48 & 86.0 ± 7 & 80.5 ± 7.8 & 41.5 ± 48.8 & 62.0 ± 18.4 \\
12 & 24 & 88.5 ± 6.4 & 86.0 ± 5.7 & 73.0 ± 18.4 & 76.0 ± 9.9 \\
12 & 48 & 87.5 ± 9.2 & 88.5 ± 4.9 & 55.5 ± 40.3 & 78.0 ± 11.3 \\
24 & 24 & 83.5 ± 10.6 & 86.5 ± 3.5 & 82.5 ± 0.7 & 81.5 ± 0.7 \\
24 & 48 & 83.5 ± 7.8 & 85.0 ± 4.2 & 64.0 ± 22.6 & 77.5 ± 4.9 \\
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84% ± 6.5% (48 h after a 4, 12 or 24 h lock). The activity of 12 h L-AMB locks was therefore at least equivalent to those of micafungin and caspofungin obtained in a previous study using the same strains of *C. albicans* and *C. glabrata*. This suggests the usefulness of L-AMB against *Candida* biofilms. Mukherjee et al., investigating AMB lipid complex (ABLC) lock solutions using a rabbit model, showed that it was possible to eradicate *C. albicans* biofilms on catheters using 5000 mg/L ABLC locked for 4 or 8 h each day for 7 days. This concentration was slightly higher than ours, which could explain why overall eradication was never reached under our conditions. In addition, it is always difficult to compare *in vitro* and *in vivo* results. Moreover, a case of *C. glabrata* fungaemia cured with intraluminal conventional AMB (5000 mg/L, 6 h daily, 14 days) in addition to systemic therapy with intravenous fluconazole has been previously described.

In conclusion, L-AMB used as a 1000 mg/L solution would be useful to inhibit *Candida* spp. biofilms for up to 48 h on silicone catheters using short (4 h), medium (12 h) or long (24 h) lock times. L-AMB as a 200 mg/L lock solution was not efficient against *C. parapsilosis* mature biofilms, but demonstrated a sustained activity against 12-h-old *C. parapsilosis* biofilms. Lastly, 200 mg/L L-AMB lock solutions were able to inhibit *C. albicans* and *C. glabrata* biofilms for up to 48 h, regardless of the lock duration. However, as previously observed for echinocandins, overall eradication was never obtained. Thus, in combination with systemic medication, L-AMB (1000 mg/L) lock solutions could contribute to the control of candidiasis in catheterized patients.

**Acknowledgements**

We would like to thank Gilead Sciences for supplying standard antifungal powders of L-AMB.

**Funding**

This work was supported by a grant from Gilead Sciences.

**Transparency declarations**

None to declare.

**References**


