N-chlorotaurine, a natural antiseptic with outstanding tolerability

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N-chlorotaurine, the N-chloro derivative of the amino acid taurine, is a long-lived oxidant produced by activated human granulocytes and monocytes. Supported by a high number of in vitro studies, it has mainly anti-inflammatory properties and seems to be involved in the termination of inflammation. The successful synthesis of the crystalline sodium salt (Cl-HN-CH2-CH2-SO3Na, NCT) facilitated its development as an endogenous antiseptic. NCT can be stored long-term at low temperatures, and it has killing activity against bacteria, fungi, viruses and parasites. Transfer of the active chlorine to amino groups of molecules of both the pathogens and the human body (transhalogenation) enhances rather than decreases its activity, mainly because of the formation of monochloramine. Furthermore, surface chlorination after sublethal incubation times in NCT leads to a post-antibiotic effect and loss of virulence of pathogens, as demonstrated for bacteria and yeasts. Being a mild oxidant, NCT proved to be very well tolerated by human tissue in Phase I and II clinical studies. A 1% aqueous solution can be applied to the eye, skin ulcers, outer ear canal, nasal and paranasal sinuses, oral cavity and urinary bladder, and can probably be used for inhalation. Therapeutic efficacy in Phase II studies has been shown in external otitis, purulently coated crural ulcerations and keratoconjunctivitis, so far. Based upon all presently available data, NCT seems to be an antiseptic with a very good relation between tolerability and activity. Recently, C-methylated derivatives of NCT have been invented, which are of interest because of improved stability at room temperature.

Keywords: chloramines, active chlorine compounds, monochloramine, biocide, disinfection

Introduction

It has been known for >40 years that oxidants are involved in the action of the human defence system. Among them, so-called long-lived oxidants (N-chloro derivatives of amino acids and peptides) were identified to contribute not only to the killing of pathogens invading the body, but also to controlling the inflammatory response.

An important representative of this class of compounds is N-chlorotaurine (NCT), which plays an essential role in vivo because of both its relatively high concentration and its superior stability. The compound is also cited as ‘taurine chloramine’, a term that, however, does not conform to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature recommendation. It was a logical challenge to try to make NCT available for practical use, because its well-documented occurrence in the human body and proved germicidal activity suggest it as an antiseptic, which implies a minimal potential of intolerance. Subsequent to the successful synthesis of NCT in form of the sodium salt, an endogenous compound was available in ample quantities, which, indeed, revealed an optimal compromise between sufficient disinfecting power and good tissue compatibility. Both are basic requirements for an antiseptic in human medicine.

Historical survey and functions of NCT in vivo

The first extended studies on active chlorine compounds (bearing O-Cl or N-Cl functions) date from the First World War, and refer to the activity of chloramine T (CAT) and hypochlorous acid in vitro and in vivo. In 1930, the killing of spores of Bacillus anthracis by N-chloroglycine in its dichlorinated form was described. The first explicit quotation of NCT (denoted as ‘taurine chloramine’) was in 1971 by researchers from Poland, who found the chlorination of amino acids by the myeloperoxidase system.

In the following years, the properties and possible functions of NCT as the main long-lived oxidant produced by leucocytes were investigated, which comprise the killing of pathogenic organisms and immune-modulatory reactions. The first systematic quotation of NCT’s killing activity was in 1987, against larvae of the helminth Schistosoma mansoni.

