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Tolerability of N-chlorotaurine plus ammonium chloride in the rabbit and human eye - a phase 1 clinical study

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Abstract
Background N-chlorotaurine (NCT), an endogenous mild antiseptic, is well-tolerated by application to the human conjunctiva and has been shown to offer beneficial effects in infectious conjunctivitis. Animal tests revealed improved efficacy of a combination of NCT with ammonium chloride in adenoviral conjunctivitis. The aim of this study was to evaluate the tolerability of NCT plus ammonium chloride in the healthy rabbit and human eye.
Methods First, a tolerability study was performed in rabbits. In a blinded and randomized fashion, one eye was treated with the test medication, the other one with 0.9% saline. Twenty-one animals (three per concentration) were treated with one drop every 2 hours for 6 days. Second, in two volunteers one drop of a defined concentration was applied to one eye every 15 min for 1 hour, saline to the control eye. Four different concentrations were tested on different days. Third, a double-blind, randomized phase 1 study in 13 healthy volunteers was performed. One drop of 0.1% NCT plus 0.1% NH_4Cl versus saline was applied every 15 min within the first hour, followed by four drops every 2 hours. This regimen was done daily for 5 days.
Results In rabbits, no side effects were seen with 0.1% NCT plus 0.1% NH_4Cl, while higher concentrations sometimes caused short-time and minimal conjunctival injection and secretion after dosing. By 1% NCT plus 1% NH_4Cl, these effects were moderate, but disappeared again without any detectable residues. In the pilot study with two volunteers, treatment with 0.5% NCT plus 0.1% NH_4Cl caused medium-scale eye burning for 30 seconds, while 0.1% NCT plus 0.1% NH_4Cl was very well-tolerated, with no or minimal burning for a few seconds. In the subsequent phase 1 study, 0.1% NCT plus 0.1% NH_4Cl was well-tolerated by all subjects except for minimal eye burning for a few seconds after dropping. No objective signs of eye changes could be detected in the human beings.
Conclusion The results of this study clearly demonstrate the good tolerability of a promising NCT formulation with improved activity.

Keywords N-chlorotaurine · Monochloramine · Ammonium chloride · Active chlorine compounds · Conjunctivitis · Oxidants · Tolerability · Rabbit model · Clinical trial

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Introduction

N-chlorotaurine (Cl-HN-CH_2-CH_2-SO_3^-, NCT) is a mild long-lived oxidant produced by human granulocytes during the oxidative burst [3, 24]. The chemical synthesis of the crystalline sodium salt (Cl-HN-CH_2-CH_2-SO_3Na) was successful a few years ago [2]. It acts like an antiseptic and is bactericidal against Gram-positive and Gram-negative bacteria [6, 7, 10, 11], virucidal [12] and

fungicidal [7, 9, 13]. Activity against a broad spectrum of adenoviruses causing epidemic keratoconjunctivitis has been proved [22, 23].

Tolerability of 1% NCT (55 mM) by human tissue, for instance in the healthy human eye [14], is very good, so the substance seems to provide an optimal compromise between antimicrobial activity and tolerability. In a phase 2a study on bacterial conjunctivitis, the substance was also very well tolerated, and the inflammation could be healed rapidly [17]. Recent phase 1 and 2 studies revealed very good tolerability and good efficacy in local therapy of crural ulcers [15] and external otitis [19]. Moreover, NCT was very well tolerated upon irrigations of recurrent sinusitis (Phase 2a) [18]. In one of three patients suffering from cystitis caused by omniresistant Pseudomonas aeruginosa, it was possible to eradicate the pathogen [16, 26].

Viral conjunctivitis, particularly epidemic keratoconjunctivitis (EKC) which is caused by adenoviruses, is a highly infectious, in part severe eye disease, for which no well-tolerated causative therapy exists to date [5]. Even in viral conjunctivitis, efficacy of 1% NCT is very probable in the sense of attenuation of severe courses of inflammation, according to the results of our recent double-blind, randomized phase 2 study [25]. This was confirmed in an animal model using rabbits whose eyes were infected with adenoviruses [23]. However, the development of subepithelial infiltrates in man can probably not be inhibited by NCT. These infiltrates are frequent (ca. 40% in the test and control group in our study [25]), and may lead to vision disorders for several months. Because of this fact, there is the need to further improve the efficacy of NCT in viral conjunctivitis.

