Tolerability of \( N \)-chlorotaurine in chronic rhinosinusitis applied via yamik catheter

Andreas Neher \(^a^*, \) Hannes Fischer \(^a^, \) Elisabeth Appenroth \(^a^, \) Cornelia Lass-Flörl \(^b^, \) Astrid Mayr \(^b^, \) Andreas Gschwendtner \(^c^, \) Hanno Ulmer \(^d^, \) Thaddeus F. Gotwald \(^e^, \) Michaela Gstöttner \(^a^, \) Vladimir Kozlov \(^f^, \) Markus Nagl \(^b^)

\(^a^\) Department of Otorhinolaryngology, Innsbruck Medical University, Anichstr. 35, Innsbruck, Austria
\(^b^\) Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria
\(^c^\) Department of Pathology, Innsbruck Medical University, Innsbruck, Austria
\(^d^\) Department of Medical Statistics, Informatics, and Health Economics, Innsbruck Medical University, Innsbruck, Austria
\(^e^\) Department of Radiology, Radiology 2, Innsbruck Medical University, Innsbruck, Austria
\(^f^\) Department of Otorhinolaryngology, Yaroslavl State Medical Academy, Yaroslavl, Russia

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Abstract

Objectives: A rational approach in the treatment of chronic rhinosinusitis (CRS) is the intranasal application of antiseptic agents, due to the pathogenetic role of bacteria and fungi. \( N \)-Chlorotaurine (NCT), a mild endogenous oxidant with broad-spectrum antimicrobial activity, has been tested for the first time in CRS.

Methods: This one-arm phase IIa clinical study is the first step in the clinical development of this promising substance for local therapy of CRS. The nasal and paranasal cavities of 12 patients were rinsed with 10–20 ml of 1% aqueous NCT solution, applied via a novel catheter system (YAMIK). Treatment consisted of three lavages per week for 4 weeks.

Results: NCT caused neither alterations of the mucosa nor burning pain during application. Nevertheless, the insertion of the catheter, the insufflation of the posterior cuff and the overpressure inside the sinuses after infiltration led to moderate pain in some patients. Mucosal swelling decreased in all subjects, nasal breathing could be improved in nine patients and impaired olfaction in seven. Polyps did not disappear within the 1-month period of the study.

Conclusions: The good tolerability and possible beneficial effects of NCT encourage its further investigation in CRS. Despite some limitations the YAMIK catheter proved to be a convenient and safe device for rinsing the nasal and paranasal sinuses.

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Keywords: \( N \)-Chlorotaurine; Chronic rhinosinusitis; YAMIK catheter

1. Introduction

Chronic rhinosinusitis (CRS) is characterised by inflammatory changes of the nasal and paranasal sinus mucosa ranging from mucosal thickening to nasal polyps [1]. It has become one of the most common chronic diseases in the recent years [1]. The pathogenic mechanisms of CRS are not yet understood very well. Beside bacteria as the causing agent [2], overreaction of the immune system against fungal organisms within the nasal mucus has been reported to be associated [3]. According to these findings, local antiseptic therapy can be considered as a new approach in CRS [4]. Because of the life-long exposure of the nasal and paranasal mucous membranes to fungal antigens, long-time treatment has to be taken into account. Therefore, any medication used must be free of severe toxic side effects.

\( N \)-chlorotaurine (Cl–HN–CH\(_2\)–CH\(_2\)–SO\(_3\)\(^{-}\)), an endogenous long-lived oxidant generated by leukocytes during inflammation [5,6], might meet these requirements. It can be synthetised as a crystalline sodium salt (NCT, Cl–HN–CH\(_2\)–

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* Corresponding author. Tel.: +43 512 504 23141; fax: +43 512 504 23144.
E-mail address: Andreas.Neher@uibk.ac.at (A. Neher).
necessary. Solutions were stored at 2–4°C, no preservatives or buffers, respectively, were used. The above-mentioned broad-spectrum activity against pathogens, no preservatives or buffers, respectively, were necessary. Solutions were stored at 2–4°C, which warrants a high stability of 90.7% per year [7], and allowed to reach room temperature before application to the patients.

2.3. Study design

An open one-arm phase IIa study was performed in 12 patients suffering from CRS. All subjects were treated with 1% NCT. The study was in accordance with the Declaration of Helsinki and it was approved by the ethical committee of the University of Innsbruck. All patients gave written informed consent.

2.4. Patients

The study population comprised eight female and four male patients, ranging in age from 21 to 63 years, median 33.5 years, 25% percentile 30, 75% percentile 38. Inclusion criterion was the presence of CRS. Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa that is present for longer than 3 months and is associated with inflammatory changes ranging from polyoid mucosa thickening to gross nasal polyps [20]. Mucosal swelling in the nasal cavity was observed in all patients. In two cases we found polyps in the middle nasal meatus on both sides, in one patient only on one side.

