JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 21, Number 2, 2005

Tolerability and Efficacy of N-Chlorotaurine in Epidemic Keratoconjunctivitis—a Double-Blinded Randomized, Phase-2 Clinical Trial

BARBARA TEUCHNER,[1]* MARKUS NAGL,[2]* AXEL SCHIDLBAUER,[1] HIROAKI ISHIKO,[3] ERNST DRAGOSITS,[1] HANNO ULMER,[4] KOKI AOKI,[5] SHIGEAKI OHNO,[6] NOBUHISA MIZUKI,[5] WALDEMAR GOTTARDI,[2] and CLARA LARCHER[2]

ABSTRACT

The aim of this study was to assess the tolerability and efficacy of N-chlorotaurine (NCT), an endogenous antimicrobial agent, in epidemic keratoconjunctivitis. In a prospective double-blind, randomized phase 2b study, the infected eyes were treated for 7 days with eye drops containing 1% aqueous solution of N-chlorotaurine (33 subjects) or gentamicin (27 subjects, control group). Adenovirus types 3, 4, 8, 19, and 37 were detected in 39 subjects (65%), enteroviruses in 8 (13.3%), and staphylococci in 5 (8.3%). Subjective and objective symptoms were scaled and added to a subjective and objective score, respectively, on day 1 (baseline), day 4, and day 8. Analyzing the whole study population, the subjective score on day 8 was lower in the NCT group ($P = 0.016$), whereas there were no differences in the objective score. However, in severe infections caused by adenovirus type 8 ($n = 20$) both the subjective and objective score were lower in the NCT group on day 4 ($P = 0.003$ and 0.015, respectively), which was also true for the subjective score on day 8 ($P = 0.004$) in this subgroup. The frequency of subepithelial infiltrates was similar in both groups. N-chlorotaurine was well-tolerated, shortened the duration of illness, and seems to be a useful causative therapeutic approach in severe epidemic keratoconjunctivitis.

INTRODUCTION

Epidemic keratoconjunctivitis is the most common viral infection of the human eye in many parts of the world. It is caused by adenoviruses, mainly types 3, 8, 19, 37, but it has also been reported with multiple serotypes, including types 2–5, 7–11, 14, 16, and 29.[1,2] Viruses survive on surfaces for weeks, and infectivity is extremely high, resulting in epidemic appearance—particularly in autumn, winter, and spring.[3] Clinically, the disease usually attacks young adults and is bilat-

[1]Department of Ophthalmology, Innsbruck Medical University, Innsbruck, Austria.
[2]Department of Hygiene, Microbiology and Social Medicine, Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria.
[3]Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo, Japan.
[4]Department of Medical Statistics, Informatics, and Health Economics, Innsbruck Medical University, Innsbruck, Austria.
[5]Department of Ophthalmology, Yokohama City University School of Medicine,Yokohama, Japan.
[6]Department of Ophthalmology and Visual Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan.
*These authors contributed equally to this work.

eral in one third of patients. The course of disease is, in general, severe, and subepithelial infiltrates which impair the visual acuity occur in 20%–50% of patients.

Because of the viral origin, only very few specific therapeutic approaches exist. Antiviral drugs, such as trifluorothymidine, have not been very successful[4,5] or have considerable side-effects (cidofovir,[6,7]). In addition, rapid viral diagnosis is not available in most cases, limiting the administration of antiviral therapy. Corticoids may inhibit subepithelial infiltrates but they prolong viral shedding and do not shorten the duration of illness.[4,6] So far, the best results have been found using the antiseptic povidone-iodine.[5,8] Because of low efficacy or the side-effects of conventional drugs, new causative therapeutic approaches are needed.

N-chlorotaurine (NCT) (Cl-HN-CH_2-CH_2-SO_3), a mild active chlorine compound produced by granulocytes and monocytes during the oxidative burst,[9] might be of interest for treatment of infectious conjunctivitis. Because of to its unspecific reaction mechanism (i.e., oxidation of amino groups, thio and aromatic compounds[10]) it has broad-spectrum microbicidal activity similar to antiseptics. The sodium salt solution of N-chlorotaurine (Cl-HN-CH_2-CH_2-SO_3Na, NCT) has been shown to kill *in vitro* bacteria and fungi.[11–13] In addition, a virucidal effect has been demonstrated against adenovirus types 1, 2, 3, 4, 5, 7, 8, 19, and 37, as well as *Herpes simplex* virus 1 and 2.[14,15]

