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NVC-422: Towards Developing Preclinical Infected Tissue Models

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Abstract

Background *P. aeruginosa* and *S. aureus* are opportunistic human pathogens implicated in many clinical diseases of the eye and skin, including keratitis and impetigo, respectively. Infection can involve destruction of underlying tissue by toxins to exacerbate tissue damage, leading to erosive disease. Developing infected tissue models provide a platform for assessing antimicrobial efficacy and toxicity in that the tissues mimic the *in vivo* environment; yet maintain the controlled advantages of an *in vitro* system. *N,N*-dichloro-2,2-dimethyltaurine (NVC-422) has broad spectrum activity against bacterial pathogens. This study aimed to develop practical *in vitro* models of primary human cells in tissues superficially infected with bacteria to examine the antimicrobial efficacy of NVC-422. **Methods** Two tissue models were developed: 1) human dermal (EpiDerm™), and 2) ocular (EpiOcular™) epithelial cells (MatTek Corp). Tissues were cultured on 10 mm transwell inserts and infected with 10^6 CFU of *S. aureus*, or *P. aeruginosa* for 1 h at 37ºC. Infected tissues were treated apically with test compounds, rinsed and excised by punch biopsy. Microbial kill was determined by CFU analysis. Toxicity was tested by MTT assay. Infection levels and susceptibility of pathogens to test compounds was confirmed by cryosectioning and immunofluorescence microscopy. **Results** Both tissues yielded quantifiable and reproducible superficial infections: $1.5x10^6$ cfu/cm^2 *P. aeruginosa* (EpiOcular), and $1.6x10^6$ cfu/cm^2 *S. aureus* (EpiDerm™)1h topical treatment of 1.5% NVC-422/AA1 and 3h treatment of 0.5% NVC-422/acetate resulted in a 3.2 log and 4 log reduction of *S. aureus* and *P. aeruginosa,* respectively. **Conclusions** These results demonstrated the utility and reproducibility of MatTek tissues for modeling superficial infections of human tissue *in vitro*, and for testing efficacy and toxicity of topical antimicrobials. NVC-422 showed significant bacterial efficacy in both models and support the continued development of NVC-422 as a topical antimicrobial.

Introduction

A new method has been developed to assess topical antibacterial efficacy in an infected tissue model, with reproducible and quantifiable results. The utility of the infected EpiOcular™ and EpiDerm™ tissue models is to create a more *in vivo*-like environment to identify topical bactericidal drugs.
MatTek's EpiOcular™ corneal model and EpiDerm™ dermal model consist of normal, human-derived epithelial keratinocytes which have been cultured to form a stratified, squamous epithelium similar to the structure and morphology of the cornea or epidermis, respectively. These *in vitro* tissue models exhibit *in vivo*-like growth and morphological characteristics which are uniform and highly reproducible (1, 2).
A member of a new class of stable *N*-chlorotaurines (*N,N*-dichloro-2,2-dimethyltaurine sodium salt; NVC-422) with improved long term stability was recently described (3). NVC-422 exhibits efficacy in both tissue models against *S. aureus* and *P. aeruginosa*, with a difference in efficacy seen between dose, exposure, and formulation components.

Materials & Methods

Preparation of Bacteria *S. aureus* (ATCC 29213, Eurofins 1674619) and *P. aeruginosa* (MCC 4438) were grown from single colonies in TSB to log-phase growth (~2-3hrs).

Tissues EpiOcular™ and EpiDerm™ (MatTek Corporation, Ashland, MA) *in vitro* tissues were grown in phenol-free, antibiotic/antifungal-free media according to MatTek specifications.

NVC-422 solutions were prepared in unbuffered saline pH 4, or polycarbophil hydrogel (AA) pH 5. All formulation components are proprietary and confidential.

Tissue Infection Procedure Tissues were infected 1hr with 10^7 cfu/ml bacteria, then rinsed with PBS. 100ul/tissue of drug was added for specified treatment time at 37ºC. Following treatment, tissues were rinsed and removed by 8mm punch biopsy. The tissue was vortexed for 30s and 10-fold serial dilutions were made in Dey Engley broth to neutralize NVC-422, then plated onto TSA. CFU analysis in triplicate was performed the following day.

Tissue Toxicity Procedure Tissues were treated with NVC-422 solutions according to the protocol supplied by MatTek entitled (4).

