In Vivo Efficacy of Anidulafungin against Mature Candida albicans Biofilms in a Novel Rat Model of Catheter-Associated Candidiasis

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The present study demonstrates the efficacy of anidulafungin on mature Candida albicans biofilms in vivo. One hundred fifty-seven catheter fragments challenged with C. albicans were implanted subcutaneously in rats. After formation of biofilms, rats were treated with daily intraperitoneal injections of anidulafungin for 7 days. Catheters retrieved from treated animals showed reduced cell numbers compared to those retrieved from untreated and fluconazole-treated animals. Systemic administration of anidulafungin is promising for the treatment of mature C. albicans biofilms.

Fungal biofilms represent a persistent source of disseminated infections in high-risk patients and are recalcitrant to antifungal therapy (11). Two classes of agents, the lipid formulations of amphotericin B and the echinocandins, appear to have a unique activity against Candida biofilms. Intraluminal lock therapy with caspofungin alone (4) or combined with systemic therapy (10) was shown to be effective against Candida biofilms in two intravascular catheter models in rabbits and mice. Anidulafungin, active against Candida biofilms in vitro (6), seems a very attractive antifungal agent to employ for a lock therapy approach since this drug was shown to induce fewer paradoxical growth effects than caspofungin and micafungin (2). Recently, we reported a novel in vivo subcutaneous Candida biofilm model in rat (8), in which biofilms develop inside catheter fragments implanted under the skin. We here report on the ability of anidulafungin to strongly reduce the number of viable cells in mature Candida biofilms in such animal model, using more than 150 infected catheters.

For all experiments, the sequenced Candida albicans SC5314 strain (3) was used. Fluconazole and anidulafungin, provided by Pfizer (Grotton, CT), were prepared in sterile water and dimethyl sulfoxide (DMSO), respectively. In vitro biofilm drug susceptibility assays were performed using 1-cm pieces (20 pieces per tested concentration) of serum-coated polyurethane catheters (Arrow International Reading) as previously described by Říčcová et al. (8). Biofilms were subjected to fluconazole or anidulafungin at concentrations ranging from 0.125 μg/ml to 64 μg/ml or to antifungal-free medium for 24 h. The metabolic activity of the biofilms was measured using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazoli- um-5-carboxanilide inner salt] reduction assay as previously described (7). Biofilm MICs were determined as the minimal drug concentration that caused ≥50% reduction in the metabolic activity of the biofilm compared to the level for the controls. In vivo biofilms were grown subcutaneously in a rat model as described by Říčcová et al. (8). Briefly, female Sprague-Dawley rats (200 g) were immunosuppressed by the addition of 1 mg/liter of dexamethasone to their drinking water. Polyurethane catheter pieces incubated overnight in serum sonicated before biofilm quantification by CFU counting. Results were analyzed using the Mann-Whitney test (Analyze-it software).

Fluconazole did not cause any reduction of metabolic activity of in vitro biofilms formed in the polyurethane catheter model even at the highest concentration of 64 μg/ml, whereas the in vitro biofilm MIC of anidulafungin was 0.25 μg/ml, with no paradoxical growth at higher concentrations. The numbers of Candida cells recovered from the implanted catheters in vivo are given in Fig. 1. Despite the illustrated variation, it is noteworthy that more than 70% of catheters retrieved from treated animals contained fewer than 2 log10 cells, which is below the diagnostic threshold for catheter-related infections (5). Additionally, 14 catheters (17%) retrieved from 7 out of 11 anidulafungin-treated animals were sterile. Finally, the few catheters that contained as many cells as the control biofilms were retrieved from only 2 animals out of 11, highlighting the animal-dependent variability. The mean number of CFU ± standard deviation (SD) obtained per catheter fragment of fluconazole-treated animals (3.01 ± 0.1 log10 CFU/catheter fragment) was not significantly different (P = 0.94) from the mean number of
FIG. 1. Effect of antifungal intraperitoneal treatment on mature Candida biofilms formed on the catheter’s lumen in a rat model. The log_{10} numbers of CFU of Candida albicans cells cultured from each catheter fragment (2.92 ± 0.34 log_{10} CFU/catheter fragment) compared to the level for the control animals (P < 0.0001). We report here that systemic administration of anidulafungin in rats resulted in a significant reduction of C. albicans cells living within biofilms in vivo. The activity of caspofungin was previously described for two intravascular catheter animal models (4, 10). In both models, the drug was instilled intraluminally. In the subcutaneous model, anidulafungin was administered intraperitoneally. Despite the fact that this is not the most clinically relevant mode of administration, therapeutic levels were achieved, as shown by the complete killing of the fungal population in 17% of the implanted catheters. The variability in number of CFU recovered from catheters of anidulafungin-treated animals was rather large. This might be a limitation of the model. Otherwise, this might be a reflection of the variability in clinical response that could occur while patients are treated. Intravenous treatment needs to be tested in such subcutaneous model but may lead to an even higher and more reproducible rate of killing. Our data show that the in vivo Candida albicans biofilm subcutaneous model system is very attractive for in vivo testing of the activity of antifungal drugs. In addition, the lack of in vivo activity of fluconazole on Candida biofilms reported by other groups (1, 9) was confirmed in our model. The results of this study support the use of anidulafungin for the treatment of biofilms that are not located in the intravascular compartment, but confirmation of these results in other in vivo models is certainly warranted. In conclusion, we demonstrated the activity of anidulafungin on mature Candida biofilms in an animal model. Our results are promising for the treatment of Candida biofilms on devices that cannot be readily removed from the patient.

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REFERENCES