As a mild, long-lived oxidant, its toxicity against human cells is very low, compared to powerful oxidants like hypochlorite.[9] Koyama et al. demonstrated in a rabbit model that taurine was effective to prevent tissue damage by hypochlorite in the eye, explained by reaction of hypochlorite with taurine to NCT.[16]

These findings encouraged us to test the tolerability of NCT *in vivo*. A 1% aqueous solution of NCT applied as eye drops up to 10 times per day for 5 days proved to be very well tolerated in the healthy rabbit and human eye.[17] The only side-effects were a temporary mild burning and itching after dosing in some subjects, which never required a discontinuation of dosing. The same was true for a pilot study in conjunctivitis, where bacterial infections were improved rapidly and cured between 3 and 5 days, while the course of illness was longer in viral infections.[18]

Based on the *in vitro* virucidal activity and on the good tolerability in the human eye, we designed this study to investigate the tolerability and efficacy of NCT in epidemic keratoconjunctivitis.

METHODS AND PATIENTS

Reagents

Pure NCT as a crystalline sodium salt (molecular weight 181.57*g* mol^{-1}, Gatt-Koller Inc.; Absam, Austria) was dissolved in sterile and pyrogen-free distilled water to a concentration of 1% (55 mM NCT). Purity of NCT was verified by iodometric titration and spectrophotometry.[10] Solutions were stored at 2–4°C, where they show high stability (9.3% loss per year).

Gentax® eye drops (containing 3 mg/mL gentamicin as sulfate and 0.1 mg/mL benzalkonium chloride) were provided by Agepha GesmbH (Vienna, Austria). NCT was filled in similar flasks than Gentax to warrant double-blinding, and all eye drops were numbered consecutively in accordance with the randomization code.

PCR protocols

Cotton swabs taken from the inflamed lower palpebral conjunctiva were stirred manually in Eppendorf eprouvettes containing 0.5 mL of RPMI 1640 medium plus 0.2 M sucrose. Samples were stored at −70°C. All reagents were from Amersham Pharmacia Biotech (Vienna, Austria), except for primers.

Adenovirus PCR

The protocol was set up with reference to that of Kinchington et al.[2] After DNA lysis (10 minutes, 98°C), (PCR) was performed with primers T1 (GCC GCA GTG GTC TTA CAT GCA CATC, position 18858 to 18882) and T2 (CAG CAC GCC GCG GAT GTC AAA GT, position 19136 to 19158) from TIB Molbiol Synthesis Laboratory (Berlin, Germany). PCR was started with 94°C for 5 minutes, followed by 40 cycles consisting of 94°C for 1 minute, 50°C for 1.5 minutes, 72°C for 1 minute, and terminated by 72°C for 10 minutes. The size of the PCR product was 300 bp. Nested PCR was performed using primers T3 (GCC ACC GAG ACG TAC TTC AGC CTG, position 18936 to 18960) and T4 (TTG TAC GAG TAC GCG GTA TCC TCG CGG TC, position 19051 to 19079) also from TIB Molbiol. Amplification was started at

AU1

94°C for 5 minutes, followed by 10 cycles consisting of 94°C for 1 minute, 70°C minus 1°C per cycle for 1 minute, and 72°C for 1 minute, and by 25 cycles consisting of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, and terminated by 72°C for 10 minutes. The size of the product was 143 bp. Positive controls of adenovirus type 5 (ATCC VR-5) and type 8 (ATCC VR-1368), 10^6 plaque-forming units/mL each, and 2 negative controls consisting of master mix and water, respectively, without additives were performed in parallel.

Enterovirus PCR

The protocol was set up with reference to that of Rotbart et al.[19] RNA was extracted using QIAamp® Viral RNA Mini Kit, Qiagen Ltd. (Hilden, Germany). PCR was performed with primers Entero 1 (CCC CTG AAT GCG GCT AAT CC) and Entero 2 (CAA TTG TCA CCA TAA GCA GCC A) from MWG Biotech (Ebersberg, Germany). PCR was started with 42°C for 20 minutes (reverse transcription), followed by 40 cycles consisting of 94°C for 20 seconds, 58°C for 20 seconds, 72°C for 20 seconds, and terminated by 72°C for 10 minutes. The size of the PCR product was 149 bp. Nested PCR was done with primers Entero 3 (TGA ATG CGG CTA ATC C(CT)A AC) and Entero 4 (TGA AAC ACG GAC ACC CAA AGT). PCR was started with 95°C for 5 minutes, followed by 40 cycles consisting of 94°C for 15 sec, 58°C for 15 sec, 72°C for 15 seconds, and terminated by 72°C for 10 minutes. The size of the PCR product was 113 bp. Two positive controls of coxsackie virus B 3 (strain Nancy, ATCC VR-30, 5×10^4 and 5×10^2 plaque-forming units/mL, respectively) and two negative controls consisting of master mix without additives and pooled human serum negative for enterovirus, respectively, were performed in parallel.

