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Tolerance of N-Chlorotaurine, a New Antimicrobial Agent, in Infectious Conjunctivitis – A Phase II Pilot Study

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Key Words

N-chlorotaurine · Antimicrobial agent · Infectious conjunctivitis · Tolerability

Abstract

N-Chlorotaurine (NCT) is an endogenous microbicidal oxidant. This open pilot study (phase IIa) with 9 patients was done to gain first knowledge on the tolerance of NCT in infectious conjunctivitis. By application of 1% NCT 5 times a day, no adverse effects could be observed. All 6 subjects with bacterial conjunctivitis were cured within 3–5 days. Two subjects with epidemic keratoconjunctivitis were treated for 7–10 days and 1 subject with herpes simplex blepharitis for 3 days with no rapid improvement but probable mitigation of inflammation. Therefore, NCT seems to be useful in the treatment of infectious conjunctivitis, and further investigation on its therapeutic efficacy is suggested.

This study has been presented in part at the 26th Conference of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP) on May 26, 1998, in Millstatt, Austria.

Introduction

N-Chlorotaurine (Cl-NH-CH_2-CH_2-SO_3H, NCT), the N-chloro derivative of the amino acid taurine, is a weak oxidant produced in high amounts (20–50 μM) by stimulated human granulocytes and monocytes [1–4]. It is thought to be involved in destruction of pathogens during inflammation [5]. Recently, its microbicidal activity against bacteria *(Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa)*, yeasts *(Candida albicans)*, viruses (herpesvirus type 1 and 2, adenovirus type 5) [6–8] and worms *(Schistosoma mansoni)* [9] has been confirmed.

On the other hand, cytotoxicity of NCT against leukocytes and erythrocytes as well as endothelial cells proved to be low compared to the more powerful oxidant hypochlorite [1, 10, 11]. Moreover, NCT may minimize damage to cartilaginous joint structure in inflammatory arthritis [12].

Because of its microbicidal activity and low cytotoxicity, NCT was conceived to be useful as an antimicrobial agent in human medicine. Therefore, a randomized, double-blind and placebo-controlled clinical phase I study has been performed recently, which demonstrated a good tolerance of 1% NCT solution by application to the healthy rabbit and human eye [13].

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Based upon these results, the aim of the present phase II pilot study was a first evaluation of the tolerability of NCT in infectious conjunctivitis.

Materials and Methods

Materials

NCT was prepared as the crystalline sodium salt (MW = 181.52 g/mol) [6], and its purity was verified by iodometric titration (calculated 19.53% Cl^+, found 19.3% Cl^+, which equals 99% purity). A solution of 1.0% NCT (55 m*M*) in sterile double-distilled water was dispensed into 8-ml pipette tubes. Since aqueous solutions of NCT exhibit a pH ≈ 8 and, moreover, a broad spectrum of activity against pathogens [6–9], no preservatives and buffers, respectively, were felt to be necessary. Stock solutions stored at 2–4 °C show high stability (9.3% loss per year [7]).

Study Design

The study was designed as an open, one-armed and descriptive pilot study (clinical phase IIa) to examine the tolerability of NCT in infectious conjunctivitis. The Ethics Committee of the University of Innsbruck approved the study, which was performed in accordance with the Declaration of Helsinki. All patients gave written informed consent. Ten patients participated in the study (7 male, 3 female, ranging in age from 13 to 76 years, mean 42 years, standard deviation 24.1).

Inclusion criteria were acute bacterial or viral conjunctivitis. Exclusion criteria were conjunctivitis by another origin than mentioned above, pretreated conjunctivitis, pregnancy, participation in another study at the same time, ophthalmologic medication and medication with possible adverse effects to the eye.

Medical status was determined by evaluation of the medical history and medication and by detailed ophthalmological examination. All parts of both eyes were examined by usage of a slitlamp and indirect ophthalmoscope, and the following *objective* signs of illness were evaluated: exudation, conjunctival injection, caruncular injection, chemosis, palpebral edema, corneal stippling and infiltration of the corneal stroma. *Subjective* signs evaluated were eye burning, eye itching, lacrimation and photophobia.

Diagnosis was made clinically, and bacterial conjunctivitis was confirmed by cultural and biochemical characterization of pathogens gained from a conjunctival swab (see below).

Treatment

Subsequent to examination and inclusion of the patient, a swab from the conjunctiva of both eyes was taken for bacterial culture. The infected eye or both eyes in the case of bilateral infection were treated for 1 h daily with aqueous 1% NCT solution, 1 drop as a single dose. Five serial single doses at an interval of 15 min were applied at the same time each day under ambulatory observance of the patient. This mode of treatment was chosen to warrant continuous oxidative efficacy for more than 1 h according to the 15-min detectability of oxidative capacity subsequent to 1 drop of 1% NCT and was expected to be suitable for sufficient microbicidal action [13].

Patients were examined as described above just before treatment each day. On the third day of therapy, a second swab from the conjunctiva was taken *before* application of the eye drops. Duration of treatment was planned for at least 3 days, and for a longer time dependent on clinical course and tolerability. Follow-up examinations were done 4 weeks after the last day of treatment in patients with viral conjunctivitis to detect possible long-term adverse effects of NCT.

Evaluation and Statistical Analysis

Statistical analysis was limited to descriptive analysis due to the pilot character of the study. Subjective symptoms and objective signs were scaled 'absent, mild, moderate and severe' and rated 0, 1, 2 and 3 points, respectively. Subjective and objective scores of inflammation were calculated by addition of these points daily in each patient to elucidate the time course of conjunctivitis. Special attention was paid to sensations not corresponding to conjunctivitis but to NCT, such as rapid worsening of eye burning and of conjunctival injection following drug application. Absolute frequencies of such adverse effects of NCT were calculated, and their severity was determined as above.

