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Therapeutics

Tolerability and efficacy of *N*-chlorotaurine in comparison with chloramine T for the treatment of chronic leg ulcers with a purulent coating: a randomized phase II study

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Summary

Background The well-known active chlorine compound chloramine T (CAT) with broad-spectrum antimicrobial activity is in common therapeutic use for leg ulcers with purulent coatings; however, this treatment is painful. The tolerability of the less aggressive *N*-chlorotaurine (NCT), an endogenous compound also produced *in vivo* by stimulated human granulocytes, could be superior.

Objectives To assess the tolerability and efficacy of NCT in the cleaning of purulent coatings in chronic leg ulcers in comparison with CAT.

Methods In a double-blind, randomized phase IIb clinical study 40 patients were treated for a median of 7 days (range 3–14) with a 1% aqueous solution of either NCT (20 subjects) or CAT (20 subjects) by twice-daily application of dressings soaked in the test solutions. Criteria for evaluation of tolerability were intensity and duration of pain caused by the ulcer therapy and scores of tissue toxicity (necrosis, granulation tissue and re-epithelialization). Therapeutic efficacy was graded as scores of intensity of purulent coating of the ulcers.

Results The concentration tolerated *in vitro* by human epidermoid carcinoma cells was at least 10-fold higher for NCT (0·01%) compared with CAT (0·0001–0·001%). There was significantly less pain caused by NCT compared with CAT ($P < 0·05$) on days 1 and 4 and a trend for a shorter duration of pain ($P = 0·093$). The scores of intensity of coating improved without difference in both treatment groups, whereas granulation and re-epithelialization appeared earlier in the NCT group ($P < 0·05$). Non-quantitative microbiological cultures from ulcer smears revealed persistence of colonization by bacterial species in approximately half of both treatment groups.

Conclusions Both active chlorine compounds were helpful in reducing purulent coatings. Because of its lower toxicity and better tolerability, NCT is of advantage in the treatment of leg ulcers.

Key words: chloramine T, leg ulcer, *N*-chlorotaurine, randomized phase II study, wound healing

Most leg ulcers are of venous or arterial origin (85%). Neuropathy, diabetes, autoimmunity or trauma may also play a role.[1,2] Bacterial contamination of ulcers is frequent, while infection characterized by apparent cellulitis and the associated host reaction is rarer.[3] However, intense contamination with bacteria leads to clinically relevant putrid membranes, which do not respond to systemic antibiotic therapy. Although the role of bacteria in the pathogenesis of leg ulcers is controversial, beneficial effects of local therapy with disinfectants on the healing time have been demonstrated for povidone-iodine.[3,4] Antimicrobial treatment has to target on predominant pathogens, i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Enterobacteriaceae.[5]

The disinfectant chloramine T (CAT), a well-known active chlorine compound, has been demonstrated to inactivate Gram-positive and Gram-negative bacteria

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in vitro,[6] and is also bactericidal *in vivo* when applied to contaminated wounds.[7,8] Topical application of CAT at a concentration of 1% in aqueous solution has been used successfully for decades in the Department of Dermatology and Venereology, University Hospital of Innsbruck (Innsbruck, Austria) to treat intensely contaminated leg ulcers with purulent coatings. However, because of significant pain caused by this treatment, novel disinfectants with improved tolerability would be advantageous.

N-chlorotaurine (NCT) as a mild endogenous active chlorine compound (Cl-HN-CH_2-CH_2-SO_3^-) might meet these requirements. It is generated by the reaction of the amino acid taurine with hypochlorous acid, a highly reactive oxidant produced by activated human granulocytes and monocytes.[9] One of its main functions may be the destruction of pathogens,[10,11] and NCT has been demonstrated to exert broad-spectrum bactericidal,[12–14] fungicidal[15,16] and viricidal[17] activity. In addition, it is thought to be involved in downregulation of proinflammatory cytokines such as tumour necrosis factor (TNF)-α, nitric oxide, prostaglandins, nuclear factor-κB and some interleukins (IL).[18–23]