AU2

PCR-RFLP analysis for serotyping of adenovirus

A partial hexon sequence was amplified, as described previously (20). Viral DNA was extracted using a Sumitest EX-R&D kit (Genome Science Laboratories Co., Ltd.; Fukushima, Japan). The 1004 bp of the hexon gene was amplified with primers AdTU7 (20,734-20,753 5′-GCCACCTTCTTCCCCATGGC-3′) and AdTU4' (21,718-21,737 5′-GTAGCGTTGCCGGCCGAGAA-3′). The positions of the primers for PCR were numbered according to the complete nucleotide sequence of the AdV-2 strain (GenBank Accession No. J01917). Nested PCR was performed to amplify the 956-bp DNA fragment with primers AdnU-S′ (20,743-20,762 5′-TTCCCCATGGCNCACAACAC-3′) and AdnU-A (21,679-21,698 5′-GCCTCGATGACGCCGCGGTG-3′). PCR was carried out for 36 cycles in a Cetus 9600 thermal cycler (PE-Applied Biosystems; Foster City, CA). Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and primer extension at 72°C for 2 minutes. Purified DNA was digested with restriction endonucleases (REs) *Eco*T14 I, *Hae* III, and *Hinf* I (Takara Shuzo Co., Ltd.; Shiga, Japan), and the fragments were separated by electrophoresis. The serotypes of AdVs were determined by comparison of the restriction patterns to those of the prototypes.

Study design

A double-blind, prospective, randomized phase IIb study was performed in 60 outpatients suffering from epidemic keratoconjunctivitis. Thirty-three patients were treated with NCT eye drops (test group) and 27 patients with gentamicin (Gentax) eye drops. The antibiotic as a control was required by the Ethics Committee of the University of Innsbruck (Innsbruck, Austria) to provide sufficient treatment in case of bacterial infections that cannot be distinguished clinically from viral infections in all cases. Patients were numbered consecutively and assigned to a group in accordance with the randomization code. The study was in accordance with the Declaration of Helsinki and it was approved by the Ethics Committee of the University of Innsbruck. All patients gave written, informed consent.

Patients

The characteristics of the study population are summarized in Table 1. The inclusion criterium was clinically diagnosed viral conjunctivitis. Exclusion criteria were known allergy against gentamicin or benzalkonium chloride, herpetic keratitis, medication with side-effects on the eye, contact lenses, other ophthalmologic therapy, pregnancy, and participation in another study at the same time.

T1

No patients had to be withdrawn after randomization. In 7 patients, the application of the eye drops was finished early after 4 days because of the disappearance of infection (3 patients of the

TABLE 1. STUDY POPULATION AND CAUSATIVE PATHOGENS

	NCT	*Gentax®*	*Total*
Patient profile			
age[a]	33 (10–76)	39 (16–77)	34.5 (10–77)
gender	17 female	15 female	32 female
	16 male	12 male	28 male
duration of study therapy (days)[a]	8 (4–8)	7/5 (4–8)	8 (4–8)
previous period of illness (days)[a]	1.5 (1–10)	3 (1–14)	2 (1–14)
Pathogens in the whole study population			
adenovirus	22	17	39 (65.0%)
enterovirus	5	3	8 (13.3%)
Staphylococcus aureus	3	2	5 (8.3%)
no pathogen detected	4	6	10 (16.7%)
adenovirus subtypes			
type 3	1	1	2
type 3v	1	3	4
type 4v	2	1	3
type 8	12	8	20
type 19a	1	3	4
type 37	2	1	3
undefined	3	0	3
Number of double-sided infections	12	157	27
Pathogens in double-sided infections			
adenovirus	10	12	22
adenovirus type 8	5	6	11
enterovirus	1	2	3
no pathogen detected	1	1	2

[a]Values are shown as median (minimum–maximum); $P > 0.05$ between both test groups for all parameters. NCT, N-chlorotaurine.

NCT group, 2 patients of the gentamicin group), eye burning (1 patient of the NCT group, see results), worsening of symptoms (1 patient of the gentamicin group). Three patients (2 patients of the NCT group, 1 patient of the gentamicin group) failed to appear after the first day. Values gained from all of these patients were included in the results (intention to treat).