Diagnosis was done clinically, histologically and by computed tomography (see below). Exclusion criteria were other local therapy to the nasal and paranasal sinuses, systemic therapy with corticoids, antihistamins or antibiotics, latex allergy, minority and pregnancy. No patients had to be withdrawn after inclusion, and all completed the trial.

2.5. Application of NCT

Before introduction of the YAMIK catheter, a 3% cocaine gauze was placed for 4 min into the nasal cavity for decongestion and local anesthesia of the mucosa. After blocking the anterior and posterior cuffs of the introduced catheter, the paranasal sinuses were rinsed with Ringer's lactate solution ( Fresenius Kabi Austria Inc., Graz, Austria) in order to evacuate mucus for microbiological investigations. Then 10 ml of NCT were instilled and remained in the sinuses for 10 min. The patients were kept in lying position, bending the head toward the shoulder of the treated side. After 10 min the NCT solution and mucus were sucked off.

TREATMENT was performed on Monday, Wednesday and Friday during four consecutive weeks, i.e. 12 days of application in total. All patients were treated bilaterally.

2.6. Evaluation of symptoms

Diagnosis was established by anamnesis and by detailed otorhinolaryngological examination. Before the irrigations with NCT a coronary computed tomography (CT) of the nasal and paranasal sinuses and a Zurich smelling test were performed on Monday, Wednesday and Friday during four consecutive weeks, i.e. 12 days of application in total. All patients were treated bilaterally.

The YAMIK sinus catheter is made of natural latex (Revultex™, Yartec Medical Inc., Yaroslavl, Russia, [19]). It consists of a body with two inflatable cuffs with valves. A flexible rod inside the catheter facilitates the insertion into the nasal cavity. The posterior cuff seals the patient’s choana, whereas the mobile anterior cuff is located in the nasal vestibulum. Insufflation of both cuffs creates a self-contained space. Through a working canal the instillation of fluid or the evacuation of mucus with a syringe is possible.
performed [21]. CT scans were graded using the Lund–Mackay radiologic score [22]. For each sinus region, i.e. frontal sinuses, osteomeatal complex, maxillary sinuses, anterior ethmoid sinuses, posterior ethmoid sinuses and sphenoid sinuses, either 0 (no opacification), 1 (1–90% opacification), or 2 points (>90% opacification) were summarised to obtain a score of 0–24 points. The smelling test consisted of eight smell substances, which had to be identified by the patient (maximum of eight points).

The nasal mucosa was investigated by endoscopy for the presence of swelling or polyps. Patients were asked about the tolerability of the procedure, evaluated by visual analogue scale (0 = no pain, 10 = intolerable pain), on each day of dosing. These documented discomforts included pain caused by introduction of the catheter, by insufflation of the posterior cuff, and by instillation of liquid into the sinuses under pressure. The values of the more painful side were documented. The smelling tests were performed on treatment days 1, 6 and 12, CT control scans 4 weeks after the end of therapy.

2.7. Microbiology

Fungal cultures were grown from the Ringer’s solution samples. To solve the fungi from the mucus, a 2.5% N-acetyl-l-cystein aqueous solution was tenfold diluted with the samples, and they were incubated in a rotatory shaker for 20 min at a frequency of 400/min. After centrifugation at 1800 \( \times g \) for 10 min the supernatant was withdrawn. Pellets were suspended in 1 ml of sabouraud-glucose broth (Merck, Darmstadt, Germany), stirred three times vigorously, and 200 \( \mu l \) were spread on Sabouraud agar plates in duplicate. Both the plates and the remaining broth were grown for 10 days at 30°C. Aliquots of the broth were transferred to plates in the case of fungal growth. Fungi were identified according to Raper and Fenell [23].

2.8. Histology

Mucus-samples were obtained in 11 cases. In nine cases cytology was taken before and after the therapy. May Gruenwald Giemsa stain was applied for the detection of eosinphils and Grocott silver stain for the detection of fungi. Histology (two cases) was performed on 5 \( \mu m \) sections from formalin fixed and paraffin embedded tissue excised from polyps of the nasal mucosa removed surgically one month after the end of the study. Sections were hematoxylin–eosin as well as Grocott silver stained.

