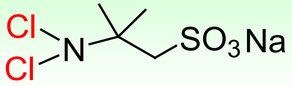
Poster D-1029

# *In vitro* Microbicidal Activity in Tears of *N,N*-dichloro-2,2-dimethyltaurine (NVC-422, AL-46383A), a Novel Topical Ophthalmic Agent

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## Abstract

**Purpose**: To evaluate the microbicidal activity and chemical stability in synthetic tears of *N,N*-dichloro-2,2-dimethyltaurine ( NVC-422), which is a synthetic analog of *N*-chlorotaurine (NCT). NCT is a relatively longer-lived endogenous antimicrobial component produced during oxidative burst by neutrophils and monocytes.

**Methods**: Stability of NVC-422 in 10% synthetic tears containing lysozyme, IgG, albumin and other components was tested using HPLC with UV detection. Bacterial time kill kinetics was studied in 5 mM acetate saline, pH 4 and in the presence of synthetic tears. Antiviral activity was determined by incubating viruses with serial dilutions of test compounds in 5 mM acetate saline, pH 4 followed by cell infection. The titer of virus was calculated based on the number of wells that show cytopathic effect (CPE). The $TCID_{50}$ was determined from the dilution where 50% of the cultures were positive for CPE.

**Results**: NVC-422 showed stability in 10% synthetic tears (97.5% remaining) after a 2 hr incubation. 0.3% NVC-422 in acetate saline pH 4 in the presence of synthetic tears was bacteriocidal against Gram-positive and Gram-negative bacteria (Table 1). Antiviral activity of NVC-422 was retained in 10% synthetic tears. 0.1% NVC-422 reduced all viral titers to the limit of detection (Table 2). See tables below.

Table 1:

| Bacteria | Excipient (% Synthetic tears) | Time to 4 Log Kill (min.) |
|---|---|---|
| S. aureus 6538 | 0 | 5 |
| S. aureus 6538 | 20 | 60 |
| S. aureus 6538 | 50 | 90 |
| MRSA 33591 | 0 | 5 |
| MRSA 33591 | 10 | 15 |
| S. marcescens 13880 | 0 | 5 |
| S. marcescens 13880 | 20 | 15 |
| S. marcescens 13880 | 50 | 30 |

Table 2:

| | $Log_{10}$ Reduction ($TCID_{50}$/mL) | | |
|---|---|---|---|
| Virus | 0.1% NVC-422 | 0.01% NVC-422 | 0.001% NVC-422 |
| Ad5 | > 4.2 | > 4.3 | 2.8 |
| Ad5 + 10% tears | > 3.8 | 1.7 | 1.0 |
| HSV-1 | > 3.5 | > 4.5 | > 4.5 |
| HSV-1 + 10% tears | > 3.8 | > 4.8 | > 4.7 |

**Conclusions**: NVC 422 retains its chemical stability, virucidal and bactericidal activity in presence of synthetic tears.

## Introduction

With the threat of multi-drug resistant bacteria and viruses facing the world's population and healthcare system, there is a growing need for effective antimicrobial products with little or no potential to develop resistance. An ideal agent would be effective against all viruses, bacteria and fungi including resistant strains. NovaBay Pharmaceutical's first-in-class non-antibiotic antimicrobials harness the unique chemical properties found in compounds produced by activated neutrophils in the ever increasing battle against resistant organisms. Based on the innate microbicidal molecules [1,2], *N, N*-dichloro-2,2-dimethyltaurine (NVC-422) is a shelf stable synthetic small molecule suitable for pharmaceutical use [3] Broad spectrum activity against Gram-positive bacteria including MRSA, Gram-negative bacteria including MDR *P. aeruginosa* [4], as well as viruses, wide therapeutic index and a very low likelihood of developing resistance[5] support the attractive therapeutic potential of NVC-422 as a broad spectrum antimicrobial agent.

NVC-422 promises the potential for a real paradigm shift in the prevention and treatment of ophthalmic infections.

## Methods

**HPLC analysis**. NVC-422 ± 10% synthetic tears was analyzed at t = 0 and 2 hrs at room temperature for stability using a C18 reverse phase isocratic method using 30% acetonitrile/$H_20$ gradient with 7mM TBAH eluent. At a flow rate of 0.7 mL/min NVC-422 eluted at approx. 6.5 min.

**CLSI Method Modifications.** Due to the rapid cidal nature of NVC-422 and its reactivity to certain components of Cation-Adjusted Mueller Hinton Broth (CAMHB) 5mM acetate saline pH 4 was substituted as diluent and the time kill assays conducted within 2 hrs at room temperature.[6]

**Time Kill (TK).** Standard microorganisms from ATCC were cultured to mid-log phase, resuspended in 5mM acetate saline pH 4 and added to test conditions at a final inoculum of $10^5$ – $10^6$ CFU/mL in a total volume of 1 mL. Aliquots were removed at 0, 1, 5, 15, 30, 60 and 90 min, neutralized in D/E (Dey Engley) broth, and drop plated for quantitation. Results of triplicate independent assays were analyzed and graphed using Prizm© plot.