Clinical evaluation

Medical status was determined by evaluation of the medical history and medication and by detailed ophthalmological examination using a slit-lamp and indirect ophthalmoscope. Three investigators (B.T., A.S., E.D.) performed the clinical examinations. The baseline investigation was performed on day 1 before the beginning of treatment. The following *objective* signs of keratoconjunctivitis were evaluated: palpebral edema, conjunctival hyperemia, petechial hemorrhages, exudation, chemosis, pseudomembranes, and corneal stippling. *Subjective* signs evaluated were pain, tearing, itching, foreign body sensation, and photophobia. Objective signs and subjective symptoms were scaled "absent, mild, moderate, and severe" and rated 0, 1, 2, and 3 points, respectively, similar as reported previously.[21] Particularly to evaluate the tolerability of NCT, the patients were asked for worsening of subjective symptoms immediately after dosing, and special attention was paid to possible worsening of objective symptoms or adverse effects to any visible part of the eye. Objective and subjective scores of inflammation were calculated by the addition of the mentioned single points of symptoms on days 1, 4, and 8 as the primary criteria of evaluation. Occurrence of subepithelial infiltrates was noted as a secondary criterium.

Way of treatment and time course

After the baseline examination on day 1, patients were instructed in the application of the drops, equipped with a scheme of dosing, and given the eye drops. The infected eyes (1 or both eyes) were treated for maximally 7 days with 1 drop of NCT or Gentax, hourly during the 1st day, and every 2 hours on the following 6 days. Dosing was terminated prematurely in case of earlier healing. Clinical controls with slit-lamp examination and performance of swabs were

done on day 4 (range, 3–5), day 8 (range, 7–9), and day 14. The onset of treatment was between 1 and 7 days after the beginning of the symptoms.

Statistical analysis

Baseline characteristics were compared between the treatment groups using a chi-square, *t* test or Fisher's exact test for categorical variables and a *t* test or Mann-Whitney test for continuous variables, as appropriate. Repeated objective and subjective scores were analyzed using a three-factorial analysis of variance (ANOVA) with the factors group, virus, and day of measurement. To ensure normal distribution for the score variables, a logarithmic transformation was applied. *P* values were calculated for the main-effects group, virus, and days, as well as for interaction effects, such as group by virus. *P* values smaller than 0.05 were considered to indicate statistical significance. Because of the early stage of treatment testing, no corrections for multiple comparisons were performed.

RESULTS

Causative pathogens and general course of inflammation

The study population of both groups was comparable (Table 1). As evaluated by virus PCR and bacterial cultures, respectively, approximately two thirds of the infections were caused by adenoviruses (65%), 13.3% by enteroviruses, 8.3% by *Staphylococcus aureus*, and in 16.7% no pathogen could be detected. Both adenovirus and *S. aureus* were found in 2 patients. RFLP-PCR analysis revealed that adenovirus type 8 was the prevailing type, followed by types 3, 19, 4, and 37 (Table 1).

Symptoms scaled by the subjective score and clinical signs of inflammation scaled by the objective score improved significantly ($P < 0.01$) within 8 days in both groups. Adenovirus type 8 caused a very severe inflammation for more than 8 days. All other types of adenoviruses and all other pathogens caused relatively mild infections which were largely cured within 8 days.

Tolerability of NCT

The tolerability of both NCT and gentamicin was very good. In the NCT group, 8 of 33 patients (24.2%) noted mild eye burning for a few minutes after dosing. When the symptoms of illness had markedly improved after 4 days, burning by NCT became moderate in 2 of these patients, and in 1 patient (3%) therapy was terminated previously because of this reason after consulting the study physician.

Efficacy of NCT

Summing up all eyes, on day 4 there was no difference in both scores. On day 8, the subjective score was significantly lower in the NCT group