It is known that addition of ammonium chloride (NH_4Cl) markedly enhances the activity of NCT against bacteria and fungi [7, 8, 13, 16]. This can be explained by transfer of the oxidative chlorine atom to ammonium, at which monochloramine is formed in equilibration, which is more lipophilic than NCT and penetrates pathogens better [2, 3].

$$\mathrm{Cl-HN-CH_2-CH_2-SO_3^- + NH_4^+} \leftrightarrow \mathrm{H_3N^+ - CH_2 - SO_3^- + NH_2Cl} \quad (1)$$

This is a natural process, as well, which occurs during every inflammation upon which a mixture of chloramines is formed after the oxidative burst. The chlorine transfer leads to an improved microbicidal activity of NCT in body fluids and inflammatory exudates compared to buffer solution [7, 13, 16]. This can be regarded as an advantage, in the sense that a mild substance is enhanced exactly at the place of action. Our clinical studies prove clearly that these processes do not cause toxic effects.

Although the direct virucidal effect of NCT is enhanced by ammonium chloride only to a small extent [Nagl M, unpublished], the combination may be of advantage in viral conjunctivitis because of the following facts. Plain NCT hardly permeates the cornea [23], and attack of subepithelially placed viruses might be difficult. In vitro, significantly more oxidative activity permeates the cornea if ammonium chloride is added [23]. Actually, in the EKC rabbit model the efficacy of NCT plus ammonium chloride proved to be significantly higher than that of NCT alone, demonstrated by higher in vivo antiviral activity and shorter periods of viral shedding. Concentrations of 0.1% NCT +0.1% NH_4Cl were equally effective to 1% NCT +0.1% NH_4Cl or 0.1% NCT +1% NH_4Cl [23]. This seems surprising, but may be explained by the fact that the concentrations of the formed monochloramine (NH_2Cl) differ, at maximum, by about a factor of 3, with 31.5, 91.2, and 75.0 ppm respectively [1]. It was the aim of this study to evaluate the tolerability of NCT plus ammonium chloride in the healthy eye in the rabbit and in man.

Materials and methods

Reagents

Pure NCT as a crystalline sodium salt (molecular weight 181,57) was prepared according to [2]. Purity was proved by spectrophotometry and standardized quality controls. Ammonium chloride at the highest available purity was purchased from Merck (Darmstadt, Germany). Both reagents were dissolved in sterile and pyrogen-free distilled water to a final concentration of 0.1% NCT (5.5 mM) and 0.1% NH_4Cl (18.7 mM). In a pilot study, higher concentrations were applied also (see below). Solutions were prepared before the beginning of the study and stored at 2–4°C. They were contained in flasks of similar appearance to ensure double-blinding, and all eye drops were numbered consecutively in accordance with the randomization code.

Animal study

The animal experiments were approved by the Austrian Federal Government Department for Science and Research and followed the "Principles of laboratory animal care". Twenty-one rabbits (from SAVO, Kissleg/Germany) were randomized and divided into groups of three. One eye was treated with the test medication, the control eye with 0.9% NaCl. The schedule of application was one single dose of one drop five times per day (every 2 hours) for 6 consecutive days in total.

To find out well-tolerated and irritating concentrations, the following test medications were applied to three animals each: 1% NCT +1% NH_4Cl, 1% NCT +0.1% NH_4Cl, 0.1% NCT +1% NH_4Cl, 0.1% NCT +0.1% NH_4Cl, and 1% NH_4Cl (control without NCT). One week later, 0.2% NCT +0.2% NH_4Cl as well as 0.3% NCT +0.3% NH_4Cl were tested.

Applications and examinations were performed in a blind fashion. The external parts of the eye (conjunctiva, cornea, sclera, iris) were examined daily by usage of a slit lamp and magnifying glasses. The grading of adverse effects was similar to that used in human subjects described below. Examinations were performed daily during the treatment period, 1 and 2 weeks later and 4 months later to exclude long-time adverse effects.

Phase 1 - study design

Both a previous pilot study in two healthy volunteers and the main study in 13 healthy volunteers were designed as a double-blind, randomized, placebo-controlled phase 1 study. This university trial was in accordance with the Declaration of Helsinki, and it was approved by the Ethics Committee of the University of Innsbruck. All subjects gave written informed consent.

