**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 infections and burns. Infections can involve destruction of underlying tissue by toxins to exacerbate tissue damage, leading to ensues. Developing infected tissue models provides a platform for assessing antimicrobial efficacy and toxicity in that the tissues mimic the in vivo environment while maintaining the advantages of an in vitro system. A N,N-dimethyltaurine dimethyltaurine (NVC-422) has broad spectrum activity against bacterial pathogens. This study aimed to develop practical in vitro models of non-helical human in tissue superficially infected with bacteria to assess the antimicrobial efficacy of NVC-422.

**Methods**

Two tissue models were developed: 1) human dermal (EpiDermTM) and 2) ocular (EpiOcularTM) tissues (MatTek Corp). Tissues were cultured on 10 mm transwell inserts and infected with *10^5 CFU* of *P. aeruginosa* or *S. aureus* at 37°C. Infected tissues were treated with test compounds, rinsed and excised by punch biopsy. Microbial kill was determined by CFU analysis.

**Results**

Both tissues exhibited a dose- and time-dependent antibacterial efficacy with NVC-422 showing significant bacterial killing efficacy in both models and support the continued development of NVC-422 as a topical antimicrobial.

## Materials & Methods

**Preparation of Bacteria**

*S. aureus* (ATCC 29213, Furnish 106187) and *P. aeruginosa* (MC 4403) were grown from single colonies in TSB to log phase growth (3-3.5h).

**Tissue Preparations**

Tissues EpidermalOcular and EpidermalTM MatTek Corporation, Ashland, MA in vitro tissues were grown in phenol-free, antibiotic/culture free media according to MatTek specifications. NVC-422 solutions were prepared in unbuffered saline pH 4, or polypropylene hydrogel (AP) pH 5. All formulations are proprietary and confidential.

**Tissue Infection Procedure**

Tissues were infected with 10^5 CFU/ml of *P. aeruginosa* bacteria, then rinsed with PBS. 100µl of tissue was added for specified treatment time at 37°C. Following treatment, tissues were rinsed and removed by 3mm 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 the following day.

**Tissue Toxicity Procedure**

Tissues were treated with NVC-422 solutions according to the methods described above.

**Histology**

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

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**Summary**

- NVC-422 demonstrates dose-dependent efficacy in both infection models, with complete bacterial kill by 4x1min treatment at a concentration of 40mM in the infected EpDermalTM tissue (Fig. 7).
- In EpDermalTM 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 (40mM) shows minor toxicity in cornea at 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 EpDermalTM and EpidermalTM 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 top antimicrobials.
- Both dermal and corneal tissue models, NVC-422 shows time- and concentration-dependent antibacterial efficacy.

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**References**


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**Figure 1. Tissue Infection Assay Schematic**

<table>
<thead>
<tr>
<th>Concentration NVC-422</th>
<th>Log CFU/tissue</th>
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<th>3 hours</th>
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<tr>
<td>Saline 6mM</td>
<td>-</td>
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**Figure 2. H&E stained, paraffin embedded sections of corneal tissue after NVC-422 treatment (no infection). NVC-422 treatment does not affect corneal morphology. Note: NVC-422 shows no morphological changes in tissue with associated toxicity at 3 hours exposure (data not shown).**

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

**Figure 4. Treatment of *P. aeruginosa* infected corneal tissue shows time-dependent log CFU (Fig. 7). There was no tissue kill (no 3-log kill up to 480mM at 30min exposure; data not shown).**

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

**Figure 6. The infected corneal tissue model shows different efficiencies between formulations and dosing conditions. Note: the gel without NVC-422 showed activity.**

**Figure 7. NVC-422 demonstrates dose and time-dependent bacterial activity in the infected corneal tissue model.**