The chemical synthesis of the sodium salt of NCT facilitated clinical studies using a concentration of 1% NCT in aqueous solution for topical application;[14] systemic use is impossible because of inactivation in the blood. When applied to the human and rabbit eye, it was very well tolerated in a phase I study,[24] and first signs of efficacy in bacterial conjunctivitis were observed in a phase IIa study.[25] The same was true for urinary tract infections caused by omniresistant *P. aeruginosa*, where NCT demonstrated rapid bactericidal activity,[26] and it was able to eradicate this pathogen in a case of localized cystitis.[27] In a mouse model, NCT was applied to the middle ear without severe side-effects.[28] Because of these encouraging findings we compared in the present double-blind, randomized phase IIb study the clinical efficacy and tolerability of the new compound NCT with CAT in bacterially contaminated chronic leg ulcers with purulent coatings. Both disinfectants were used as local adjuvant treatment to reduce purulent coating. In addition, their *in vitro* toxicity was investigated in a keratinocyte culture.

Materials and methods

Reagents

For clinical application, pure NCT as a crystalline sodium salt (molecular weight 181·57 g mol^{-1}, high purity grade; Gatt-Koller Inc., Absam, Austria) and CAT (trihydrate, molecular weight 281·69 g mol^{-1}, reagent grade; Merck, Darmstadt, Germany) were dissolved in sterile distilled water to a concentration of 1% (55 mmol L^{-1} NCT and 36 mmol L^{-1} CAT). Both solutions are colourless. The purity of NCT was verified by iodometric titration (calculated 19·53% Cl^+, observed 19·3% Cl^+, which equals 99% purity) and spectrophotometry showing a peak at 252 nm characteristic for the R-NHCl group. As aqueous solutions of NCT exhibit a pH of 8, no buffer was needed. Similarly, no preservatives were required as solutions stored at 2–4 °C showed high stability (9·3% loss per year). Solutions were allowed to reach room temperature before application. For *in vitro* testing, NCT and CAT were dissolved in 0·01 mol L^{-1} phosphate-buffered saline (PBS; pH 7·2), and serial 10-fold dilutions were performed in the same solvent.

In vitro *toxicity tests*

Human epidermoid carcinoma cells (line A 431, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; kindly provided by Christine Heufler-Tiefenthaler, Department of Dermatology, Innsbruck University Hospital) were grown at 37 °C and 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine, penicillin and streptomycin for 24 h. Subsequently, they were washed in PBS, suspended with trypsin, washed in RPMI + supplements and transferred to 96-well microtitre plates (90 μL of 2·5 or $5{\cdot}0 \times 10^3$ cells mL^{-1} to each well). After a further 24 h at 37 °C the medium was replaced by 90 μL of PBS.

NCT or CAT (10 μL each) previously diluted in PBS were added to final concentrations of 0·0001%, 0·001%, 0·01%, 0·1% and 1%. Controls were treated with PBS and RPMI, without additives. Cells were incubated at 37 °C for 30 min. Subsequently, test solutions were replaced by RPMI, and the cells were grown for 24 h. Growth and morphology of the cells were evaluated under a light microscope. Morphological criteria for damage were change of shape (fusiform to spherical), fragmentation and absence of growth. Viability was assessed by trypan blue (Sigma, Vienna, Austria) exclusion tests immediately after the 30-min incubation in the test solution and again 24 h later. All experiments were performed in duplicate.

In vivo *study*

Design. A double-blind, randomized phase IIb study was performed in 40 patients with bacterially contaminated

chronic leg ulcers with coatings. Twenty subjects were treated with 1% NCT (NCT test group, encoded as solution B) and 20 with 1% CAT (CAT comparison group, encoded as solution A). The study was in accordance with the Declaration of Helsinki and was approved by the ethical committee of the University of Innsbruck. All patients gave written informed consent.

Patients. The study population comprised 40 inpatients, 22 women and 18 men, age range 29–87 years, median 72, 25% percentile 53, 75% percentile 78 (Table 1). The main inclusion criterion was the presence of purulent coatings on the leg ulcers. Exclusion criteria were a known intolerance of CAT, ulcers without coatings, age under 18 years, pregnancy and participation in another study at the same time. The duration of disinfectant application was planned to be 5–14 days, until cleaning of the purulent coating from the ulcers was achieved, which was a prerequisite for further treatment strategies performed after the end of this study in order to obtain healing of the ulcers.