**$TCID_{50}$ assay.** Dilutions of NVC-422 at 0.1, 0.01, and 0.001% were prepared in sterile 5mM acetate saline pH 4 in sterile tubes. Ad5 or HSV at ~ $10^7$ IU/mL was added with or without 10% synthetic tears to NVC-422 dilutions, and incubated at RT for 1 hr. Reactions were terminated by addition of 1 mL complete culture medium with 20% FCS. Each reaction was titrated by six serial 10-fold dilutions in culture medium with 10% FCS. Medium was removed from polystyrene TC plates seeded with A549 (for Ad5) or Vero cells (for HSV-1) overnight; 100 uL of each virus/NVC-422 dilution, along with diluent controls was added to each well with replicates of at least four per dilution. After 1 hr incubation at 37°C, plates were aspirated, and plates were incubated at 37° C 5% $CO_2$ for 7 to 9 days. Cultures were observed microscopically for cytopathic effects (CPE). At the end of the incubation period, titers were calculated using Kärber method. $TCID_{50}$ was determined from the dilution where 50% of cultures were positive for CPE. $Log_{10}$ reductions in viral titer were calculated for each NVC-422 formulation compared to diluent controls.

**Table 1:** Composition of Synthetic tears

| Component | Concentration |
|---|---|
| Lysozyme | 0.05% |
| IgG | 0.05% |
| Albumin, human | 0.05% |
| $CaCl_2$ | 0.03% |
| Sodium phosphate | 0.036% |
| Sodium citrate | 0.14% |
| Citric acid, monohydrate | 0.02% |
| Sodium chloride | 0.9% |

Adjusted to pH 7.4, 0.2 µM PES sterile filtered

**Table 2.** NVC-422 + tears is stable over the course of the 2 hr. assay

| Study Solution | Δt (hours) | % of Initial |
|---|---|---|
| 0.3% NVC-422 in 5mM acetate saline pH 4 | 2 | 100 |
| 0.3% NVC-422 in 5mM acetate saline pH 4 + 10% **synthetic** tears | 2 | 97.7 |

**Table 3.** Effect on pH of synthetic tears in various diluents

| Diluent | pH (measured) |
|---|---|
| 5mM acetate saline pH 4 | 4.0 |
| 5mM acetate saline pH 4 + 10% tears | 4.6 |
| 5mM acetate saline pH 4 + 50% tears | 6.0 |

**Figure 1:** Effect of synthetic tears on the time kill of 0.3% NVC-422 in 5mM acetate saline pH 4

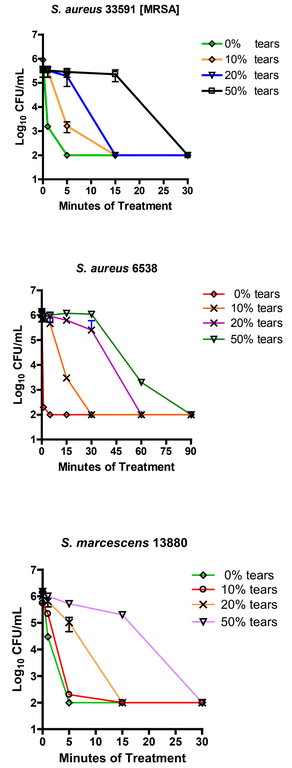



**Figure 2.** $TCID_{50}$ Ad5 Quantitation

- Infection at higher concentrations of virus cause cytopathic changes (CPE) to cells in morphology detectable by microscopy
- Uninfected cells maintain confluent monolayers
- Treatment with NVC-422 inactivates virus and abrogates cell infection

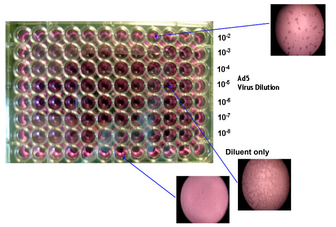
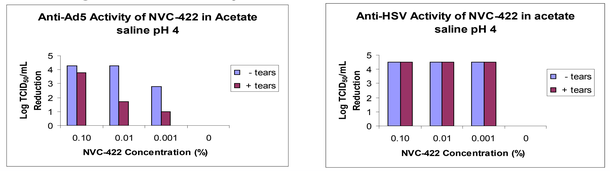



**Figure 3.** Antiviral testing: Reduction in Viral Titers Post-Treatment




## Conclusions

- NVC 422 retains its chemical stability in the presence of 10% synthetic tears for at least 2 hours
- NVC 422 retains bactericidal activity against Gram-positive bacteria including MRSA and Gram-negative bacteria in the presence of 10 - 50% synthetic tears
- NVC 422 retains virucidal activity against adenovirus and HSV in the presence of 10% synthetic tears

## References

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