Development of Novel Human-Derived Hybrid Host Defense Peptides for the Treatment of Bacterial Keratitis

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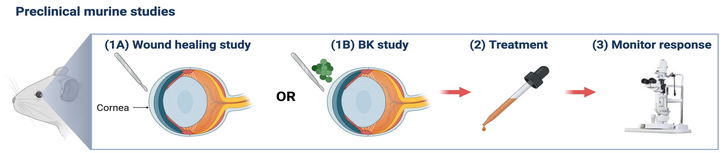
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INTRODUCTION

- Bacterial keratitis (BK) is the leading cause of corneal blindness globally.
- Topical antibiotic treatment is the main treatment for BK, but its efficacy is being challenged by emerging antimicrobial resistance (AMR).
- Host defense peptides (HDPs) are important components of innate immune system. At the ocular surface, LL-37 and human beta-defensins (HBDs)-1 to -3 have been shown to play important roles during infection.
- HDPs exhibit rapid and broad-spectrum antimicrobial activity with low risk of developing AMR, serving as a potential solution for AMR.
- **Aim:** To generate potent human-derived hybrid HDPs (HyHDPs) as novel topical antimicrobial therapy for BK.

METHODS

- **Design of HyHDPs:** HyHDPs were rationally designed based on the native templates of LL-37 and HBDs-1 to -3.
- **In vitro antimicrobial efficacy and toxicity:** Minimal inhibitory concentration (MIC), time-kill kinetics, lactate dehydrogenase (against human corneal epithelial cells), and haemolysis assays were performed.
- **Evaluation of mechanism of action:** SYTOX green dye uptake assay and molecular dynamics simulations (MDS) were performed.
- **In vivo antimicrobial efficacy and safety: (A)** Corneal wound healing and **(B)** *S. aureus* (ATCC SA29213) keratitis murine models were used.



RESULTS

- All 3 single, short-sequenced HDPs and 6 first-generation HyHDPs did not exhibit any antimicrobial efficacy (MIC<200μg/ml).
- A second-generation HyHDP (CaD23), based on LL-37 and HBD-2, exhibited good efficacy against methicillin-sensitive and methicillin-resistant *S. aureus* (MIC=12.5μg/ml) and *P. aeruginosa* (MIC=50μg/ml).
- At 200μg/ml (16x MIC), CaD23 was shown to be relatively safe against corneal epithelial cells (<30% toxicity) and erythrocytes (<10% toxicity).
- CaD23 (25.0μg/ml) achieved 100% killing of *S. aureus* within 30 mins, which was 8 times faster than amikacin (25.0μg/ml). It also expedited the action of amikacin when used in combination (**Figure 1**).
- The rapid antibacterial action was attributed to its membrane-permeating activity, supported by SYTOX green dye assay (70% membrane permeation indicated by SYTOX green release) and MDS (**Figure 2**).
- CaD23 0.05% (500μg/ml) achieved a median reduction of *S. aureus* bacterial viability by 94% (or 1.2 $\log_{10}$ CFU/ml; **Figure 3**).

DISCUSSIONS

- Rational hybridisation of HDPs has led to the generation of an efficacious CaD23 for treating Gram-positive BK.
- The rapid antimicrobial action is likely attributed to the membrane-permeating property, with a low risk of developing AMR.
- Peptide-antibiotic synergism can help reduce the toxicity of the treatment.
- MDS study provides further insight into the key membrane-interacting residues for further optimisation of CaD23.
- Future studies will examine the potential for treating other types of infection.

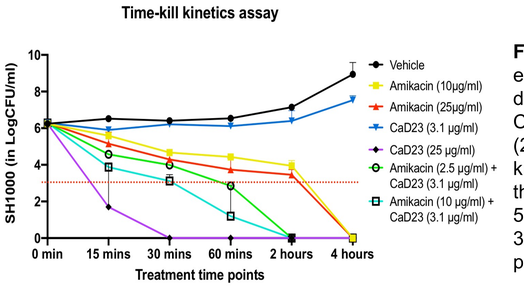


Figure 1. Time-kill kinetics assay examining the concentration- and time-dependent antibacterial efficacy of CaD23 against *S. aureus*. CaD23 (25.0μg/ml; 2xMIC) achieved complete killing within 30mins. It also expedited the antibacterial action of amikacin by 50% when used in combination (at 3.1μg/ml; 0.25x MIC), highlighting the peptide-antibiotic synergism.

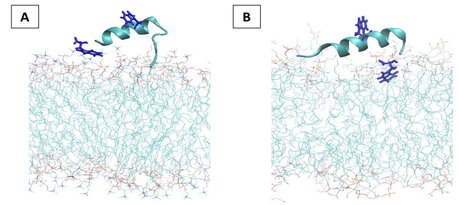


Figure 2. MDS evaluating the interaction of CaD23 with (A) mammalian mimetic membrane and (B) bacterial mimetic membrane. CaD23 is more alpha-helical (important for antimicrobial effect) on bacterial membrane than mammalian membrane. The Trp residue (highlighted in blue) at C-terminal penetrates deeper into the bacterial membrane.

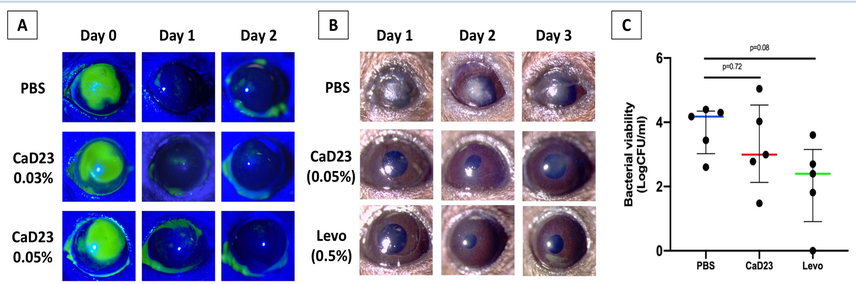


Figure 3. Pre-clinical studies examining the safety and efficacy of topical CaD23. **(A)** Topical CaD23 (0.05%; 4x/day) did not delay corneal wound healing (n=4 mice/group). Fluorescein-stained epithelial defect healed within a day, similar to PBS group. (**B-C**) Topical CaD23 (0.05%; 4x/day) reduce *S. aureus* bioburden by 1.2 logCFU/ml at day 3 post-treatment (=94% reduction), comparable to topical levofloxacin 0.5% (commonly used for BK). Note the clear corneas after treatment with CaD23 and levofloxacin 0.5%.