**N,N-dichloro-2,2-dimethyltaurine, N-monochloro-2,2-dimethyltaurine, and N-chlorotaurine are Safe and Effective Bactericidal Agents in Corneal Models**


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**Abstract**

**Background:** N-chlorotaurine, NVC-422 (N,N-dichloro-2,2-dimethyltaurine), and NVC-612 (N-monochloro-2,2-dimethyltaurine) are potent anti-infective agents useful for the treatment of conjunctivitis and keratitis. The aim of this study is to show that these compounds are safe in in vitro and in vivo with P. aeruginosa and S. aureus.

**Methods:** Corneal discs were prepared and bacterial suspensions were added to investigate the bactericidal activity of the compounds. Different concentrations of the test compounds were applied to the cornea in phosphate buffer at pH 7.1. Discs were homogenized followed by quantitative bacterial cultures or subjected to histological analysis. Corneal integrity was tested using the EpiOcular™ tissue system (Mattek Corporation).

**Results:** In histological sections, bacteria attached to the surface and accumulations of bacteria in the upper third of the stroma could be seen. All test compounds: 1% (55 mM) NCT, 1.35% (55 mM) NVC-422, 1.15% (55 mM) NVC-612, 0.2% (11 mM) NCT+NH_4Cl (1% (55 mM) NCT+NH_4Cl reduced the bacterial count by approximately 5 log/log after 60 min (P: aeruginosa) and 120 min (S. aureus) incubation. Significant cell death occurred after only 5 min incubation and increased over time. While surface bacteria were killed by using 70% ethanol for 0.5 min giving a 0.5-1 log10 kill, additional treatment with the test compounds resulted in kill of the test organisms suggesting that the test compounds killed tissue resident bacteria. Using an EpiOcular™ tissue model, we further show that NCT, NVC-422 and NVC-612 do not or very low irritancy potential to corneal tissue in the in vivo experiments. Conclusions: The results demonstrate that NCT, NVC-422, and NVC-612 have the ability to kill P. aeruginosa and S. aureus, are non-irritating in cornea, and are potential therapeutic agents for the treatment of conjunctivitis and keratitis.

**Materials & Methods**

**Bacterial strains:** Pseudomonas aeruginosa ATCC 27833 and Staphylococcus aureus ATCC 25923 were grown in tryptic soy broth for 48 hours. Preparation of pig cornea discs: Eyes were enucleated from slaughtered Tyrolean farm pigs, the center of the corneal epithelium was carefully abraded with a hockey knife in some of the experiments, and a corneal disc around this artificial erosion was cut out with a 10 mm trephine and small scissors.

**Bacterial infection of pig corneal discs:** 0.1 mL of the bacterial suspensions each were added to 1 corneal disc in 1.5 mL medium consisting of MEM plus antibiotics, 2% FCS and 5% serum. Discs were incubated at 37°C for 24 h. They were then washed twice with 2 mL 450 μL. Washed discs were incubated at 37°C under continuous agitation at 100 rpm for different times in the test solutions containing 1% (55 mM) NCT, 1.35% (55 mM) NVC-422, 1.15% (55 mM) NVC-612 and 0.2% (11 mM) NCT+NH_4Cl (1% (55 mM) NCT+NH_4Cl). In special experiments, a preincubation in 70% ethanol was performed to kill bacteria attached to the surface of the discs.

**Evaluation of microbicidal activity:** Subsequently to incubation in the test solutions and washing in phosphate buffer, the discs were homogenized using a Waring Ultra-Torque homogenizer (Waring Co., Inc., Groton, CT, USA) on level 5. Quantitative cultures from the homogenate and appropriate dilutions were performed on Mueller-Hinton agar plates using an automated spiral platter (model WASP2, Don Whitley Scientific Limited, Shipley, UK). The detection limit was 10 cfu/mL.

**Table 1:** In the EpiOcular™ tissue irritancy model developed by Mattek, 1% NCT, 1.35% NVC-422 and 1.15% NVC-612 have minimal or no irritancy. Tolerability and efficacy of N-chlorotaurine in epidemic keratoconjunctivitis – a double-blind randomized phase 2 clinical trial. *Pharmacol Ther.* 2005; 21: 157-65. Pharmacol Ther. 2006; 47: 2021-6.

**Arrows indicate clusters of bacteria within the corneal stroma.**

**EpiOcular™ tissue irritancy assay:** EpiOcular tissues (Mattek Corp.) were placed in 900 µL cell culture media, and 100 µL solution for 3 hours. Tissues were extracted overnight and viability was determined by MTT absorbance. Tissue viability was correlated with a Draize-type score for tissue irritancy according to Mattek's instructions.

**Results**

In histological sections, bacteria attached to the surface and accumulations of bacteria in the upper third of the stroma could be seen. All test compounds: 1% NCT, 1.35% NVC-422, 1.15% NVC-612 and 0.2% NCT+NH_4Cl reduced the bacterial count by approximately 5 log10 after 60 min (P: aeruginosa) and 120 min (S. aureus) incubation. Significant cell death occurred after only 5 min incubation and increased over time. While surface bacteria were killed by using 70% ethanol for 0.5 min giving a 0.5-1 log10 kill, additional treatment with the test compounds resulted in kill of the test organisms suggesting that the test compounds killed tissue resident bacteria. Using an EpiOcular™ tissue model, we further show that NCT, NVC-422 and NVC-612 do not or very low irritancy potential to corneal tissue in the in vivo experiments. Conclusions: The results demonstrate that NCT, NVC-422, and NVC-612 have the ability to kill P. aeruginosa and S. aureus, are non-irritating in cornea, and are potential therapeutic agents for the treatment of conjunctivitis and keratitis.