No patients were withdrawn after randomization. In seven patients the study was finished early, after 3–4 days, because of rapid disappearance of the purulent coating (three in the NCT group, two in the CAT group), pain (one in the NCT group, three in the CAT group) or for reasons not connected with the study (one in the NCT group). Values gained from these patients were included in the results (intention-to-treat). Three subjects with ulcers on both legs received NCT on the one and CAT on the other side in a double-blind and randomized fashion.

Table 1. Patient profile

	NCT	CAT
Age (years)[a]	73 (30–87)	68 (29–86)
Sex	10 F/10 M	12 F/8 M
Type of ulcer (*n*)		
Venous	9	10
Arterial	5	1
Diabetic	3	3
Pyoderma gangrenosum	2	2
Decubitus	0	2
Ecthyma gangrenosum	1	1
Haemorrhagic erysipelas	1	0
Traumatic	1	0
Primary chronic polyarthritis	0	1
Combustion	1	0
Area of ulcer (cm^2)[a]	35 (4–300)	25 (2–225)
Duration of study therapy (days)[a]	7 (3–14)	7 (4–14)
Previous ulceration period (months)[a]	4 (0·1–180)	4 (0·3–36)
Previous treatments (*n*)	11	13
Erysipelas (*n*)	7	8
C-reactive protein on day 0 (mg dL^{-1})[a]	1·6 (0–11·2)	3·2 (0–22)

NCT, *N*-chlorotaurine; CAT, chloramine T. [a]Values are shown as median (range). $P > 0{\cdot}05$ between both test groups for all parameters.

Evaluation of symptoms. Medical status was determined by evaluation of the medical and drug history and by detailed dermatological examination. As a subjective symptom of tolerability the intensity and duration of pain caused by the study medication were evaluated by a visual analogue scale (VAS): 0 = no pain, 10 = intolerable pain.

The following objective conditions were scored: (i) intensity of coating, a measure of therapeutic efficacy, as assessed by the appearance of inflammatory membranes (none = 0, fibrous = 1, thin and purulent = 2, thick and purulent = 3); and (ii) local toxicity, as assessed by the presence or absence of granulation tissue (yes = 0, no = 2), re-epithelialization (yes = 0, no = 2) and necrosis (none = 0, 0–1 cm = 1, > 1 cm = 2).

Bacterial colonization was determined by qualitative cultural and biochemical characterization of pathogens gained from swabs from the ulcer. The clinical diagnosis of the ulcers (venous, arterial, diabetic, other), the localization, the approximate size in centimetres, the duration of ulceration before the beginning of the study, previous treatments, concomitant erysipelas, and C-reactive protein level were noted. In cases of additional cellulitis, systemic antibiotic treatment was started.

Treatment and time course. All the patients were treated on the ward by the same team of nurses according to strict hygienic standards (sterility) under the supervision of one single experienced dermatologist (R.H). Subsequent to a baseline evaluation of pain and recording of objective findings, 8 × 8 cm cotton swabs were soaked with 1% NCT or CAT, placed directly on to the ulcers and fixed with a bandage. This procedure was performed twice daily (morning and afternoon). Overnight swabs soaked with 2% saline were used in both groups. The surrounding of the ulcers was protected with vaseline.

Patients were examined daily with the collection of score data just before and after application of the solutions on days 1, 3–5, and the last day of the study depending on improvement as described above. The intensity of pain was evaluated twice on these days by the nurses just before and shortly

after application of disinfectant. The duration (in minutes) of pain caused by NCT or CAT was documented.

Statistical analyses

Intensity and duration of pain as well as the inflammation and toxicity scores were compared between both treatment groups using the Mann–Whitney *U*-test. Wilcoxon's test was applied to compare these parameters before and after treatment separately for each group. Baseline characteristics such as age of patients, duration of study treatment, size and duration of ulcer, and C-reactive protein were also compared using the Mann–Whitney *U*-test. For categorical baseline characteristics, i.e. gender, type of ulcer, previous treatments and concomitant erysipelas a χ^2 test was applied. $P < 0{\cdot}05$ was considered to indicate statistical significance. Descriptive statistics were used for the *in vitro* results.