Some preliminary data indicating antibacterial and antifungal activity were published by Thomas and Wagner in 1987. Comprehensive studies followed, facilitated by the availability of NCT as a pure sodium salt since 1989 and by the assessment of its intrinsic chemical properties. In vitro, NCT proved to kill bacteria, fungi (yeasts and moulds), and protozoa.
In the 1990s, it was found that concentrations of NCT of up to 0.5 mM were not cytotoxic but caused a down-regulation of mainly proinflammatory chemokines, cytokines and enzymes, i.e. prostaglandin E₂, tumour necrosis factor α, nitric oxide, interleukins 1β, 2, 6, 8 and 12, collagenase, and neopterin. As one basic mechanism, Met45 oxidation of iκB-α followed by inhibition of the activation of NF-κB have been described. Another mechanism is the induction of heme oxygenase-1, a stress-inducible enzyme with anti-inflammatory properties, at mRNA and protein levels. Additionally, NCT induces a proliferative arrest independent from the cell cycle, followed by apoptotic cell death. All these reports support the concept that NCT is involved in the termination of inflammation, although activation of the complement system has also been reported.

### Chemical issues

#### Formation in vivo

By a cascade of enzyme-catalysed reactions (O₂ → O₂⁻ → H₂O₂ → HOCl) known as the respiratory (or oxidative) burst, special leukocytes (neutrophilic and eosinophilic granulocytes, monocytes and, in small amounts, probably macrophages) are able to synthesize the very strong oxidant and chlorinating agent hypochlorous acid (HOCl) from oxygen, hydrogen ions and chloride ions.

In turn, HOCl reacts with N-H compounds occurring in the intra- and extracellular environment of leukocytes:

\[
R-\text{NH}_2 + \text{HOCl} \rightarrow R-\text{NHCl} + \text{H}_2\text{O}
\]  

Because taurine amounts to ~50% of the amino acid pool of neutrophilic granulocytes, NCT is the main reaction product, which, moreover, exhibits surprising stability. The above reaction (Equation 1) is also understood as a detoxification of the very strong oxidant HOCl, which would destroy living cells.

#### Synthetic approach

Although aqueous solutions of NCT can be obtained easily by reaction of taurine with chlorinating agents such as hypochlorite, the isolation of the pure substance did not succeed in the aqueous system. Unexpectedly, this was possible by a heterogeneous one-step reaction of taurine with an ethanolic solution of CAT. It yielded the only slightly soluble sodium salt of NCT (NCT-Na, mol. wt 181.57), which could easily be separated from the well-soluble by-product toluenesulphonamide:

\[
\begin{align*}
\text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^- + \text{CH}_3\text{C}_6\text{H}_5\text{SO}_2\text{NCla} &\rightarrow \\
\text{ClHNCH}_2\text{CH}_2\text{SO}_3\text{Na} + \text{CH}_3\text{C}_6\text{H}_5\text{SO}_2\text{NH}_2
\end{align*}
\]  

The reaction implies the simultaneous transfer of sodium and chloride from CAT to taurine, and represents a special feature, since it failed with connatural compounds such as 2,2-dimethyltaurine, β-alanine and γ-aminopropanesulphonic acid.

### Chemical characteristics

NCT is the N-chloro derivative of the β-aminosulphonic acid taurine, a conditionally essential amino acid not involved in the set-up of peptides. The substitution of the amine function of taurine by a chlorine atom, e.g. by reaction with HOCl (Equation 3), results in the abolition of its basic properties, and is why it loses the betaine structure. The emerging acid NCT-H disproportionates very fast, forming taurine and the strong acid N,N-dichlorotaurine (NDCT-H) (Equation 4):

\[
\text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^- + \text{HOCl} \rightarrow \text{ClHNCH}_2\text{CH}_2\text{SO}_3\text{H} + \text{H}_2\text{O}
\]  

\[
2\text{ClHNCH}_2\text{CH}_2\text{SO}_3\text{H} \leftrightarrow \text{Cl}_2\text{NCH}_2\text{CH}_2\text{SO}_3\text{H} + \text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^-\
\]

Completely different is the sodium salt of NCT, whose anion undergoes hydrolysis (Equation 5), forming initially a weak alkaline solution (pH ~9) that soon, partly by the action of airborne CO₂, is neutralized, equilibrating between pH 8.2 (1% NCT) and pH 7.1 (0.1% NCT).

\[
\text{ClHNCH}_2\text{CH}_2\text{SO}_3^- + \text{H}_2\text{O} \leftrightarrow \text{ClH}_2\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^- + \text{OH}^-
\]

Since disproportionation is largely suppressed in neutral and, particularly, in weak alkaline solution, NCT self-actingly produces the right milieu (pH 7–8) for an optimal stability.

