ABSTRACT

Objectives: Candida biofilms are responsible for many nosocomial infections. These biofilm-associated infections are often resistant to commonly used antifungal agents. Thus, discovery of effective therapies to prevent and treat real-life associated infections is critical. CD101 is a new extracellular with extended half-life that is being developed for the treatment of invasive Candida infections. In this study, we determined the effect of CD101 on in vitro prevention and treatment of biofilms formed by Candida albicans in vitro, and (2) on formation of biofilms in real-time using Time-Lapse Microscopy (TLM).

Methods: Biofilms were grown in vitro using an in vitro biofilm model and the effect of CD101 on adhesion phase biofilms (to assess biofilm prevention) or mature phase biofilms (biofilm treatment) was determined. CD101 was tested at 0.25, 1, or 2 µg/ml. Disks incubated with fluconazole (Flu) or media alone were used as controls. At the end of drug exposure in both adhesion and mature phase biofilms, biofilms were quantified by measuring their metabolic activity using an XTT assay. Separate batches of biofilms were treated with fluconazole (0.25 µg/ml) and observed under Confocal Scanning Laser Microscopy (CSLM) to evaluate biofilm architecture and thickness. CD101 concentrations used in the above experiments were monitored to ensure its effect on biofilm formation in real-time using TLM, allowing temporal monitoring of the interactions occurring between the drug and Candida biofilms.

Results: Metabolic activity and CSLM results showed that CD101 prevented formation of Candida biofilms at both concentrations tested (0.25 and 1 µg/ml). Assessment of metabolic activity revealed that C. albicans treated with CD101 significantly lost less biofilm biomass compared to untreated C. albicans (P = 0.05). CSLM images showed highly heterogeneous architecture of biofilms with cells/hyphae embedded within extracellular matrix for untreated control. Exposure to both concentrations of CD101 showed only remnants of adherent cells, and no biofilm maturation. Additionally, exposure to CD101 significantly reduced the thickness of biofilms compared to untreated control (µm). Fluconazole treatment showed that mature biofilms treated with CD101 were eradicated, leaving only bulged, deformed/fragmented yeast cells remaining. Additionally, CD101 significantly reduced thickness of mature biofilms compared to the untreated control (µm). In contrast, Flu did not affect adhesion or mature phase Candida biofilms. Time-lapse microscopy showed that untreated biofilms formed a highly heterogeneous architecture with cells/hyphae embedded within extracellular matrix. In contrast, biofilms exposed to 0.25, 1, or 2 µg/ml CD101 showed reduced growth, which failed to grow into mature biofilms.

Conclusion: We herein demonstrate that CD101 possesses anti-biofilm activity against both adhesion phase and mature phase biofilms formed by C. albicans. Therefore, CD101 may have utility in both prevention and treatment of fungal biofilm infections.

INTRODUCTION

- fungi, particularly Candida, have been shown to form biofilms on intravascular catheters and other foreign materials, creating a nidus of organism dissemination, which is often associated with persistent infections (1).
- biofilms are complex three-dimensional structures formed by microorganisms, entrapped within an extracellular matrix and are often resistant to antibiotics (2, 3).
- recent emergence of resistance (4) has underscored the importance of developing new antifungal agents that are effective against biofilms. In this study, we determined: (1) the effect of CD101 (a long-acting novel echinocandin that is being evaluated as a once-weekly IV infusion for the treatment of invasive candidiasis), against prevention and treatment of biofilms formed by C. albicans, and (2) the temporal effect of CD101 on formation of biofilms using TLM. Fluconazole was used as a comparator and media-alone was used as control in all experiments.

METHODS

Effect of CD101 Against Candida Biofilms:
Biofilms were formed on polystyrene (2%) discs using our catheter-associated-biofilm model (2, 3). To evaluate the ability of CD101 to prevent and treat biofilms, Candida SC5314 cells were adhered to 2% catheter discs for 90 min, incubated for 24 h with treated or untreated (0.25 or 1 µg/ml concentrations) and were allowed to form biofilms in yeast nitrogen base media. Separate batches after adhesion phase were allowed to mature into biofilms and then exposed to CD101 (0.25 or 1 µg/ml concentrations) for 24 h. A2Fluron (1 or 4 µg/ml) was used as comparator, and media-alone was used as control in all experiments. These concentrations were selected for testing the activity of CD101 or Flu against Candida biofilms based on Minimum Inhibitory Concentration (MIC) data for CD101 or Flu (0.25 or 1 µg/ml, respectively), performed according to CLSI, M37- A3 method. We elected to use 4 µg/ml to test activity of Flu against Candida biofilms based on previous studies of Flu that showed it has a poor antibiofilm activity (2).