Results

In vitro *toxicity*

Human epidermoid carcinoma cells tolerated a concentration of NCT of 0·01% for 30 min without any visible damage ($n = 7$). For CAT the concentration was 10–100 times lower (0·001–0·0001%) ($n = 7$). In the presence of 1% NCT or 0·01%, 0·1% and 1% CAT, almost all cells changed in shape from fusiform to spherical. As evaluated 24 h after exposure to these high concentrations, they did not grow any more and became fragmented. With 0·1% NCT, all cells were destroyed in two of seven tests, while cell numbers were reduced to $\approx 50\%$ in three tests and to $< 5\%$ in two tests. In two of seven tests, 10–20% of cells were damaged by 0·001% CAT.

Trypan blue exclusion tests showed a $> 95\%$ loss of viability immediately after incubation in 0·1–1% CAT and 1% NCT ($n = 4$). The values ranged between 50% and 100% for 0·01% CAT, and between $< 5\%$ ($n = 2$) and 50% ($n = 2$) for 0·1% NCT. All cells ($> 95\%$) treated with NCT at a concentration of 0·01% or lower remained viable, while 10–20% of those treated with 0·001% CAT appeared dead and had changed their shape in two of four experiments.

In vivo *study*

There was no baseline difference between the study groups treated with NCT or CAT (Table 1). With both disinfectants the ulcer pain increased significantly after

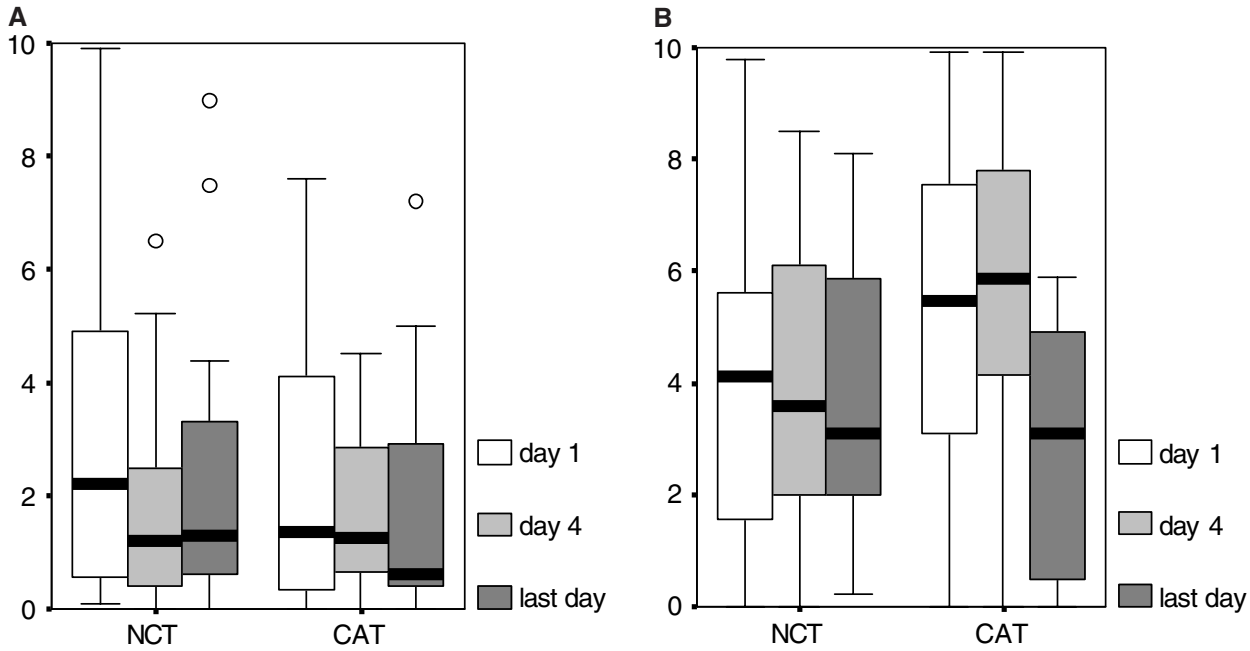

Figure 1. Ulcer pain (A) before and (B) after application of swabs soaked with *N*-chlorotaurine (NCT) or chloramine T (CAT), evaluated by visual analogue scale. Results are given as box plots showing median (bold line), 25th and 75th percentiles, inner fences and outliers (o). The absolute intensity of pain on day 4 ($P = 0{\cdot}033$) and the increase of pain caused by therapy on day 1 ($P = 0{\cdot}024$) and day 4 ($P = 0{\cdot}049$) were lower in the NCT group.

application in comparison with the baseline score ($P < 0{\cdot}01$). At the end of therapy, when the purulent coating had disappeared, this effect became less obvious (Fig. 1). The absolute intensity of pain after application of disinfectant was lower in the NCT group than in the CAT group on day 1, and on day 4 this finding was significant. The increase of pain after dosing was significantly lower in the NCT patients on both days 1 and 4 (Fig. 1). At the end of the treatment period pain was in general less pronounced, and no statistical difference between NCT and CAT was detected. The duration of increased pain caused by disinfectant tended to be longer in the CAT group, but this trend did not reach significance (Fig. 2).