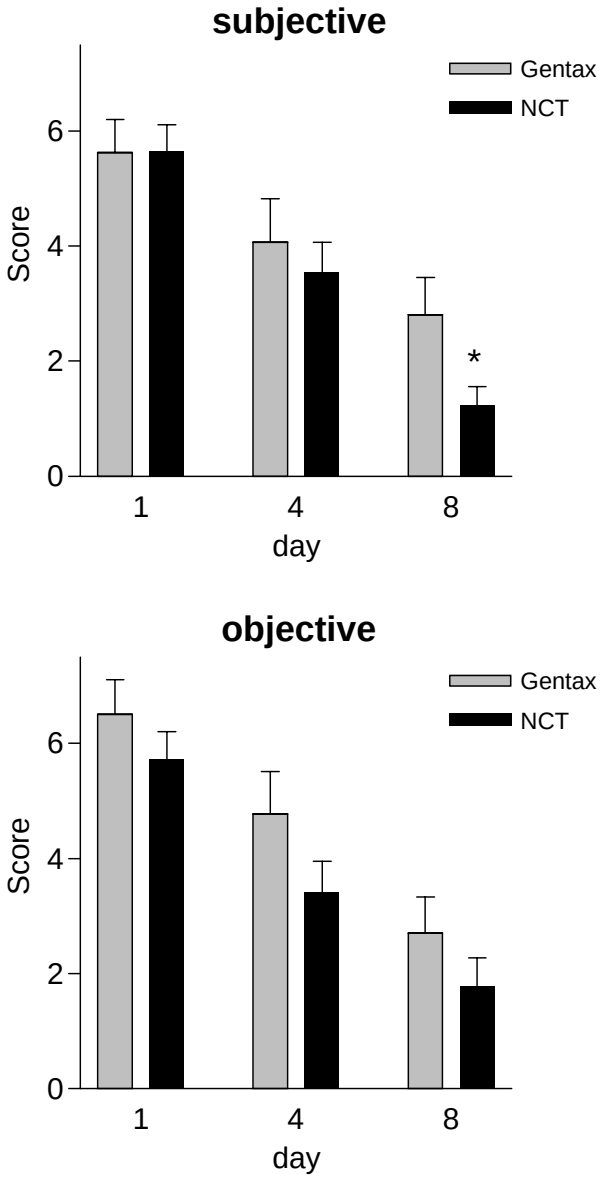

FIG. 1. Subjective and objective ocular inflammation scores of all patients. Scores are the sum of scaled single symptoms. Day 1 represents the baseline before beginning treatment. Mean values ± standard error of the mean. *$P = 0.016$ compared to the control group. $P > 0.05$ for all other compared values.

than in the control group ($P = 0.016$) (Fig. 1), while still no significant difference in the objective score occurred. In subgroups of patients suffering from enteroviruses, staphylococci, and unknown pathogens, respectively, both scores on all days were similar in the test and control subjects. In infections caused by adenoviruses other than type 8, only the subjective score on day 8 was lower in the NCT group ($P = 0.031$).

In severe infections caused by adenovirus type 8, however, the subjective symptoms of keratoconjunctivitis became, in general, much better in the NCT group with $P = 0.003$. Also, the clinical examination showed a trend to less inflammation ($P = 0.055$) with NCT. The mean values of both scores on day 1 (baseline before the beginning of treatment) were, by chance, lower in the NCT patients, but this was not significant (Fig. 2). On day 4, the symptoms rather decreased in the NCT group, while they did not weaken in the control patients (Fig. 2). Therefore, the difference between NCT and gentamicin became significant for both scores on day 4 and for the subjective score also on day 8 (Fig. 2).