NCT sodium salt forms colourless crystals that decompose at 135–140°C. Already, at the temperature of a normal refrigerator (2–4°C) both the salt and aqueous solution demonstrate sufficient stability for practical use. The decrease of oxidation capacity [c(Ox)] was only 10% within 12 months.

### Oxidizing properties

The oxidizing properties of NCT can be attributed to the N-Cl function according to the general equation:

\[
\text{NCl} + \text{H}^+ + 2\text{e}^- \rightarrow \text{NH} + \text{Cl}^- 
\]

A typical oxidizing reaction of NCT, e.g. with a thiol (Equation 6), reads as:

\[
2\text{R}-\text{SH} + \text{NCT} \rightarrow \text{R}-\text{SS}-\text{R} + \text{taurine} + \text{Cl}^- 
\]

and shows that the final products of its reduction are taurine and chloride.

Against activated aromatic (Ar) C-H functions (phenols, histidine), NCT acts as a chlorinating agent according to Equation (7):

\[
\text{Ar-H} + \text{NCT} + \text{H}^+ \rightarrow \text{Ar-Cl} + \text{taurine} 
\]

NCT is a weak oxidant and lies on the lower end of the following sequence, which is ranked according to decreasing oxidizing potency: HOCl > chloroisocyanuric acids > chloramides > chloramines.

Accordingly, unlike HOCl or chloroisocyanuric acids, NCT does not react with amides and alcohols. Of particular importance are reactions with amines, which are discussed below.
Transhalogenation equilibria

An essential property of N-chloro compounds, which was named ‘transhalogenation’ and ‘transchlorination’, designates the transfer of the halogen of an N-chloro compound to another N-H compound:

$$R\text{-NHCl} + R'\text{-NH}_2 \leftrightarrow R\text{-NH}_2 + R'\text{-NHCl} \quad (8)$$

In general, it deals with an equilibrium that is reached at room temperature within minutes and that needs no catalyst. It suggests that in the presence of N-H compounds (amino acids, peptides) not only one but diverse chlorinating agents can be responsible for a defined reaction. The following three transhalogenation reactions of NCT are important for understanding its activity.

Disproportionation

The above-mentioned Equation (5) discloses that a hydroxyl ion is produced with the effect that the pH of the solution increases and disproportionation is additionally slowed down. This reaction brings about a dynamic pH regulation important for the long-term stability of NCT solutions, e.g. by neutralizing the hydrochloric acid that emerges at the intramolecular redox decay reaction shown in Equation (10).

Equilibration with amino acids

An important feature of NCT concerns the increase of microbicidal activity in the presence of N-H compounds such as glycine and α- and β-alanine, which can override consumption and degradation effects. The origin of this effect, which increases in the order glycine≈α-alanine<β-alanine, is the formation of the corresponding N-chloro derivatives, whose bactericidal activity is higher than that of NCT alone. One can derive that at pH 7 the portion of not dissociated, i.e. not charged, free amino acid is 400 times higher for glycine (pKa=2.36) and α-alanine (pKa=2.35) and 7000 times higher for β-alanine (pKa=3.55) compared with NCT (pKa=-0.3). This could explain a fostered penetration of the uncharged molecules, resulting in an improved bactericidal activity.

Monochloramine formation

A similar reaction, however, with a really drastic increase in bactericidal and fungicidal activity takes place in the presence of ammonium, whereby monochloramine is produced (Equation 9):

$$\text{ClHNCH}_2\text{CH}_2\text{SO}_3^- + \text{NH}_4^+ \leftrightarrow \text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^- + \text{NH}_3\text{Cl} \quad (9)$$

The mass-law constant of this reaction \((5.8 \pm 1.2 \times 10^{-3})\) shows that the equilibrium of Equation (9) lies on the left side.

