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-dimethyltaurine) and NVC-612 (*N*-monochloro-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 an EpiOcular model and effective in corneas infected *ex vivo* with *P. aeruginosa* and *S. aureus*.
Methods: Corneal discs were punched with a Trepan from eyes from Tyrolean farm pigs and scarified to produce artificial erosions. Discs were incubated with bacteria and subsequently washed with saline. The different test compounds were applied to the corneas in phosphate buffer at pH 7.1. Discs were homogenized followed by quantitative bacterial cultures or subjected to histological preparation. Ocular irritancy 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+ 0.2% (37 mM) NH_4Cl, or 0.1% (5.5 mM) NCT+ 0.1%(18.5 mM) NH_4Cl reduced the bacterial counts by approximately 5 log10 after 60 min (*P. aeruginosa*) and 120 min (*S. aureus*) incubation. Significant cidal activity 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 to the limit of detection suggesting that the test compounds killed tissue resident bacteria. Using an EpiOcular™ tissue model developed for *in vitro* irritancy testing, we further show that NCT, NVC-422 and NVC-612 have no or very low irritancy potential to corneal tissue in this study.
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

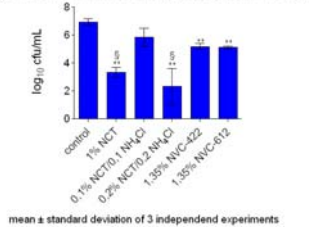
Bacterial strains: *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were grown in tryptic soy broth overnight to 1 - 5 x 10^9 log_{10} cfu / mL.
Preparation of pig cornea discs: Eyes were enucleated from slaughtered Tyrolean farm pigs, the center of the corneal epithelium was carefully abraded with a hockey knife in some of the experiments, and a corneal disc around this artificial erosion was cut out with a 10mm 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 glutamine, 2% FCS and 5% dextran. Corneas were incubated at 37°C for 24 h. They were then washed twice and stirred for 2 min at 400/rpm. Washed discs were incubated at 20°C under continuous agitation at 100/min 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+ 0.2% (37 mM) NH_4Cl, or 0.1% (5.5 mM) NCT+ 0.1%(18.5 mM) 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: Subsequent to incubation in the test solutions and washing in phosphate buffer, the discs were homogenized using an IKA Ultra Turrax Tube Drive® (IKA Works Inc., Staufen, Germany) on level 9. Quantitative cultures from the homogenate and appropriate dilutions were performed on Mueller-Hinton agar plates using an automated spiral platter (model WASP 2, Don Whitley Scientific Limited, Shipley, UK). The detection limit was 10 cfu/mL.
EpiOcular™ tissue irritancy assay: EpiOcular tissues (Mattek Corp.) were placed in 900 µL cell culture media, and 100 µL compound was added to the apical side of the tissue for varying exposure times. Tissues were rinsed with PBS and placed in an MTT 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+ 0.2% NH_4Cl, or 0.1% NCT+ 0.1% NH_4Cl reduced the bacterial counts by approximately 5 log_{10} after 60 min (*P. aeruginosa*) and 120 min (*S. aureus*) incubation (**Figure 1-6**). Significant cidal activity occurred after only 5 min incubation and increased over time. While surface bacteria were inactivated by using 70% ethanol for 0.5 min giving a 0.5-1 log_{10} reduction, additional treatment with the test compounds resulted in a kill to the limit of detection suggesting that the test compounds killed tissue resident bacteria (**Figure 4**). Using an EpiOcular™ tissue model developed for *in vitro* irritancy testing (Draize score), we further show that NCT, NVC-422 and NVC-612 have no or very low irritancy potential to corneal tissue in this study (**Table 1**).