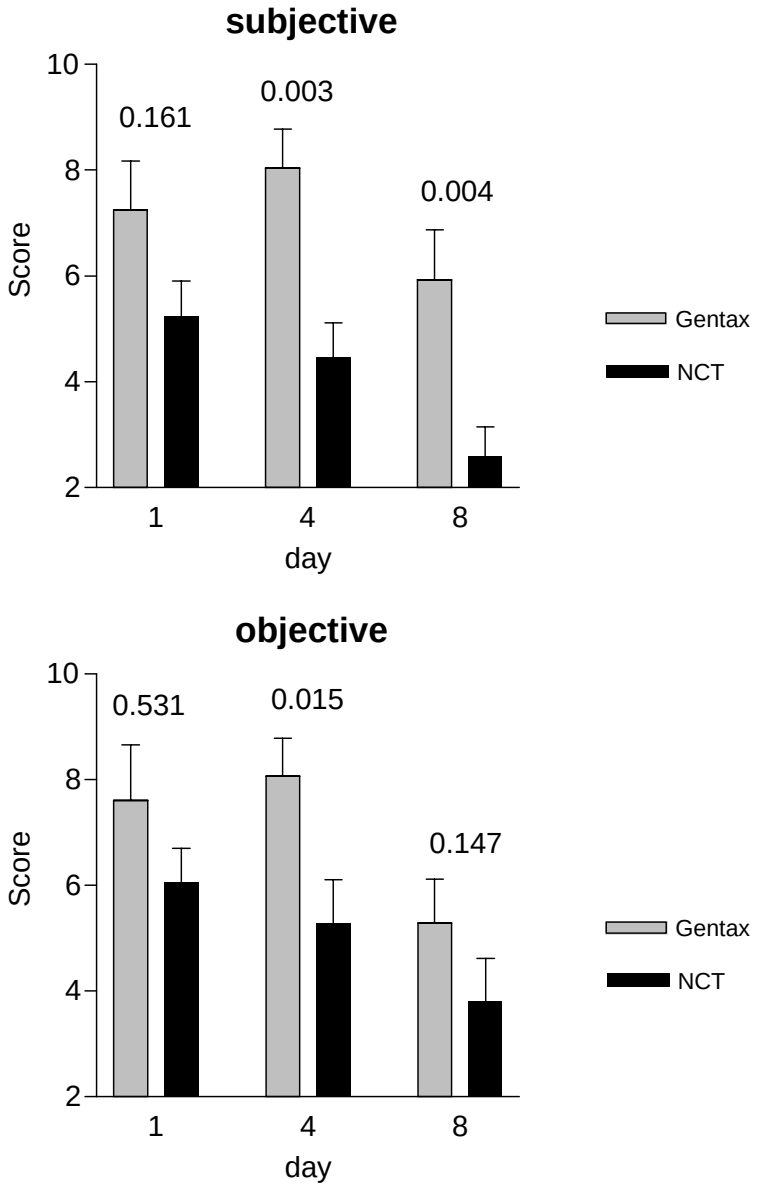

FIG. 2. Subjective and objective scores of inflammation of all eyes of patients suffering from infections caused by adenovirus type 8. Scores are the sum of scaled single symptoms. Day 1 represents the baseline before beginning of treatment. Mean values ± standard error of the mean. The P value for the difference between N-chlorotaurine (NCT) and Gentax® is indicated above each bar pair.

Table 2. Occurrence of Subepithelial Infiltrates (Nummuli)

	NCT	*Gentax®*	*Sum*
all	14	20	24
adenoviral	12	9	21
AV-8	10	5	15
enteroviral	1	0	1
unknown	1	1	2
S. aureus	0	0	0

NCT, N-chlorotaurine.

Bilateral infections were observed mostly in adenoviral inflammations (Table 1). Four patients per group developed adenoviral inflammation of the 2nd eye during the first 3 to 4 days of treatment, and they were treated on both sides in the following days. Remarkably, the symptoms of the 2nd eye became only mild in these NCT subjects, compared to moderate in the control ones.

Subepithelial infiltrates occurred in 24 patients (60%), most of them again in adenoviral infections ($n = 21$). Fourteen patients (42.4%) treated with NCT and 10 patients (38.5%) of those treated with Gentax developed infiltrates ($P = 0.8$, Table 2). In all cases, the infiltrates developed after onset of the study therapy, except for 2 eye in 1 patient who had already suffered from the disease for 10 days before inclusion.

DISCUSSION

The majority of the conjunctival infections in this study turned out to be caused by adenovirus subtypes (3, 4, 8, 19, and 37), as found in previous reports.[2,20] Type 8 prevailed and caused particularly severe and prolonged infections with subepithelial infiltration, which is a well-known fact.[5]

Gentamicin was chosen as a control to be sure to provide sufficient treatment for bacterial infections, as well as to prevent bacterial superinfections. It should be mentioned that it contains benzalkonium chloride at a concentration of 0.01% (0.1 mg/mL), which has been reported to be virucidal against adenovirus types 5 and 20 *in vitro*.[22,23] Therefore, although never proposed to date, we cannot exclude an antiviral effect of the control medication *in vivo*.

Both medications used were well tolerated, despite the frequent application. The slight eye burning noted sometimes for a few minutes after dosing in the test group was expected because of the mild oxidative properties of NCT.[17] Similar to our previous pilot study,[18] this sensation was largely masked by the symptoms of infection in the acute stadium and was noticed as soon as the symptoms of infection disappeared and the medication was terminated anyway. The absence of any visible toxic effects of NCT in our patients confirms the previous finding of Koyama et al. that the addition of taurine protects the ocular surface from damage by hypochlorite by conversion to NCT.[16] All these results underline that the application of NCT to the infected eye is very safe. The short period of oxidative activity (15 minutes) after a single dosing in the eye explains the absence of protracted side-effects.[17]

