Exploring the antimicrobial peptide mimetic, Brilacidin, as novel therapeutic for fungal keratitis

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Introduction

- Fungal keratitis is a potentially blinding infection of the cornea affecting 1-2 million people annually.
- The main causative agents of fungal keratitis is *Aspergillus fumigatus, Aspergillus flavus,* and members of the *Fusarium solani* species complex.
- Natamycin remains the only FDA drug approved for treating FK and fails to resolve infection 40% of cases.
- **Brilacidin** is a new small molecule host defense peptide mimetic that has been used as a broad-spectrum antimicrobial for a variety of applications. Recently, Reis *et al* have shown that combinations of Brilacidin with other antifungals can improve the treatment outcome of Invasive Pulmonary Aspergillosis.
- **Here we investigate the antifungal activity of Brilacidin against various FK agents as well as its safety and therapeutic potential in a murine model of *A. fumigatus* keratitis.**

In vitro activity of Brilacidin

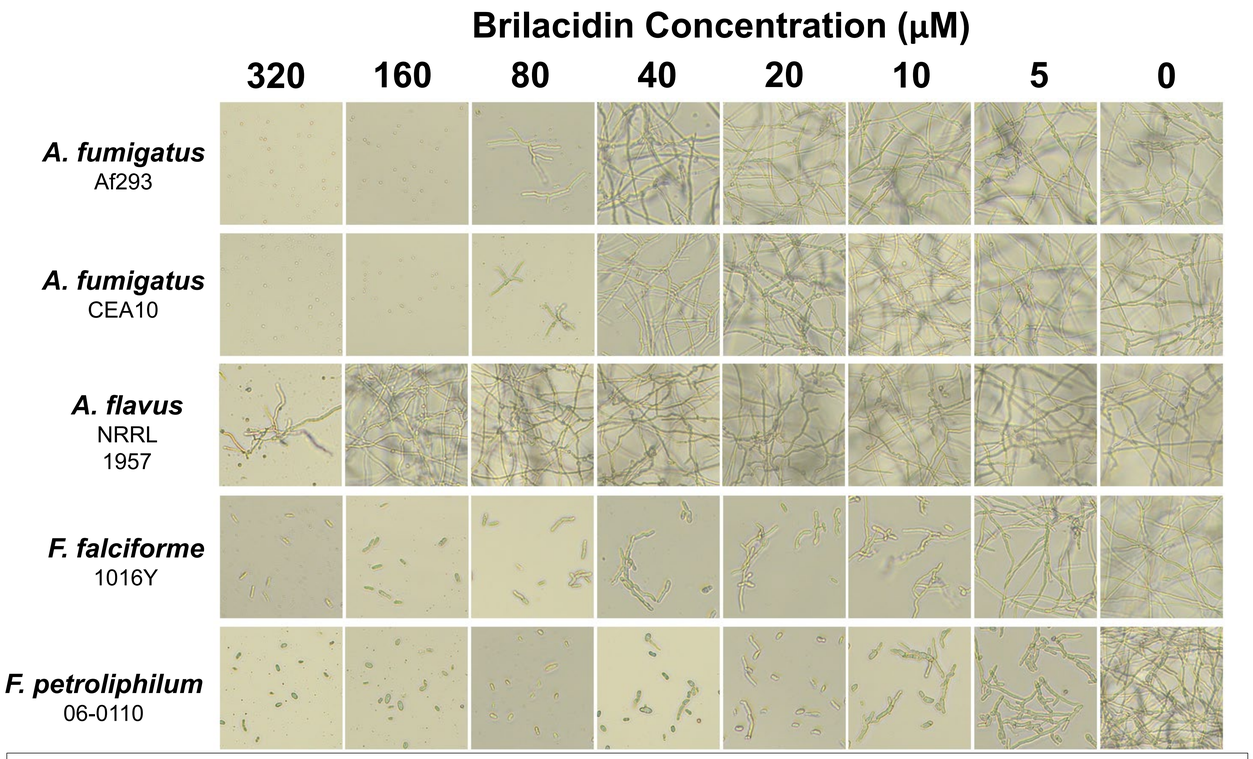

Figure 1: Brilacidin shows activity against major causative agents of fungal keratitis. $1x10^5$ conidia were inoculated into RPMI+2% glucose and Brilacidin at varying concentrations. These were incubated at 35 °C for 24h. Images were captured on via inverted microscope and absorbance was read at 600nm.