Pilot study

A pilot study with two male subjects (39 and 71 years old) was done to identify the tolerated dose and to get pharmacodynamic data. Because it had been tolerated without any side effects in the rabbit study, we started with a test concentration of 0.1% NCT +0.1% NH_4Cl. Subsequently, 0.5% NCT +0.1% NH_4Cl, then 0.3% NCT +0.1% NH_4Cl, and finally 0.1% NCT +0.2% NH_4Cl were applied to one eye, isotonic saline solution (0.9%, placebo) to the other one. It was the intention to perform frequent dosing connected with practicable and close monitoring. Therefore, each concentration was dripped on one different day, and five single doses of one drop were administered within 1 hour, i.e. one drop every 15 min. Both subjects were examined as described below 3 to 4 hours after the last single dose on each day of dosing.

For qualitative examination of pharmacodynamics, lacrimal fluid was collected 1, 3, 5, 10, and 15 min subsequent to application of 0.1% NCT +0.1% NH_4Cl using a strip of filter paper (Schleicher & Schuell, width of 5 mm) placed onto the conjunctiva bulbi and palpebrae inferioris for 5 seconds. One drop each of potassium iodide and starch solution was applied onto the wet strip, which caused the formation of blue color [21] in the presence of NCT (NCT $+2I^- + H^+ \rightarrow$ taurine $+I_2 + Cl^-$; I_2+starch $\rightarrow$ blue complex).

Phase 1 study

Subjects

Thirteen volunteers (six female, seven male, ranging in age from 22 to 71 years, mean 33.3 years, standard deviation 15.1) participated. Medical status was determined by evaluation of the medical history and medication and by detailed ophthalmologic examination by usage of a slit lamp and an indirect ophthalmoscope. Included were healthy volunteers who particularly did not suffer from a disease of the eye. Exclusion criteria were nonage (<19a), eye disease, any acute disease, chronic disease with involvement of the eye, medication with side effects on the eye, other treatment of the eye at the same time, contact lenses during the study, participation in another clinical study at the same time, and pregnancy. Subjects were numbered consecutively, and test and control eyes were assigned in accordance with the randomization code. No subjects had to be withdrawn after randomisation.

Treatment

The test eye of each subject was treated with 0.1% NCT plus 0.1% NH_4Cl, the concentration which was best tolerated in the rabbit study and human pilot study and which had shown efficacy in the EKC rabbit model. The control eye was dripped with 0.9% saline in a randomized and double-blind manner. The dosing schedule was planned to simulate treatment for viral conjunctivitis, to warrant a high frequency and to be practicable for the volunteers. Therefore, the period of dosing was 5 days. On each day, treatment was started with an intensive phase of five single doses at an interval of 15 minutes to warrant oxidative action for 1 hour, followed by four single doses at an interval of 2 hours starting 1 hour later.

Clinical evaluation

All subjects were examined daily subsequent to the last single dose during the treatment cycle, as well as 14 days later. The baseline investigation was performed on day 1 before the beginning of treatment. The following parts of the eye were evaluated: conjunctiva, caruncula, cornea, sclera, glandula lacrimalis, iris, lens, choroidea, and retina. Any changes were rated as *objective* signs of adverse effects (palpebral edema, conjunctival hyperemia, petechial hemorrhages, exudation, chemosis, pseudomembranes, corneal stippling, iritis, cataract, choroiditis, and changes of the ocular fundus). *Subjective* symptoms evaluated were pain (eye burning, headache, pain of the eyelid), tearing, itching, foreign-body sensation, and impaired vision (unsharp vision, photophobia, colored vision, impaired visual field) and other impairments, as well as their intensity, duration and time. Objective signs and subjective symptoms were scaled “absent, mild, moderate, and severe” and rated 0, 1, 2, and 3 points respectively, similar to what has been reported previously [4, 20]. *Mild* were hardly noticeable subjective effects like short-time minimal eye-burning, and objective ones like discrete corneal stippling or discrete

vascular conjunctival injection. *Moderate* were subjective effects with impairment of condition such as more pronounced eye burning, and more pronounced objective effects, but self-limited and not threatening health. *Severe* were intense subjective effects such as hardly tolerable eye-burning, and objective effects threatening health or requiring therapeutic intervention. Objective and subjective scores were calculated by addition of the mentioned single points of signs and symptoms on all days as the primary criteria of evaluation.

To exclude long-term adverse effects, an additional clinical examination was performed 4 months after the end of the study in 12 of the 13 subjects, since one was no more available because of emigration. Because the corneal endothelium—a monolayer of hexagonally shaped cells without regenerative properties—is very sensitive to toxic effects, the corneal endothelium was photographed with an endothelial specular microscope (ESM 2001, Rhine-Tec, D-41179 Mönchengladbach, Germany) before starting treatment and 4 months later. Endothelial cell density was determined with the SeaEagle® software (Rhine-Tec, Mönchengladbach, Germany) and the values of the test and control eye as well as the values before and after the study were compared.