On day 1, the score for the intensity of coating was 2–3 in 96% of patients in the NCT group and in 90% of patients in the CAT group, and it improved with both disinfectants to the values of 0 or 1 in 40% on day 4 ($P < 0{\cdot}01$ for both substances). On the last day, the score had improved in all patients of both groups: 72% of the NCT patients had a score of 0 or 1, and 28% a score of 2, while 100% of the CAT patients had a score of 0 or 1. This difference was probably due to a higher percentage of subjects in the NCT group with a score of 3 before the beginning of treatment (52% vs. 20%). Therefore the therapeutic efficacy of both solutions was similar.

The toxicity score decreased in the NCT group on the last day of therapy compared with baseline ($P = 0{\cdot}009$), in contrast to the CAT group ($P = 0{\cdot}71$) (Fig. 3). This difference was because of increased granulation ($P = 0{\cdot}050$) and re-epithelialization ($P = 0{\cdot}034$) in the NCT group. Increased necrosis as a sign of severe toxicity was not seen with either disinfectant.

In two of three patients receiving bilateral treatment the ulcers were of similar size, enabling a direct comparison of both disinfectants. In these two subjects the intensity of pain caused by CAT was higher than that caused by NCT. The differences in VAS points were 4·1 and 4·5 on day 1, 1·9 and 6·5 on day 4, and 2·1 on the last day. In addition, the duration of pain caused by therapy was 50% longer for CAT than for NCT on day 1 (45 vs. 20 min, and 120 vs. 60 min). On day 4, the trend was the same in one patient (20 vs. 5 min), but not in the other (120 min for both NCT and CAT). The reduction of purulent coating was identical for both agents on all 3 days of evaluation. In the third patient the ulcer treated with NCT proved to be more painful, probably because of its 10-fold larger size compared with that on the other side treated with CAT.

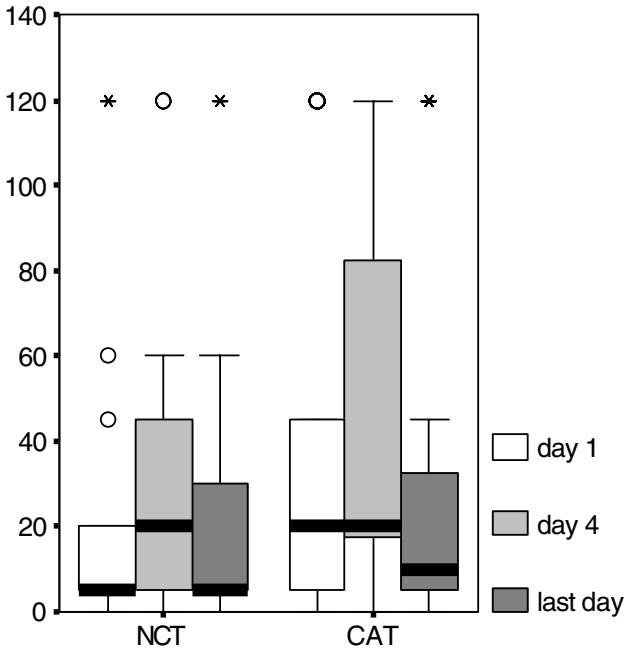

Figure 2. Duration of increased pain (min) subsequent to application of *N*-chlorotaurine (NCT) and chloramine T (CAT), respectively. $P > 0{\cdot}05$ for all differences between the substances ($P = 0{\cdot}093$ on day 1, $P = 0{\cdot}214$ on day 4 and $P = 0{\cdot}600$ on the last day). Results are given as box plots showing median (bold line), 25th and 75th percentiles, inner fences and outliers (o) and extreme values (*).