In the case of a mild course of infection, the scores of inflammation were rather similar in both study groups, with a small advantage for NCT (subjective score on day 8). However, patients suffering from severe infections (adenovirus 8) benefited from antiviral therapy insofar as the symptoms decreased already within 4 days, compared to an increase in the control group (Fig. 2). Despite the (not significantly) lower average scores, the advantage of NCT appeared clearly, particularly regarding the subjective symptoms, which makes a therapeutical effect very probable. Direct antiviral effects of NCT which have been shown previously[14,15] could reduce the viral load on the conjunctiva. This might influence not only the course of infection but also its transmissibility, as indicated by the finding of only mild inflammation of the 2nd eye after the beginning of treatment of the other eye. Although a milder course of the 2nd eye is known, we found a clear difference between the test and control group. Furthermore, immune-modulatory effects of NCT described *in vitro* (i.e., down-regulation of proinflammatory cytokines [e.g., tumor necrosis factor (TNF) alpha, prostaglandins, nitric oxide, some interleukins[24,25]]) could contribute to the amelioration of the clinical symptoms.

In this study, the occurrence of subepithelial infiltrates could not be inhibited by NCT. This might be explained by its hydrophilic nature,[10] which limits corneal penetration to within the first 15 minutes of activity after dosing. More frequent dosing could possibly counteract this problem. Another explanation could be the late onset of treatment, which was approximately 3 days, on average, after the outbreak of disease. Therefore, treatment should be started in the earliest

stages of infection. Because the frequency of subepithelial infiltrates was similar in both groups and within the usual range in epidemic keratoconjunctivitis, they are attributed to the disease and not to the medication.

CONCLUSION

Summing up, NCT proved to be well tolerated in viral conjunctivitis, and it ameliorated the symptoms in severe inflammation. Further evaluation of this promising causative therapy is justified.

ACKNOWLEDGMENTS

This study was supported by the Austrian Science Fund, grant no. P15240, and the Jubilee Research Fund of the Austrian National Bank (grant no. 8366). The patient's insurance and Gentax were kindly provided by Dr. Mydlar (Agepha GesmbH; Vienna, Austria), and SAS™ Adeno Test by Shah J. Khan (SA Scientific; San Antonio, TX).

We are grateful to the nursing staff of the Department of Ophthalmology of the University Hospital of Innsbruck (Innsbruck, Austria) for excellent assistance. We thank Eva-Maria Lemberger for her excellent technical assistance and the staff of the Department of Bacteriology of the Institute of Hygiene and Social Medicine for identification of microbial cultures.