Results

Diagnoses and duration of treatment in each patient are shown in table 1. One patient failed to appear again after the first day of treatment, so that 9 patients (6 male, 3 female) finished the study and could be evaluated.

Adverse Effects of NCT

Patient No. 3 noted a minimal increase in eye burning for 1 min subsequent to application of NCT on the second day of treatment. Besides that, no subjective or objective findings could be connected with NCT. No long-term adverse effects appeared during the follow-up.

Course of Conjunctivitis

Subjective and objective scores of inflammation and their time course in each patient are summarized in table 2. There were significant differences between bacterial and viral conjunctivitis.

Bacterial infections were improved markedly after 1 to 2 days of treatment with NCT and cured to restitutio ad integrum between 3 and 5 days. Cultures from ocular swabs were sterile on the third day in all cases.

Two patients with adenoviral infections were treated for 7 and 10 days, respectively, and suffered from conjunctivitis for more than 10 days. Nevertheless, symptoms graded 'severe' were all mitigated after 3–5 days of treatment. In patient No. 9, therapy was changed from NCT to acyclovir after 3 days because of the occurrence of herpetic vesicles at the inferior eyelid and failing of a rapid curative effect of NCT.

Based upon these results, the aim of the present phase II pilot study was a first evaluation of the tolerability of NCT in infectious conjunctivitis.

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Discussion

Usage of disinfectants in the treatment of conjunctivitis is limited because of irritative effects so that highly reactive chloro compounds like hypochlorite and chloramine T are not applied. The weak oxidant NCT, however, may be considered in a different way.

A recent phase I study on the tolerability of 1% NCT solution in the healthy rabbit and human conjunctiva [13] found no side effects except minimal eye burning and eye itching immediately after application. In consideration of the present study, no difference in tolerability of NCT between healthy and inflamed tissue can be found. Especially, a significant increase in eye burning, which could have been assumed by application of NCT to the infected conjunctiva, was not noted. Adverse effects are obviously of such small extent that they are not added but superposed and masked by symptoms of infectious origin. Extended treatment for 10 days seems not to change tolerability and not to induce long-term adverse effects.

Concerning a curative effect, no definite statements are allowed because of the pilot character of the study. However, all symptoms of bacterial conjunctivitis were improved rapidly which makes a significant therapeutic effect very probable. In viral conjunctivitis, no rapid healing, but a mitigation of symptoms seems possible.

Advantages of NCT in the treatment of infectious conjunctivitis may be: NCT is a weak endogenous oxidative amino acid; as no additives are necessary for storage and application, occurrence of allergic reactions is improbable; the unspecific mode of action (oxidation of SH and NH groups) provides broad-spectrum activity against pathogens with low probability of occurrence of resistance [7]. Finding the range of its therapeutic efficacy in bacterial and viral conjunctivitis will be the task of further studies.

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References

1 Grisham MB, Jefferson MM, Melton DF, Thomas EL: Chlorination of endogenous amines by isolated neutrophils. J Biol Chem 1984;259: 10404–10413.

2 Lampert MB, Weiss SJ: The chlorinating potential of the human monocyte. Blood 1983;62: 645–651.

3 Test ST, Lampert MB, Ossanna PJ, Thoene JG, Weiss SJ: Generation of nitrogen-chlorine oxidants by human phagocytes. J Clin Invest 1984;74:1341–1349.

4 Weiss SJ, Lampert MB, Test ST: Long-lived oxidants generated by human neutrophils: Characterization and bioactivity. Science 1983;222:625–628.

5 Thomas EL: Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: Effect of exogenous amines on the antibacterial action against *Escherichia coli*. Infect Immun 1979; 25:110–116.

6 Nagl M, Gottardi W: In vitro experiments on the bactericidal action of N-chlorotaurine. Hyg Med 1992;17:431–439.

7 Nagl M, Gottardi W: Enhancement of the bactericidal efficacy of N-chlorotaurine by inflammation samples and selected N-H compounds. Hyg Med 1996;21:597–605.

8 Nagl M, Larcher C, Gottardi W: Activity of N-chlorotaurine against herpes simplex and adenoviruses. Antiviral Res 1998;38:25–30.

9 Yazdanbakhsh M, Eckmann CM, Roos D: Killing of schistosomula by taurine chloramine and taurine bromamine. Am J Trop Med Hyg 1987;37:106–110.

10 Park E, Schuller Levis G, Quinn MR: Taurine chloramine inhibits production of nitric oxide and TNF-alpha in activated RAW 264.7 cells by mechanisms that involve transcriptional and translational events. J Immunol 1995;154: 4778–4784.

11 Tatsumi T, Fliss H: Hypochlorous acid and chloramines increase endothelial permeability: Possible involvement of cellular zinc. Am J Physiol 1994;267:H1597–H1607.

12 Davies JM, Horwitz DA, Davies KJ: Inhibition of collagenase activity by N-chlorotaurine, a product of activated neutrophils. Arthritis Rheum 1994;37:424–427.

13 Nagl M, Miller B, Daxecker F, Ulmer H, Gottardi W: Tolerance of N-chlorotaurine, an endogenous antimicrobial agent, in the rabbit and human eye – A phase I clinical study. J Ocul Pharmacol Ther 1998;14:283–290.