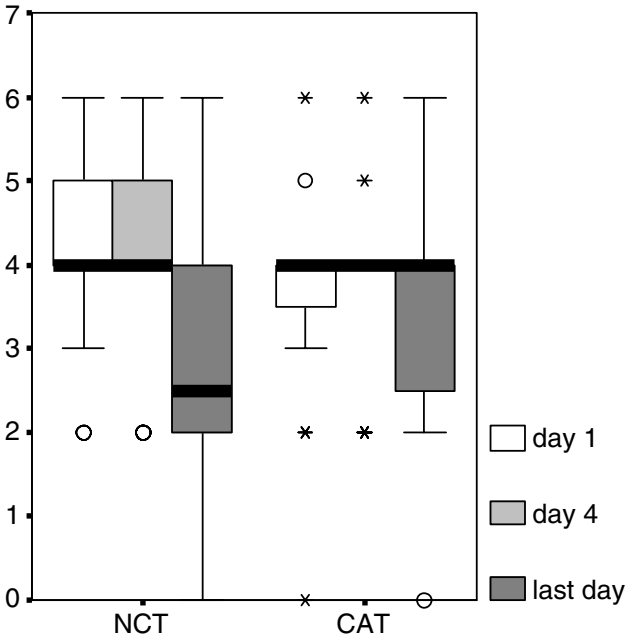

Figure 3. Toxic scores during treatment with *N*-chlorotaurine (NCT) and chloramine T (CAT). $P = 0{\cdot}009$ for NCT on the last day compared with baseline; $P = 0{\cdot}71$ for CAT. Results are given as box plots showing median (bold line), 25th and 75th percentiles, inner fences, outliers (o) and extreme values (*).

Table 2. Microbiological cultures of ulcer smears

	CAT			NCT			NCT + CAT	
	No.[a]	%	End[b]	No.[a]	%	End[b]	No.[a]	%
Staphylococcus aureus	10	50	5	10	50	5	20	50·0
Pseudomonas aeruginosa	5	25	5	12	60	9	17	42·5
Proteus sp.	2	10	1	5	25	2	7	17·5
Enterococcus sp.	2	10		3	15	1	5	12·5
Staphylococcus epidermidis	3	15	2	2	10	1	5	12·5
Streptococcus sp.	3	15		2	10	1	5	12·5
Escherichia coli	1	5	1	3	15	2	4	10·0
Klebsiella sp.	1	5		3	15		4	10·0
Enterobacter sp.	2	10	2	1	5	1	3	7·5
Serratia sp.	1	5		1	5	1	2	5·0
Stenotrophomonas maltophila	1	5		1	5	1	2	5·0
Bacteroides fragilis	0	0		1	5		1	2·5
Morganella morganii	1	5	1	0	0		1	2·5
Pasteurella aerogenes	0	0		1	5	1	1	2·5
Fusobacterium nucleatum	1	5		0	0		1	2·5
Peptostreptococcus sp.	0	0		1	5		1	2·5

NCT, *N*-chlorotaurine; CAT, chloramine T. [a]Related to the number of patients (20 per group); [b]number of positive cultures at the end of therapy.

Swabs taken from the ulcers revealed *S. aureus* and *P. aeruginosa* as the predominant bacteria, followed by Enterobacteriaceae (Table 2). In 35% we found more than one species in the same patient. Qualitative microbiological findings during or at the end of therapy revealed persistence of identical species in the majority of cases, but overall the percentage of positive cultures decreased from 87% to 54% for NCT and from 95% to 52% for CAT. Colonization with pathogens was still detectable at the end of therapy in many cases despite improvement and clinically obvious disappearance of the purulent coating (Table 2).

We made an interesting observation in one immunosuppressed subject with severe, large ulcers of pyoderma gangrenosum. This patient had previously been treated with CAT for several weeks before being switched to NCT after inclusion into the study. Within days, there was a decrease in pain with NCT in comparison with the pain reported with CAT by the patient, enabling a reduction of the pre-existing medication of pain-relieving drugs. In addition, a marked reduction of coating and activity of the pyoderma was noted, allowing successful surgical skin grafting within 2 weeks.