Statistical analyses

Adverse effects were evaluated by descriptive statistical analysis. Frequency of each side effect was calculated in relation to the number of subjects as well as to the number of days of treatment. McNemar's test was used to test for statistical significance. The number of corneal endothelial cells was calculated as the mean value from three different photographs from each eye in each subject. The mean values of all subjects were compared by Student's paired *t*-test. *P* values of less than 0.05 were considered significant.

Results

Animal study

During the treatment with five daily doses for 6 days, 0.1% NCT + 0.1% NH_4Cl caused no detectable adverse effects (Table 1). Conjunctival injection, edema, and secretion occurred in higher concentrations (Table 1). These side effects were minimal and temporary, and in no case did discontinuation of the application become a consideration.

Only the highest concentration used, i.e. 1% NCT + 1% NH_4Cl, caused moderate effects (Table 1). Therefore, dosing was stopped after 1 day in these three animals. After 2 days, effects had decreased to minimal and disappeared within 7 days without treatment.

The threshold concentration tolerated in the rabbit was 0.3% NCT + 0.3% NH_4Cl, at which temporary minimal to moderate injection, particularly of the nictitating membrane, became apparent.

The inner parts of the eye of all animals remained normal, and Tyndall tests were negative on all days. No adverse effects occurred in the control eyes treated with saline or 1% NH_4Cl. No long-term adverse effects were found, including in those rabbits challenged with 1% NCT + 1% NH_4Cl.

Table 1 Tolerability of NCT + NH_4Cl in the rabbit eyes

NCT	NH_4Cl	Animal	Side effects observed
1%	1%	1*	injection, edema, secretion (moderate each)
		2*	injection (moderate), edema and secretion (minimal)
		3*	injection and edema (moderate), secretion (minimal)
0.3%	0.3%	1	edema (minimal)
		2	injection (minimal - moderate), edema (minimal)
		3	injection (minimal - moderate), edema (minimal)
0.2%	0.2%	1	secretion (minimal)
		2	none
		3	injection (minimal)
1%	0.1%	1	none
		2	injection, edema, secretion (minimal each)
		3	injection, edema, secretion (minimal each)
0.1%	1%	1	none
		2	none
		3	injection (minimal)
0.1%	0.1%	1,2,3	none
0%	1%	1,2,3	none

*application terminated after the first day

In an extra experiment, the three rabbits who had received 0.1% NCT + 0.1% NH_4Cl were treated with one drop of this concentration every 5 min for half an hour, i.e. seven applications. This intensive treatment was tolerated virtually without side effects.

Pilot study

In the pilot test with two volunteers, different concentrations were administered every 15 min within 1 hour on 1 day each. 0.1% NCT plus 0.1% NH_4Cl was very well-tolerated, with mild eye burning for 5 seconds in the first subject and with mild foreign-body sensation for 5 to 10 seconds in the other one (subjective score 1).

0.3% NCT plus 0.1% NH_4Cl caused moderate burning for about 10 seconds. This was followed by mild foreign-body sensation for 5 min in the second subject (score 2). No objective changes were observed at the ophthalmologic examination.

0.5% NCT plus 0.1% NH_4Cl caused moderate to strong burning after the second to fifth dose for about 20 seconds immediately after application. The burning sensation disappeared after 5 min, and was rated a score of 2. A mild foreign-body sensation was present for 15 min subsequent to the last dosing. Both subjects had a minimal conjunctival injection on the nasal side (objective score 1), but in the first subject this was detected in the control eye treated with saline. No disorders came up during the following time.

0.1% NCT plus 0.2% NH_4Cl led to moderate burning for approximately 10 seconds in the second subject, while this sensation was only mild in the first subject (score 2 and 1 respectively). Minimal temporary corneal stippling could be seen in the eye treated with the test substances in the second subject (score 1).

Corneal endothelial cell counts measured 6 months after the beginning of the study did not change. Mean values ± SD of two measurements of the first volunteer were 2788± 74 before and 2718±83 after the study in the test eye (P= 0.41), and 2723±16 before and 2739±173 afterwards in the control eye (P=0.91). Values of the second volunteer were 2449±64 before and 2382±133 after the study in the test eye (P=0.57), and 2387±42 before and 2266±45 afterwards in the control eye (P=0.058).