2.9. Pharmakokinetics of NCT within the paranasal sinus

One additional volunteer was rinsed on two different days with NCT as described above, and the oxidation capacity of nasal secretion was measured for five times every 60 min. Aliquots (five samples per day in total) of 17–847 \( \mu l \) were diluted in 10 ml of distilled water and centrifuged at 1700 \( \times g \) for 10 min. After addition of excess potassium iodide the oxidation capacity in the supernatant was measured by iodometric titration [6] with 0.1 M sodium thiosulfate at pH 2–3 (acetic acid) using the automatic titration assembly TIM900 (ABU901 autoburette, Radiometer, Copenhagen).

2.10. Statistical analyses

Pain during and after treatment was compared to pain before using the Wilcoxon test. The same test was applied for the radiologic score as well as for the smelling test. \( p \)-Values < 0.05 were considered to indicate statistical significance. Due to the limited number of patients no corrections for multiple comparisons were applied.

3. Results

3.1. Fungal cultures

In total 201 mucous samples were investigated microbiologically. Fungi were detected in 11 of 12 patients. An average of 3.25 (range 0–7) different fungal species could be identified per patient, above all moulds. A survey of the species and their frequency of occurrence is provided in Table 1. Different species appeared and disappeared during the study in all patients without any regularity and often changed in single patients. The number of colony forming units per plate was \( \leq 10 \).

<table>
<thead>
<tr>
<th>Species</th>
<th>no of patients</th>
<th>no of positive cultures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria sp.</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Aspergillus tamarii</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>4</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>5</td>
<td>18 (9.0)</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>2</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>3</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>3</td>
<td>6 (3.0)</td>
</tr>
<tr>
<td>Chrysosporium sp.</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Eurotium sp.</td>
<td>3</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>3</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>8</td>
<td>24 (11.9)</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>2</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Scopulariopsis sp.</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>1</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

Total no of positive cultures 75 (37.3)
Total no of negative cultures 1 126 (62.7)
Total no of cultures 201 (100)

\( ^a \) Of 12 patients.
3.2. Histology

We observed eosinophilic granulocytes in five of nine samples (in two patients with polyps on both sides, in three patients with mucosal swelling), before and after the treatment. In the two patients with polyps on both sides histologic workup was done from mucosa samples taken during an operation 1 month after the study. It still demonstrated the presence of eosinophils.

3.3. Pharmacokinetics of NCT

Subsequent to lavage with 10 ml of 54.4 mM NCT, oxidation capacity decreased to 19–37 mM (35–67% of the start value) after 1 h, 4.1–8.7 mM (7–16%) after 2 h, 1.0–1.1 mM (2%) after 3 h, 0–0.4 mM (0.7%) after 4 h, and was no more detectable after 5 h. Values are the range of two independent lavages and measurements on different days.

3.4. Tolerability and effects of the therapy

The therapeutic procedure, which included insertion of a cocaine sauked gaze, insertion of the catheter, insufflation of its posterior cuff and instillation of NCT, caused moderate pain in five cases (p-values ranging from 0.003 to 0.018, compared to pain before treatment, Table 2). Three patients complained about pain in 2 of 12 rinsages, one patient in 1 rinsage and one patient in 9 of 12 rinsages. One of the cases with two painful instillations and the one with a single painful treatment suffered from gross polyps, so that it was impossible to introduce the catheter without touching the sensible mucosal layer, which caused pain. The other two patients with two painful episodes had a distinct septal deviation and a basal crest, so a painless introduction was also impossible. Endoscopic findings in the patient with 9 painful of 12 treatments revealed a significant septal deviation, edematous conchae and gross polyps in the middle nasal meatus. Five persons stated discomfort before the study treatment (score 2–8), caused by CRS.

During the first week of treatment rhinoscopy revealed an increase of nasal secretion. In the following the mucous membrane became rather dry, and in all patients swelling decreased as evaluated by rhinoscopy. There was no alteration of the mucosa caused by the repeated irrigations. Nasal breathing improved in nine subjects. No signs for systemic side effects could be observed which is in accordance with the absence of penetration through membranes due to the hydrophilic nature of NCT. Swallowing of small amounts of NCT that bypassed the posterior cuff caused only a chlorine taste but no discomfort.

Smelling could be improved in seven of the eight subjects with an impaired sense at the beginning of the study (p = 0.042 for day 6, and p = 0.017 for day 12). Three subjects had no smelling problems during the whole study. In one patient the smelling test was not done at the beginning and at the end of the study, in one patient not at the beginning.

The endoscopic investigation after one moth revealed a decrease of the mucosa thickening in all cases. The size of the polyps, which were found in three patients, did not change, although a deswelling of the surrounding mucosa was obvious. Before treatment the Lund–Mackay radiologic score was 14.3 ± 5.1 (mean value, S.D., range 3–22). Eight weeks later (i.e. 4 weeks after the end of the treatment) the values were 13.5 ± 6.0 (mean value, S.D., range 2–22), which means no statistical difference (p = 0.73).