The specificity of this reaction is that at room temperature and without any buffer it yields only monochloramine. If strongly chlorinating agents such as CAT are used, also di- and trichloramine, NHCl2 and NCl3, very unpleasantly smelling by-products, are formed, which precludes application in practice.

The combination of NCT and ammonium chloride offers for the first time the chance to use NH2Cl as an antiseptic agent in human and veterinary medicine. Its enhanced bactericidal activity originates from its small bulk and the absence of charge (different to NCT), which both facilitate the penetration of cell walls.

Stability

The N-chloro derivatives of α-aminocarboxylic acids (e.g. glycine and alanine) disintegrate very fast at room temperature, i.e. within hours to some days. The reasons for this are the reactions presented in the following paragraph, which finally lead to the complete loss of ClOx. In the case of the β-amino-sulphonic acid NCT the same reactions take place, but extremely slowly. Both the pure compound (sodium salt of NCT) and its aqueous solution lose only 10% activity per year if stored in a refrigerator (2–4°C). This is an acceptable value in view of application in medical practice.

Degradation and transformation products

All N-chloro compounds containing the structure element R-CH2NHCl spontaneously undergo a degradation by first splitting off HCl followed by hydrolysis of the intermediary imine, which yields an aldehyde and ammonia:

$$\text{ClHN-CH}_2\cdot\text{R} \rightarrow \text{HN=}=\text{C-}=\text{R} + \text{H}^+ + \text{Cl}^- \quad (10)$$

$$\text{HN=}\text{C-}=\text{R} + \text{H}_2\text{O} \rightarrow \text{O}=\text{CH-}=\text{R} + \text{NH}_3 \quad (11)$$

The observed order can be explained by the dissimilarity of the carboxylate and sulphonate of the isosteric molecules N-chloro-β-alanine and NCT, while the interjacent CH3 group improves the stability of N-chloro-β-alanine compared with N-chloro-α-alanine.

Another reaction affecting stability concerns transformation to NDCT by disproportionation (see above). Since NDCT exhibits antiseptic properties too, this reaction is not connected with a remarkable decrease of microbicidal activity.

General formulation of NCT use-solutions

Differing from common pharmaceutical practice, use-solutions containing NCT are made without a buffer. One reason is that NCT spontaneously brings forth a weak alkaline pH where disproportionation is widely repelled (see the ‘Chemical characteristics’ section above); a second reason is that the commonly employed phosphate buffer increases disproportionation. The same applies also to mixtures of NCT and NH2Cl. A buffer would thwart the benefits of the dynamic pH regulation illustrated in the ‘Chemical characteristics’ section and is therefore not used. Since NCT has
no buffering capacity, it exerts only a marginal change of the prevailing pH upon addition to a buffered system.

Microbicidal properties

Reactions with biological material

From the foregoing it can be derived that there are three pathways for NCT to react with biological material, which differ concerning reaction rates and the fate of c(Ox).\textsuperscript{10,58–60} NCT reacts

- with thiols very fast—c(Ox) is completely lost;
- with activated aromatic compounds (phenol, histidine) moderately fast—c(Ox) is completely lost;
- with amines (amino acids, ammonium)—an equilibrium is established within minutes, c(Ox) remains intact; but
- not with amides and alcohols (as opposed to HOCl and chloroisocyanuric acids).

All these reactions contribute not only to the microbicidal activity, but they are also the reason for unwanted reactions such as tissue irritation and consumption effects. The latter effects are only weakly pronounced, based on NCT’s moderate and restricted oxidizing power.