Murine Infection & Treatment model

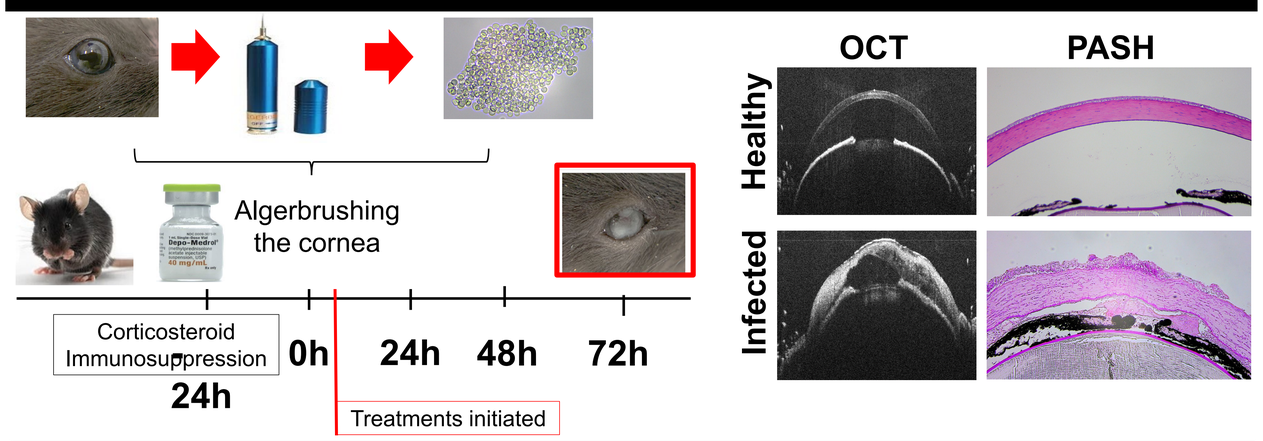

Figure 2: C57BL/6J mice were immunosuppressed with 100mg/kg of methylprednisolone the day preceding infection. On the day of infection, *A. fumigatus* Af293 conidia were swollen in rich media (YPD) for 4 hours at 35 °C, washed in PBS and normalized to an OD of 0.8 at 360nm. 5μl of these metabolically active conidia were overlaid onto the Algerbrushed cornea for 20 minutes. Treatments with either TBS, Vehicle (25% DMSO), or Brilacidin (250 mM) starting at 4h post infection (p.i.). Treatments were carried out every three hours between 8AM and 11PM for 72h p.i.. Infections were tracked progressively over the course of 72h via Optical Coherence Tomography (OCT) and Micron IV bio microscopy. At 72h p.i. the corneas were dissected for Colony Forming Unit (CFU) analysis and histology.