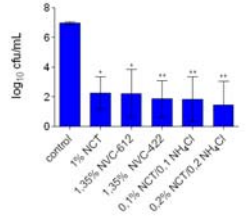
Results

Fig 1. *N*-chloro amino acids (2 mL) against *P. aeruginosa*



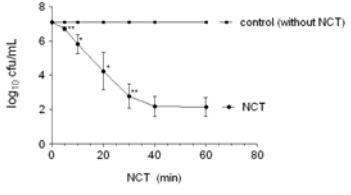
mean ± standard deviation of 3 independend experiments
** P < 0.01 versus control
§ P < 0.05 versus NVC-422, NVC-612, 0.1%NCT + 0.1% NH_4Cl
± artificial erosion, 2 mL test solution per cornea,
60 min incubation time in *N*-chloro amino acids at RT and pH 7

Fig 3. *N*-chloro amino acids (20 mL) against *P. aeruginosa*



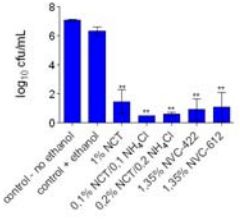
mean ± standard deviation of 3 independend experiments
* P < 0.05 versus control
** P < 0.01 versus control
± artificial erosion, 20 mL test solution per cornea,
60 min incubation time in *N*-chloro amino acids at RT and pH 7

Fig 2. Killing kinetics of 1% NCT against *P. aeruginosa*



mean ± standard deviation of 3 independend experiments
* P < 0.05 versus control
** P < 0.01 versus control
Artificial erosion, 2mL test solution per cornea,
5, 10, 20, 30, 40 and 60 min incubation time at RT and pH 7

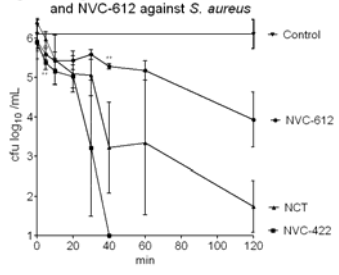
Fig 4. 70% ethanol followed by *N*-chloro amino acids against *P. aeruginosa*



mean ± standard deviation of 3 independend experiments
** P < 0.01 versus control
Artificial erosion, 30 sec incubation in 10 mL of 70% ethanol,
2 washing steps, followed by 60 min incubation time in
N-chloro amino acids at RT and pH 7

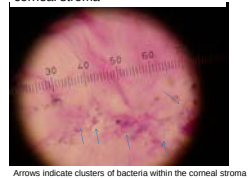
Results

Fig. 5. Killing kinetics of 55 mM NCT, NVC-422, and NVC-612 against *S. aureus*



Mean values ± SD, n = 6-8
** Threshold to P < 0.01 between control (phosphate buffer) and NCT and NVC-422 (5 min each) and NVC-612 (40 min).
Artificial erosion, 2mL test solution per cornea,
5, 10, 20, 30, 40, 60, and 120 min incubation time at RT and pH 7

Fig 6. *Pseudomonas aeruginosa* in corneal stroma



Arrows indicate clusters of bacteria within the corneal stroma

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

Compound	ET-50* [min]	Irritancy Classification
1% (55 mM) NCT	106	non/minimal
1.35% (55 mM) NVC-422	78	non/minimal
1.15% (55 mM) NVC-612	125	non/minimal
0.3% Triton X-100	20	mild
20 mM PBS pH7	> 270	non/minimal

* ET-50 is Exposure Time required to reduce tissue viability to 50%

Conclusion

- **1% (55 mM) NCT, 1.35% (55 mM) NVC-422 and 1.15% (55 mM) NVC-612:** Effective in killing *P. aeruginosa* and *S. aureus* on the pig corneal surface and interstitially (Fig.1-5).
- **Ammonium chloride:** Enhances the antibacterial activity by formation of monochloramine (Fig. 1, 3, 4).
- **1% (55 mM) NCT, 1.35% (55 mM) NVC-422 and 1.15% (55 mM) NVC-612:** Non-irritant in our ocular tissue model. (Table 1).

References

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