Summing up, no serious adverse effects occurred. Eye burning would have become a limiting factor at concentrations exceeding 0.5% NCT + 0.1% NH_4Cl. Since 0.1% NCT + 0.1% NH_4Cl was tolerated with no or minimal burning, and because this dose had shown good antiviral efficacy in the rabbit model [23], it was chosen for the main study.

Pharmacodynamics Oxidative activity was detectable for 4 to 5 min after application of one drop of 0.1% NCT + 0.1% NH_4Cl in both subjects.

Phase 1 study

All 13 subjects completed the study according to the 5-day dosing protocol. No severe adverse effects occurred. Chronological appearance of adverse effects in each subject is noted in Tables 2 and 3. The only *subjective side effect* (Table 2) significantly related to 0.1% NCT plus 0.1% NH_4Cl was mild to moderate eye-burning for a few seconds

Table 2 Chronological appearance and overlaps of *subjective* adverse effects during the five-day treatment with NCT + NH_4Cl

Subject	Test-eye					Placebo-eye				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
1	b	b, f	b	b	b	_	f	_	_	_
2	b	b	b	b	b	_	_	_	_	_
3	b	b	b	b, f	_	_	_	_	f	_
4	b	b	b	_	_	_	_	_	_	_
5	b	b	b	b	b	_	_	_	_	_
6	b	b	b	b	b	_	_	_	_	_
7	b	b	b	_	b	_	_	_	_	_
8	b	b	b	b	b	_	_	_	_	_
9	b	b, f	b	b	b	_	_	_	_	_
10	_	b, f	b	b	b, f	_	f	_	_	_
11	b	b	b	b	b	_	_	_	_	_
12	b	b	b	b	b	_	_	_	_	_
13	b	b	b	b	b	f	f	f	f	f

b burning (P<0.01)
f foreign-body sensation (P>0.1)

Table 3 Chronological appearance and overlaps of *objective* adverse effects during the five-day treatment with NCT + NH_4Cl

Subject	Test-eye					Placebo-eye				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
1	_	s	_	_	s	_	_	_	_	s
2	_	_	_	_	_	_	_	_	_	_
3	_	s	s	_	_	_	_	_	_	_
4	_	_	_	_	_	_	_	_	_	_
5	_	_	_	_	_	_	_	_	_	_
6	c	_	_	_	_	c	_	_	_	_
7	_	_	_	_	_	_	_	_	_	_
8	_	_	_	s	s	_	_	s	s	s
9	_	s	s	s	s	_	_	_	_	_
10	_	s	s	s	_	_	_	_	_	_
11	_	c, s	c, s	c	c	_	_	_	_	_
12	_	_	_	_	_	_	_	_	_	_
13	c	c	c	c	c	_	_	_	_	_

c conjunctival injection ($P<0.01$)
s corneal stippling ($P<0.01$)

immediately after dosing ($P<0.01$ compared to the control eye). Minimal foreign-body sensation occurred also in the control group at a similar frequency ($P>0.1$). Most of the time the burning was mild, and it was rated less intensive than the anesthetic eye drops used before applanation tonometry by most of the subjects, including those who rated the burning as moderate. In 12 participants, it disappeared within 5 to 10 seconds, in one (no. 13), who was known to be particularly sensitive to all kinds of eye drops, within 30 seconds. In six persons, burning was not present after the first daily dose, but appeared during the intensive phase of applications within the first hour. Besides that, it did not increase with duration of the study but sometimes disappeared. Subjective sensations were approximately similar to the entry of sweat to the conjunctiva, and of such small magnitude and short duration that no subject asked for premature treatment discontinuation.

Except for sporadic foreign-body sensation, no adverse effects occurred in the eyes treated with placebo. Impaired vision and other impairments were not noted.

Objective side effects were mild corneal stippling and mild conjunctival injection. Stippling was detected on 15 of 65 days (13 subjects x 5 days) of treatment in total (23.1%) in the test, and on four of 65 days (6.2%) in the control group ($P=0.0026$). Conjunctival injection occurred in 15.4% in the test and in 1.5% in the control group ($P=0.0077$). All these effects were minimal, self-limited and in part disappeared again even during the treatment period (Table 3). When their appearance was calculated in relation to the number of subjects, there was no statistical difference to the controls ($P=0.125$ for stippling and $P=0.5$ for injection). The control examinations 14 days after the end of dosing revealed no disorders, except for bilateral eye itching and mild conjunctival injection in one subject which was obviously related to solar irradiation and dry eyes. No further adverse effects occurred, and no alterations of the middle and internal parts of the bulbus oculi were noted. No long-term adverse effects appeared during follow-up, as confirmed at the examination 4 months after the end of treatment.