Table 1. Bactericidal and fungicidal activity of 1% NCT in phosphate buffer at pH 7.1 and 37°C

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Log(_{10}) reduction in cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus ATCC 25923}</td>
<td>2.5–5.0</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus ATCC 6538}</td>
<td>5.0</td>
</tr>
<tr>
<td>\textit{Staphylococcus epidermidis ATCC 12228}</td>
<td>3.6–3.8</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus Smith diffuse}</td>
<td>&gt;6.7</td>
</tr>
<tr>
<td>MRS\textsuperscript{a} clinical isolate</td>
<td>2.0–2.3</td>
</tr>
<tr>
<td>\textit{Streptococcus pyogenes d68}</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>\textit{Escherichia coli ATCC 11229}</td>
<td>3.8 to &gt;5.0</td>
</tr>
<tr>
<td>\textit{Proteus mirabilis ATCC 14153}</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>\textit{Pseudomonas aeruginosa ATCC 27853}</td>
<td>3.8–5.0</td>
</tr>
<tr>
<td>\textit{Candida albicans}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Candida dubliniensis}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Candida kruzei}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Candida glabrata}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Candida tropicalis}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Candida parapsilosis}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Aspergillus flavus}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Aspergillus fumigatus}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Alternaria alternata}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Fusarium moniliforme}</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Penicillium commune}</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Methicillin-resistant \textit{Staphylococcus aureus}.

\textsuperscript{b}Not significant (\(P>0.05\)).

Antimicrobial activity

NCT without additives

A summary of the log\(_{10}\) reduction of viable counts by 1% NCT (55 mM) in phosphate buffer solution is provided in Tables 1 and 2. \textit{Staphylococcus aureus}, including methicillin-resistant strains, \textit{Escherichia coli}, \textit{Proteus mirabilis}, \textit{Pseudomonas aeruginosa}, \textit{Streptococcus pyogenes}, \textit{Diplococcus pneumoniae}, Klebsiella spp. and \textit{Helicobacter pylori} are killed within a few minutes to 20 min at 37°C and within 20–60 min at 20°C\textsuperscript{21–23,67} (also M. Nagl, unpublished results). Incubation times of 7 days are needed for a 3 log\(_{10}\) reduction of mycobacteria at 20°C.\textsuperscript{63}

\textit{Candida albicans}, \textit{Candida kruzei}, \textit{Candida glabrata}, \textit{Candida tropicalis} and \textit{Candida dubliniensis} are killed within 2–5 h at 37°C.\textsuperscript{22,23,26} and \textit{Aspergillus} spp., \textit{Penicillium} spp., \textit{Fusarium} spp. and \textit{Alternaria} spp. after 4–8 h at 37°C.\textsuperscript{25} Herpes simplex virus types 1 and 2, all relevant types of adenovirus, and HIV-1 are inactivated within 5–60 min at 20°C.\textsuperscript{27–29} and influenza viruses within a few minutes according to presently performed investigations (M. Nagl, unpublished results). Acanthamoebae, leishmaniae and trichomonads are killed within ~1 h at 37°C, while cysts of acanthamoebae are more resistant.\textsuperscript{30,68}

In the study of 1987, \textit{S. mansoni} larvae were killed after 18 h by physiological concentrations of 50 \(\mu\)M NCT.\textsuperscript{17} Bactericidal activity against \textit{staphylococci}, \textit{E. coli}, \textit{P. mirabilis} and \textit{P. aeruginosa} could be found even by 12.5 \(\mu\)M.\textsuperscript{24}
Tests with bacteria and fungi performed with NCT in the presence of ammonium chloride with both compounds in the range of 0.1%–1.0% disclosed a drastic reduction of killing times.62 The most impressive results were obtained with both mycobacteria and moulds. *Mycobacterium terrae* was reduced below the detection limit within 20 min,63 while hyphae and spores of moulds were killed within surprisingly short incubation times of a few minutes.25,69 Moreover, cysts of acanthamoebae could be inactivated,30 and there is a significant reduction even of bacterial spores (M. Nagl, unpublished results). The reason for this increased activity can clearly be attributed to the formation of monochloramine, which demonstrated a high activity as a pure compound without NCT.69–71

