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Antimicrobial Effects of Adjunctive Thymosin beta-4 Therapy in Bacterial Keratitis

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Purpose: Our lab has been focused on developing thymosin beta-4 (Tβ4) as a potential therapeutic for bacterial keratitis, an extremely debilitating ocular infection that is prevalent world wide. Tβ4 is a naturally occurring g-actin sequestering peptide, which we have previously shown significantly improves disease outcome in a P. aeruginosa (PA)-induced keratitis model when used adjunctively with ciprofloxacin (cipro). Following observations of decreased bacterial load in Tβ4 +cipro-treated corneas, this project aims to investigate the synergy between Tβ4 and cipro regarding bactericidal effects and antimicrobial peptide (AMP) regulation.
Methods: Minimum inhibitory concentration (MIC) assays were used to quantify the bactericidal activity of ciprofloxacin, Tβ4, and a combination of cipro with Tβ4 to evaluate synergy. Micro serial dilutions were carried out using 96 well plates and concentrations of PA prepared for 10^5 and 10^6 CFU/mL inoculum. Additionally, we began to further elucidate the mechanism behind Tβ4 mediated effects by assessing the impact of Tβ4 on the activation of AMP pathways. Human corneal epithelial cells (HUCLs) were stimulated with LPS (25 μg/mL) for 6 and 24 hours. Experimental groups included: CTRL, Tβ4, cipro, and Tβ4 +cipro. Additionally, mRNA and protein levels of AMPs, including LL-37, S100A8, hBD-3, keratin6A, and TLR-4 were also assessed.
Results: Though we expected to see a further decrease in MIC for adjunct Tβ4 therapy in comparison with cipro alone, the two groups exhibited similar results without a significant difference in MIC. Surprisingly however, the MIC assay showed very little bacterial growth with ciprofloxacin alone, even at significantly low concentrations. Unexpectedly, Tβ4 alone did not show significant bactericidal activity. Regarding AMP expression, RT-PCR results revealed upregulation of a number of AMPs in response to Tβ4 stimulation at 6 hrs, as well as further upregulation in combo groups at 24 hrs, indicating a possible synergistic effect. This trend has been similarly observed with select lipoxygenase enzymes and SPM receptors.
Conclusion: We believe that the results from our experiments help provide evidence by which Tb4 influences the antimicrobial effects observed in the bacterial keratitis model. More importantly, suggestions of a synergistic effect between Tb4 and cipro at the gene level, as well as findings of an extremely low MIC for ciprofloxacin may allow for lower concentrations of antibiotic to be used in the clinical setting. This would not only decrease the risk for potential side effects/toxicity issues related to the use of antibiotics, but potentially reduce antimicrobial resistance.