Quantification and Visualisation of Biofilms:
After exposing to CD101, biofilms were quantified by measuring their metabolic activity using sulforhodamine B (5, 5-dimethylthiazol-2-yl)-2, 2, 5-triphenyltetrazolium chloride (MTT) of biofilms exposed to: [a] no drug, [b] 0.25 µg/ml CD101, [c] 1 µg/ml CD101, [d] 0.25, 1 µg/ml Flu or [e] 4 µg/ml Flu. Arrows show bulged/broken cells. Thickness of Candida biofilms was measured using CSLM analysis and observed under CSLM to examine the effect of CD101 on biofilm architecture and thickness.

Temporal Effect of CD101 on Candida Biofilm Formation Using TLM:
Following adherence of C. albicans suspensions (purified from biofilm) to SA for 90 min, discs were exposed to CD101 (0.25 µg/ml) incubated at 37ºC and allowed to form biofilms. Phase contrast images for this interaction were captured in real-time over a 16-h period using TLM. Discs with C. albicans biofilms incubated in growth medium alone with no antifungal was used as control.

RESULTS

Figure 1. Effect of CD101 or Fluconazole (Flu) on Adhesion Phase C. albicans Biofilms: Prevention: Effect of [a] CD101 (0.25 or 1 µg/ml) or [b] Flu (1 or 4 µg/ml) on metabolic activity of C. albicans biofilms compared to untreated control. *P-value compared to untreated control.

Figure 2. Confocal Scanning Laser Micrographs showing the Effect of CD101 or Fluconazole (Flu) on Adhesion Phase C. albicans Biofilms: Top-down three-dimensional view (top panels) and side-views (bottom panels) of biofilms formed by C. albicans treated with: [a] no drug (control), [b] 0.25 µg/ml CD101, [c] 1 µg/ml CD101, [d] 0.25 µg/ml Flu, or [e] 4 µg/ml Flu. Thickness of Candida biofilms exposed to: [f] CD101 or [g] Flu. *P-value compared to untreated control.

Figure 3. Effect of CD101 or Fluconazole (Flu) on Mature Phase C. albicans Biofilms: Treatment: Effect of [a] CD101 (0.25 or 1 µg/ml) or [b] Flu (2 or 4 µg/ml) on metabolic activity of C. albicans biofilms compared to untreated control. *P-value compared to untreated control.

Figure 4. Confocal Scanning Laser Micrographs showing the Effect of CD101 or Fluconazole (Flu) on Mature Phase C. albicans Biofilms: Top-down three-dimensional view (top panels) and side-views (bottom panels) of biofilms exposed to: [a] no drug, [b] 0.25 µg/ml CD101, [c] 1 µg/ml CD101, [d] 0.25 µg/ml Flu, or [e] 4 µg/ml Flu. Arrows show bulged/broken cells. Thickness of Candida biofilms was measured using CSLM analysis and observed under CSLM to examine the effect of CD101 on biofilm architecture and thickness.

Figure 5. Temporal Effect of CD101 (0.25 µg/ml) on Formation of C. albicans Biofilms: Images were captured immediately at 0 h and followed up to 16 h for biofilms untreated, treated with CD101 (0.25 µg/ml) at low magnification, x20, and treated with CD101 (0.25 µg/ml) at high magnification, x43. Arrows show bulging, deformed and broken cells.

Figure 6. Temporal Effect of CD101 (0.25 µg/ml) on 3 h formed C. albicans Biofilms: Drug was added after 3 h biofilm formation and images were captured immediately [A] after adding the drug and followed up to 16 h [B] magnification, x43. Arrows show bulging, deformed and broken cells.

CONCLUSIONS

Our results demonstrate that CD101 possesses anti-biofilm activity at physiologically-relevant concentrations (selected based on MIC data) against both adhesion phase and mature biofilm phases formed by C. albicans. Therefore, CD101 may have utility in both prevention and treatment of fungal biofilm infections.

REFERENCES


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