Discussion

Local antimicrobial therapy of severely contaminated chronic leg ulcers is reasonable because, in comparison with systemic treatment, higher concentrations of agents are achieved at the site of purulent membranes on the ulcers. Local infections with purulent coatings do not respond to systemic antibiotic therapy alone, and infections with *Pseudomonas* are particularly difficult to treat. Disinfectants should also be preferred to antibiotics for several reasons, such as superior microbicidal activity and absence of development of bacterial resistance. However, their toxic potential must be taken into consideration.

As expected from its higher reactivity, the concentration of CAT tolerated by human epidermoid keratinocytes *in vitro* was 10–100-fold lower (0·001–0·0001%) than for NCT (0·01%). These findings are in accordance with those of other investigators. NCT (0·5 mmol L^{-1}; 0·01%) did not affect cell viability of macrophages, dendritic cells, synoviocytes and endothelial cells.[18,21–23,29]

Regarding *in vivo* application, the absence of adverse effects of CAT on wound healing has been reported in skin wounds of guinea pigs infected with *P. aeruginosa* and in humans, where it has been used for peritoneal lavage.[7,30] On the other hand, CAT inhibited the production of collagen in skin defects of rats and arrested the capillary circulation of granulation tissue in rabbit ear chambers, both leading to a delayed repair process.[31,32] In clinical studies evaluating NCT, no adverse effects were observed when treating cystitis or conjunctivitis except for mild eye burning or eye itching.[25,26] When applied to the middle ear of the mouse, 1% NCT caused a little local irritation and a healing delay of the artificial defect of the tympanic membrane, but no residual defect was found.[28]

In the present study on leg ulcer therapy NCT was tolerated better than CAT and both oxidants were tolerated at higher concentrations *in vivo* than in the cell culture. Clinically undetectable impairment of cell functions cannot be excluded, but this observation may be explained by their hydrophilic properties, which allow oxidation of the surface only and inhibit penetration into the viable tissue. Accordingly, NCT applied to the inflamed human urinary bladder did not cause any clinically evident or other signs for systemic toxicity such as changes in the blood concentration of haemoglobin.[26] The same is true for the present study, including routine laboratory examinations continued in the follow-up period.

Satisfactory reduction of purulent coating was achieved in both groups. However, the relation between activity and tolerability seems to be superior for 1% NCT. Impairment of wound healing was less pronounced in the NCT group, as granulation tissue and re-epithelialization appeared earlier.

The major result of our study became particularly evident in the two subjects with equally large ulcers on both legs. NCT as the milder oxidant caused significantly less pain compared with CAT. All these positive observations might be due not only to the lower oxidative power of NCT but also to its known activity in the downregulation of proinflammatory cytokines (TNF-α, nitric oxide, IL-1β, IL-2, IL-6 and IL-8).[18,19,21–23] Dendritic cells incubated in the presence of NCT were shown to release decreased amounts of TNF-α, IL-6, IL-10, IL-12, nitric oxide and prostaglandin E_2, and the production of IL-10 by T cells coincubated with dendritic cells pretreated with NCT was reduced.[23] Therefore, NCT might also decrease destructive tissue effects of inflammation and accelerate wound healing. The excellent response of severe pyoderma gangrenosum to the application of NCT in our single patient suggests that such anti-inflammatory effects could be of additional advantage for its therapeutic use.

The spectrum of pathogens (Table 2) was similar to that found in previous studies, with *S. aureus*, *P. aeruginosa* and Enterobacteriaceae prevailing.[5,33,34] Positive bacterial cultures from wound smears taken in the course of therapy do not predict protracted healing but demonstrate colonization only.[3,33] Accordingly, even quantitative cultures showed no reduction in colony counts of *S. aureus*, *P. aeruginosa* and other species in ulcers treated with 0·25% CAT for 15 min.[5] Bacterial colonization of the ulcer surface seems not to be clinically relevant: only massive proliferation or invasion impairs healing. However, microorganisms invading into the tissue are not reliably detectable by swabs.[3] In other words, smears or, better, material obtained from tissue after removing purulent membranes should be taken for detection of the source of systemic infection and for susceptibility testing rather than for the monitoring of therapeutic success.

In summary, both CAT and NCT proved to be equally effective in removing the purulent coating of crural ulcers within a few days. Because of its lower cellular toxicity and significantly less painful treatment, NCT seems to be of advantage and should be preferred for the treatment of crural ulcers.

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