Table 2

<table>
<thead>
<tr>
<th>Patients</th>
<th>Pain (visual analogue scale)</th>
<th>Smelling score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. age</td>
<td>sex</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>f</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>f</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>f</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>f</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>m</td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>f</td>
</tr>
<tr>
<td>9</td>
<td>62</td>
<td>f</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>m</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>f</td>
</tr>
<tr>
<td>12</td>
<td>37</td>
<td>f</td>
</tr>
</tbody>
</table>

^aMedian (maximum) and ^bmedian (minimum; maximum), respectively, of pain before, during, and after lavage, and subsequent duration of pain; n = 12 days of treatment. n.d. not determined.
4. Discussion

As local irrigation of the nasal and paranasal sinuses is a promising therapeutic concept in CRS [4,18], the development of well tolerable topical antimicrobial substances like NCT is suggested. Devices are necessary to apply these agents into the nasal and paranasal sinuses in effective amounts. This phase IIa study demonstrated the tolerability of NCT by irrigation of the nasal and paranasal sinuses via YAMIK-catheter. First signs of efficacy were observed.

The novel YAMIK catheter proved to be advantageous for both gaining mucous material for laboratory investigations and application of solutions to the nasal, maxillary and sphenoid sinuses. After a short period of exercise it is a simple, non-invasive, quick and sufficient means for treatment circumventing puncture procedures. Despite of some inconvenience caused by the manipulation, all test subjects well tolerated the 12 sessions. Restrictions are that gross polyps, distinct septal deviation or basal crests hinder a painless introduction. In some cases the remaining liquid inside the sinuses causes pain through pressure, sometimes extending to the upper jaw. In case of strong swelling of the mucosa the lavage effect may be insufficient because of impaired access to the sinuses.

Since at maximum half of the volume applied was sucked off again, several ml of NCT remained in the maxillary sinus and oxidation capacity could be detected for 3–4 h. Within human inflammation samples of different body regions, the activity is known to decrease about 45–90% after 2 h at 37 °C in vitro [8]. By application of 1 drop of 1% NCT to the human eye, oxidative power could be detected for 15 min [24], while still 11% of the applied concentration were measured in the urine after lavage of the human urinary bladder and clamping for 1 h [11]. The present study confirms the in vivo stability of 1% NCT being by far sufficient to kill bacteria and fungi within inflammatory mucus [8,9].

The irrigations with NCT were well tolerated by the mucosa as detected in endoscopic investigations. This confirms the in vitro finding, that 1% NCT had only minimal influence on the ciliary beat frequency of nasal mucosa samples (<10% reduction) in contrast to a 50% reduction by 7% cocaine [17]. The elevated pain during lavage was caused rather by the catheter and by the instillation procedure than by NCT, since in most cases it decreased rapidly again. The increased nasal secretion during the first few days of treatment might be due to the volume of fluid applied and trapped in the sinuses and to a minimal secretory stimulus of NCT. Although the Lund–Mackay radiologic score could not be reduced significantly, we clearly saw a decrease of the mucosal swelling of the nasal cavity by endoscopic inspection. This effect was probably responsible for the improved smelling sense and the subjective impression of improved breathing in most of the patients. It remains to be clarified, if it is caused by the lavage alone or specifically by NCT.

In vitro, NCT has been reported to downregulate tumor necrosis factor, prostaglandins, nitric oxide and interleukins [25–27]. Some of these proinflammatory cytokines take part in vasodilatation. Therefore, the diminution of tumefaction observed in the second study week and later on might be explained by an inhibitory effect of NCT on these substances.

Eosinophilic granulocytes were detected in five of nine mucus samples before the treatment in patients with and without polyps. After treatment the cytologic results remained the same. This was an unexpected finding after the therapy with an antifungal substance. Maybe this was due to a too short durage of therapy. Some authors report the necessity of at least 3 months of antifungal therapy for a successful treatment in CRS [4].

Fungi could be grown from mucus in 11 out of 12 patients. Obviously, the microbiological results of Table 1 represent a spectrum of species, which are inhaled everyday by chance. Ponikau et al. observed no difference in organisms grown from rhinosinusitis patients and healthy volunteers [3]. Because of these reasons it is not suggestive to use the microbiological findings as a basis for evaluation of therapeutical success.

5. Conclusion

Summing up, the novel YAMIK catheter system is a progress in lavage of the paranasal sinuses. NCT proved to be very well tolerated by the nasal and paranasal mucosa, which encourages further investigations on this promising substance.

Acknowledgments

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