Ocular safety of Brilacidin

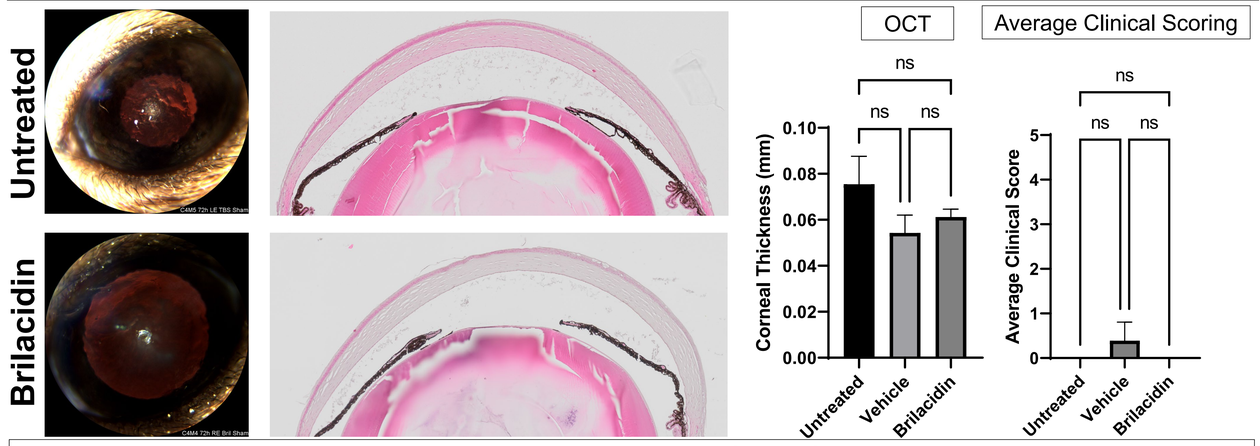

Figure 3: Brilacidin does not impact corneal clarity or architecture. Sham infected corneas were treated with either TBS, vehicle, or Brilacidin and monitored by OCT and Micron IV bio microscopy for 72h. OCT was used to measure corneal thickness across 13 points per cornea. These measurements were averaged. Images taken from Micron IV bio microscopy were scored based on 3 criteria on a scale from 0-4, with 4 being the most severe. Statistical analysis by Ordinary one-way ANOVA, P>0.05

In vivo efficacy of Brilacidin

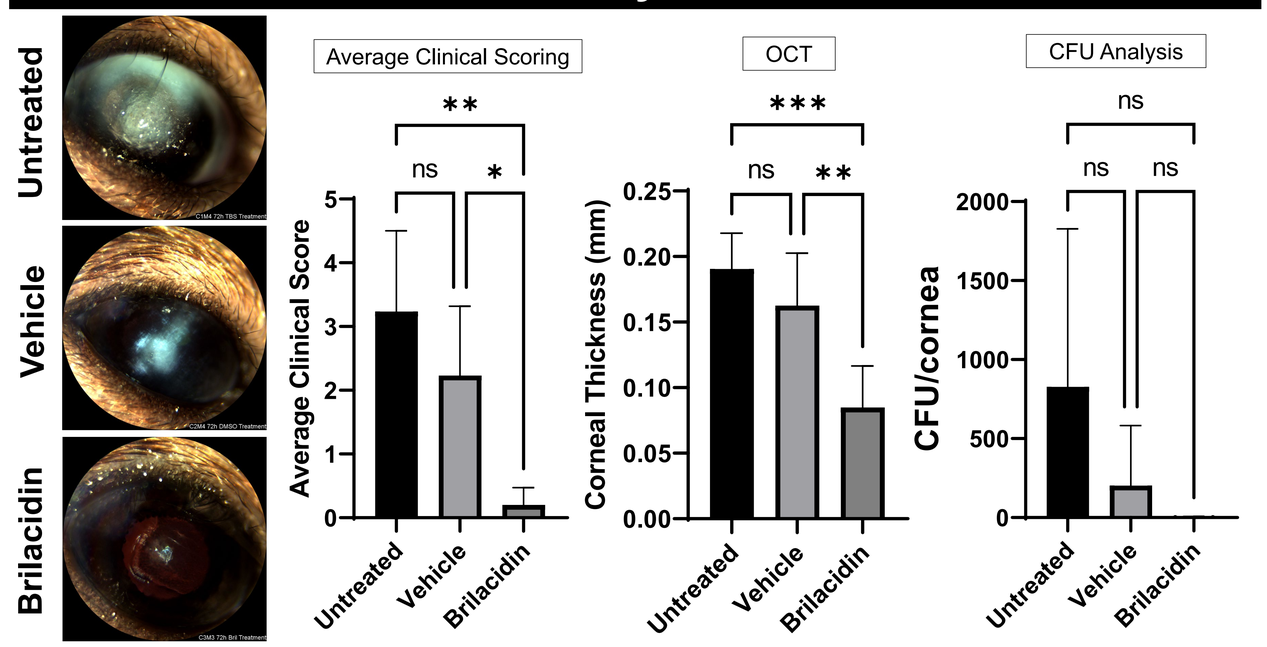

Figure 4: Brilacidin reduced fungal burden and inflammation compared to untreated controls. Clinical scoring and corneal thickness measurements were performed as described in Figure 3. CFU's were measured from homogenized corneas. Statistical Analysis by Ordinary one-way ANOVA, * P≤0.05, ** P ≤ 0.01, *** P ≤ 0.001

Discussion and Future Directions

- **<u>Brilacidin shows promise as a novel therapeutic for fungal keratitis</u>**
  - Shows *in vitro* activity against:
    - *A. fumigatus*
    - *A. flavus*
    - *F. solani species* complex
  - Shows *in vivo* activity against *A. fumigatus*
- Will Brilacidin show *in vivo* activity against *A. flavus* and members of the *F. solani* species complex?

References

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