# Modulation of antimicrobial peptide expression as a novel approach to treating infectious keratitis

Allison Shannon[1], Sanskruti Potnis[1], Theint Aung[1], Melinda Quan[1], Brian Leonard[1]

[1]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

## Purpose

- Antimicrobial peptides (AMPs) are naturally occurring antibiotics expressed by epithelial cells and leukocytes[1]
- AMPs can either be constitutively or inducibly expressed by pathogenic or inflammatory stimuli[2]
- A recent study identified andrographolide, a small molecule from natural pharmacopeia, has the ability to upregulate a single AMP, human beta-defensin 3 (hBD3)[3]
- Preliminary data suggests that andrographolide can upregulate hBD3 mRNA expression in human corneal epithelial cells (hTCEpi) by 300-fold
- The current study utilized ELISA to quantify hBD3 peptide levels in the supernatant of hTCEpi cells treated with andrographolide (10-100μM)

OPEN

## Preliminary Data

1)

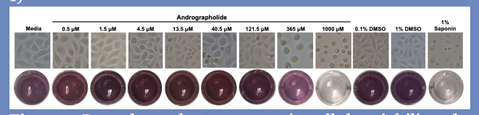


Figure 1. Dose dependent response in cellular viability of hTCEpi cells treated with increasing doses of andrographolide. hTCEpi cells exhibit an abnormal morphology when treated with andrographolide at concentrations greater than 121.5 μM. Media serves as control, 0.1% and 1% DMSO serve as vehicle controls,1% saponin serves as positive control for markedly reduced

OPEN

## Methods & Results

**Methods**

- hTCEpi cells cultured to near confluence (80-90%)
- hTCEpi cells treated with andrographolide for 48 hours at a range of doses (10-100μM) based on the MTT and qPCR assays from our preliminary data
- Quantified hBD3 peptide expression via ELISA

**Results**

3)

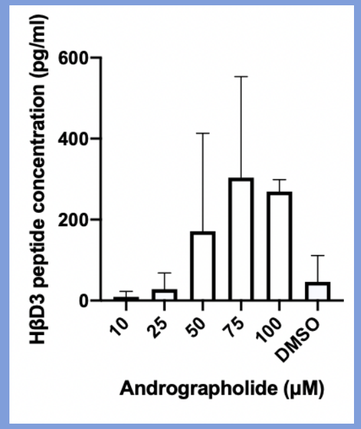


Figure 3. Andrographolide induces hBD3 peptide expression in a dose-dependent manner. DMSO used a vehicle control. Bars represent mean, error bars represent standard deviation. Results reflect two independent replicates.

## Discussion

- With increasing concentrations of andrographolide there is a dose-dependent increase in hBD3 peptide expression
- hBD3 peptide expression was markedly increased at 75 μM and 100 μM of andrographolide at 304 pg/mL and 269 pg/mL, respectively
- This study serves as a proof-of concept that andrographolide can modulate hBD3 peptide expression
- Provides the basis for translational studies with andrographolide used as a novel approach toward treatment of infectious keratitis

## Future Directions & Financial Support

- Perform additional replicates of our in vitro induction experiments
- Determine antimicrobial activity of induction supernatants on clinical bacterial isolates.

Financial support was provided by Students Training in Advanced Research (STAR) and the National Institutes of Health (NIH) Grant T35OD010956-21 (AHS) and K08EY028199 (BCL)

NIH National Institutes of Health

## References

1. Leonard, B.C., V.K. Affolter, and C.L. Bevins. Antimicrobial peptides: agents of border protection for companion animals. Vet Dermatol, 2012. 23(3): p. 177-e36.
2. Prasad, S.V., et al. Expression and Function of Host Defense Peptides at Inflammation Sites. Int J Mol Sci, 2019. 21(1).
3. Sechet, E., et al. Natural molecules induce and synergize to boost expression of the human antimicrobial peptide beta-defensin-3. Proc Natl Acad Sci U S A, 2018. 115(42): p.E9869-E9878.
4. Peterson, J.C., et al. Molecular epidemiology and resistance profiles among healthcare- and community-associated Staphylococcus aureus keratitis isolates. Infect Drug Resist, 2019. 12: p. 831-843.
5. McDermott, A.M. The role of antimicrobial peptides at the ocular surface. Ophthalmic Res, 2009. 41(2): p. 60-75.