There was no difference in the ocular pressure between test and control eyes measured on day 1 (baseline), day 5, day 19, and 4 months later ($P>0.2$ in all cases: data not shown). The baseline values were minimally higher because they were measured in the morning, the other ones in the late afternoon. Visual acuity, which was determined on the same days, did not change in any subject.

The number of endothelial cells per mm^2 did not change above the natural rate. Mean values calculated from single mean values derived from three measurements each of 13 test persons decreased from 2558.5±151.5 at the baseline to 2537.2±163.8 after 4 months in the test group ($P=0.188$) and from 2539.6±177.7 to 2498.0±169.1 in the control group ($P=0.078$). Values of the test group were not different from those of the control group at the baseline ($P=0.445$) and 4 months later ($P=0.169$).

Discussion

As an endogenous mild antiseptic, NCT has proved to be very well-tolerated and effective in clinical studies. Patients suffering from bacterial conjunctivitis were cured rapidly in a phase 2a trial [17], and also signs and symptoms of severe adenoviral conjunctivitis were attenuated by 1% NCT eye drops in a phase 2b trial [25]. Improved penetration of

activity into the cornea is desirable to avoid or successfully treat subepithelial viral infiltrates. Penetration can be improved by addition of ammonium chloride, since the formed monochloramine is more lipophilic than NCT [2, 3]. Basically it deals with a natural process, since ammonium chloride is present ubiquitously in the human body and body fluids. Artificial addition of ammonium chloride makes it possible to steer the activity of NCT in a controlled way. This was confirmed in adenoviral conjunctivitis using the Ad5/NZW rabbit ocular model, where NCT + NH_4Cl demonstrated superior reduction of viral load compared to 1% NCT [23].

In our tolerability tests in healthy rabbits, after repeated application, 0.1% NCT + 0.1% NH_4Cl was excellently tolerated, while 0.3% + 0.3% was the threshold concentration leading to mild conjunctival injection and swelling for a few hours. Since all signs (even after 1% + 1%) disappeared without long-term adverse effects, safety proved to be high enough to test the combination in man.

The results of the pilot study revealed similar tolerability of NCT + NH_4Cl in man and in the rabbit. The combination of 0.1% + 0.1% seems to be optimal because of the following reasons. First, it is tolerated without considerable adverse subjective or objective side effects. Concentrations of about 0.5% NCT + 0.1% NH_4Cl may lead to marked burning and irritation, but not to serious events, so that the safety-distance to toxic effects is high. Second, it has oxidative activity in the eye for 4 to 5 min, so that long-term adverse effects are very improbable and have actually not been observed. Third, it has excellent activity in vitro against pathogens [1], and in vivo it was as effective as are higher concentrations in the viral conjunctivitis rabbit model [23]. Fourth, it is as well-tolerated in the healthy eye as is 1% NCT alone [14]. Since the latter was tolerated very well in the inflamed human eye [17, 25], this has to be expected for 0.1% NCT + 0.1% NH_4Cl as well.

The short period of oxidative activity (a few minutes) after dosing on the one hand probably makes repeated dosing necessary to achieve sufficient efficacy; on the other hand, penetration to the inner parts of the eye and considerable side effects are avoided. Adaptations of dosing, for instance frequent application at the beginning of treatment followed by longer intervals, can be done easily and without risks of side effects.

A regimen such as this has been simulated in this phase 1 study. The subjective effects (eye burning, foreign-body sensation) are of such short duration and low extent such that they absolutely do not counteract application. By contrast, anesthetic eye drops used in daily practice seem to cause more intensive burning, as noticed by most of the subjects. The detected objective effects do not differ from everyday changes of the eye, and sometimes they even disappeared during the treatment cycle. No long-term adverse effects were found even with the highly sensitive method of in vivo microscopy of corneal endothelial cells. Therefore, the good tolerability of NCT + NH_4Cl in this study clearly confirms the expectations derived from considerations mentioned above.

Conclusion

The combination of NCT and NH_4Cl at a respective concentration of 0.1% + 0.1% proved to be very well-tolerated and safe during intensive treatment of the healthy eye for 5 days. Subjective side effects are mild and limited to seconds; objective ones are within a range of everyday minimal irritations. Further development of this medication is justified.

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