REFERENCES

1. Gordon, Y.J., Aoki, K., and Kinchington, P.R. Adenovirus keratoconjunctivitis. In Pepose, J.S., Holland, G.N., and Wilhelmys, K., eds., *Ocular infection and immunity*, 1996:877–894.
2. Kinchington, P.R., Turse, S.E., Kowalski, R.P., et al. Use of polymerase chain amplification reaction for the detection of adenoviruses in ocular swab specimens. *Invest. Ophthalmol. Vis. Sci*. 35:4126–4134, 1994.
3. Gottsch, J.D., Froggatt, J.W. III, Smith, D.M., et al. Prevention and control of epidemic keratoconjunctivitis in a teaching eye institute. *Ophthalmic Epidemiol*. 6:29–39, 1999.
4. Ward, J.B., Siojo, L.G., and Waller, S.G. A prospective, masked clinical trial of trifluridine, dexamethasone, and artificial tears in the treatment of epidemic keratoconjunctivitis. *Cornea* 12:216–221, 1993.
5. Hutter, H. Epidemic keratoconjunctivitis: Treatment results during an epidemic. *Klin. Monatsbl. Augenheilkd*. 197:214–217, 1990.
6. Gordon, Y.J., Araullo-Cruz, T., and Romanowski, E.G. The effects of topical nonsteroidal anti-inflammatory drugs on adenoviral replication. *Arch. Ophthalmol*. 116:900–905, 1998.
7. Kaufman, H.E. Treatment of viral diseases of the cornea and external eye. *Prog. Retin. Eye Res*. 19:69–85, 2000.
8. Schuhman, G., and Vidic, B. Clinical experience with povidone-iodine eye drops in patients with conjunctivitis and keratoconjunctivitis. *J. Hosp. Infect*. 6(Suppl A):173–175, 1985.
9. Grisham, M.B., Jefferson, M.M., Melton, D.F., et al. Chlorination of endogenous amines by isolated neutrophils. *J. Biol. Chem*. 259:10404–10413, 1984.
10. Gottardi, W., and Nagl, M. Chemical properties of N-chlorotaurine sodium, a key compound in the human defence system. *Arch. Pharm*. (*Weinheim*) 335:411–421, 2002.
11. Nagl, M., Lass-Floerl, C., Neher, A., et al. Enhanced fungicidal activity of N-chlorotaurine in nasal secretion. *J. Antimicrob. Chemother*. 47:871–874, 2001.
12. Nagl, M., Hengster, P., Semenitz, E., et al. The postantibiotic effect of N-chlorotaurine on *Staphylococcus aureus*. Application in the mouse peritonitis model. *J. Antimicrob. Chemother*. 43:805–809, 1999.
13. Nagl, M., and Gottardi, W. Enhancement of the bactericidal efficacy of N-chlorotaurine by inflammation samples and selected N-H compounds. *Hyg. Med*. 21:597–605, 1996.
14. Nagl, M., Larcher, C., and Gottardi, W. Activity of N-chlorotaurine against herpes simplex- and adenoviruses. *Antiviral Res*. 38:25–30, 1998.
15. Romanowski, E.G., Yates, K.A., Teuchner, B., et al. *In vitro* evaluation of the new antimicrobial agent N-chlorotaurine (NCT) against ocular isolates of adenovirus. Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Fort Lauderdale, Florida, 2002.
16. Koyama, I., Nakamori, K., Nagahama, T., et al. The reactivity of taurine with hypochlorous acid and its application for eye drops. *Adv. Exp. Med. Biol*. 403:9–18, 1996.
17. Nagl, M., Miller, B., Daxecker, F., et al. Tolerance of N-chlorotaurine, an endogenous antimicrobial agent, in the rabbit and human eye—a phase I clinical study. *J. Ocul. Pharmacol. Ther*. 14:283–290, 1998.
18. Nagl, M., Teuchner, B., Pöttinger, E., et al. Tolerance of N-chlorotaurine, a new antimicrobial agent, in infectious conjunctivitis—a phase II pilot study. *Ophthalmologica* 214:111–114, 2000.
19. Rotbart, H.A., Sawyer, M.H., Fast, S., et al. Diagnosis of enteroviral meningitis by using PCR with a colorimetric microwell detection assay. *J. Clin. Microbiol*. 32:2590–2592, 1994.
20. Saitoh-Inagawa, W., Oshima, A., Aoki, K., et al. Rapid diagnosis of adenoviral conjunctivitis by PCR and restriction fragment length polymorphism analysis. *J. Clin. Microbiol*. 34:2113–2116, 1996.
21. Notivol, R., Martinez, M., and Bergamini, M.V. Treatment of chronic nonbacterial conjunctivitis with a

cyclo-oxygenase inhibitor or a corticosteroid. Pranoprofen Study Group. *Am. J Ophthalmol*. 117:651–656, 1994.
22. Belec, L., Tevi-Benissan, C., Bianchi, A., et al. *In vitro* inactivation of Chlamydia trachomatis and of a panel of DNA (HSV-2, CMV, adenovirus, BK virus) and RNA (RSV, enterovirus) viruses by the spermicide benzalkonium chloride. *J. Antimicrob. Chemother*. 46: 685–693, 2000.
23. Valot, S., Edert, D., and Le Faou, A. A simple method for the *in vitro* study of the virucidal activity of disinfectants. *J. Virol. Methods* 86:21–24, 2000.
24. Marcinkiewicz, J., Nowak, B., Grabowska, A., et al. Regulation of murine dendritic cell functions *in vitro* by taurine chloramine, a major product of the neutrophil myeloperoxidase-halide system. *Immunology* 98:371–378, 1999.
25. Park, E., Jia, J., Quinn, M.R., et al. Taurine chloramine inhibits lymphocyte proliferation and decreases cytokine production in activated human leukocytes. *Clin. Immunol.* 102:179–184, 2002.

Reprint Requests: *Markus Nagl*
Department of Hygiene, Microbiology and Social Medicine
Division of Hygiene and Medical Microbiology
Innsbruck Medical University
Fritz-Pregl-Straße 3
A-6020 Innsbruck
Austria

E-mail: m.nagl@uibk.ac.at

TEUCHNER

AU1
Pls spell out acronym PCR.

AU2
Entero 3 and 4 also from MWG Biotech?

AU3
Pls provide city & state for Department of Bacteriology.

AU4
Pls. provide publisher and city/state for ref. 1.

AU5
Pls provide month/date of meeting.