Histology Tissues were paraffin embedded, H&E stained, and sectioned for Histology analysis, performed by MatTek Corp. (Ashland, MA)

Results

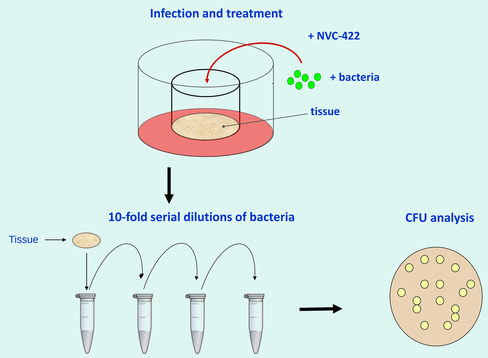

Figure 1. Tissue Infection Assay Procedure Schematic

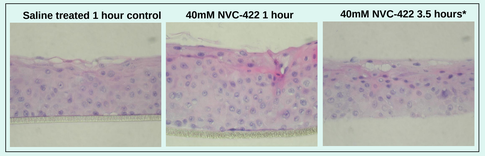

Figure 2: H&E stained, paraffin embedded sections of ocular tissue after NVC-422 treatment (no infection). NVC-422 treatment does not affect corneal tissue morphology. Note: *NVC-422 shows no morphological changes in tissue with associated toxicity at 3.5 hours exposure (data not shown)

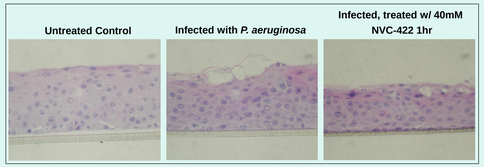

Figure 3: H&E stained, paraffin embedded sections of *P. aeruginosa* infected ocular tissue with and without NVC-422 treatment. Treatment with NVC-422 does not cause any damage to the infected tissue.

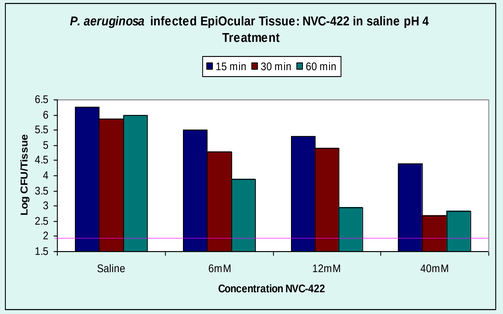

Figure 4. Treatment by NVC-422 in infected ocular tissue shows dose and time-dependent log CFU reduction. There was no tissue toxicity of NVC-422 up to 40mM at 60min exposure (data not shown).

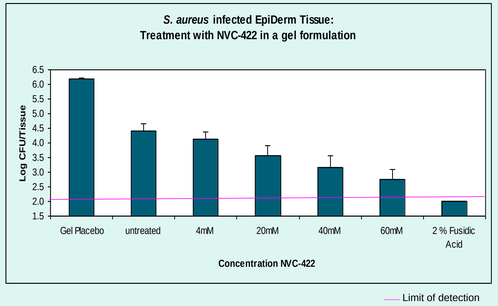

Figure 5. 1hr treatment of NVC-422 shows dose-dependent bactericidal efficacy in the *S. aureus* infected dermal tissue. At the concentrations tested, NVC-422 was shown to be non-toxic (data not shown).

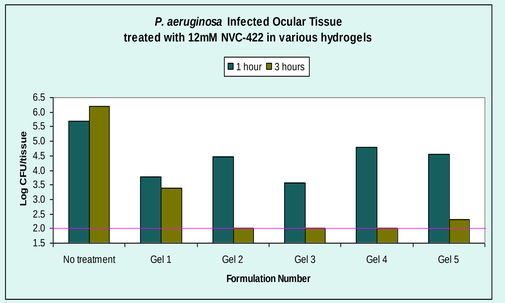

Figure 6. The infected ocular tissue model shows different efficacies between formulations and exposure times. None of the gels without NVC-422 showed activity.

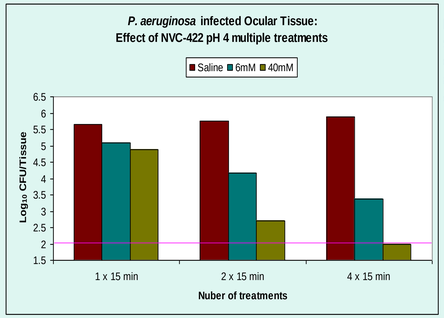

Figure 7. NVC-422 shows dose- and time-dependent bactericidal activity in the infected ocular tissue model

Summary

- NVC-422 demonstrates dose-dependent efficacy in both infection models, with complete bacterial kill by 4x15min treatment at a concentration of 40mM in the infected EpiOcular™ tissue (Fig. 7)
- In EpiDerm™ tissue, NVC-422 is safe and shows no toxicity with prolonged treatment up to 14hrs exposure (data not shown). Only the highest tested concentration of NVC-422 (40 mM) shows minor toxicity in ocular tissue at 4.5hrs exposure (data not shown)
- Several gel formulations of NVC-422 were effective in killing *P. aeruginosa* (Fig. 6).

Conclusions

- The utility of the infected EpiOcular™ and EpiDerm™ tissue models is to identify bactericidal drugs in an environment that more closely mimics *in vivo* conditions.
- Both tissue models allow to test the efficacy and toxicity of novel topical antimicrobials and formulations of topical antimicrobials.
- In both dermal and ocular tissue models, NVC-422 shows time- and concentration-dependent antibacterial efficacy.

References

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