**pH effects**

The microbicidal activity against bacteria and yeasts shows a significant pH effect, with shorter killing times at pH 4–6 compared with the normal environment at pH 7,22,24 which can be explained as follows. Increasing acidity favours the formation of the protonated form NCTH⁺ where the chlorine atom bears a positive partial charge. This species is more likely to come into reaction with nucleophilic agents such as R-NH₂ than it is the case with non-protonated NCT. The entity NCTH⁺, therefore, can be regarded as the active form in chlorination reactions.70 The increase of its portion at pH 5, at any rate, makes plausible the higher chlorinating and bactericidal activity in the more acidic milieu.

**Influence of reducing substrates**

**Body fluids**

The interaction with body fluids has an ambivalent impact, as they can both decrease and increase the anti-septic activity of NCT.23,25 On the one hand, complete consumption of c(Ox), up to a concentration of 1% NCT, is possible, e.g. in human whole blood (C. Martini and M. Nagl, unpublished results). Mainly responsible for that are the very rapidly reacting S-H and S-CH₃ functions belonging to free or protein-bound cysteine and methionine.58,59

On the other hand, with less-reducing fluids such as blood plasma and diverse inflammation samples, an increase of microbicidal activity was found against bacteria and, particularly, fungi.21,23,25 This can be explained by the formation of faster-acting chloramines by transhalogenation (see above), whereby the effect of reduction is counteracted. In large part, monochloramine is responsible for the enhancement of activity, formed from ammonium ubiquitously present in these substrates.

**Culture media**

Nutrient solutions contain considerable amounts of reducing substrates, which should not be used in killing tests with active chlorine compounds. Therefore, MIC and MBC testing according to protocols standardized for antibiotics reveals too high values and hardly reasonable results.

**Formation of bacterial chlorine covers**

Chlorine cover designates a superficial c(Ox) attached on microorganisms, which is not an adsorption phenomenon but the result of covalent N-Cl bonds fixed to the proteinaceous matrix of the surface.72,73 This phenomenon, to date proven for bacteria and fungi, was also discovered on skin surfaces after disinfection with active halogen compounds.74,75 Surprisingly, bacteria and fungi furnished with a chlorine cover are viable and can be recultivated after the oxidizing coating is reduced again, e.g. by thiosulphate, which exerts an immediate and non-destructive elimination. Upon ice cooling, the chlorine cover persisted for 8 h after a 30% reduction during the first 2 h.72 The formation of a chlorine cover without loss of viability, which typically develops upon incubation in 1% NCT for 1 min, can be considered the initial step in the antimicrobial action of active chlorine compounds. Though not lethal, it exerts a lag of regrowth76 and a loss of virulence (see the next section).

A striking effect was observed when bacteria (*S. aureus*) with a chlorine cover were incubated in saline or in 5% NH₄Cl. While in saline no decrease in cfu was observed, a decrease of >5 log₁₀

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Table 2. Virucidal and protozoocidal activity of 1% NCT in phosphate buffer at pH 7.1 and 20°C

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Log₁₀ reduction in pfu and viable cells, respectively</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Herpes simplex virus type 1</td>
<td>3.0–3.6</td>
</tr>
<tr>
<td>Herpes simplex virus type 2</td>
<td>2.9–4.1</td>
</tr>
<tr>
<td>Adenovirus 5</td>
<td>0.3–0.9</td>
</tr>
<tr>
<td>Influenza virus A (H1N1, Hong Kong, 2000)</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>HIV-1</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Acanthamoeba hatchetti trophozoites</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Acanthamoeba polyphaga trophozoites</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Leishmania donovani and infantum promastigotes</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Trichomonas vaginalis ATCC 30001 and ATCC 50138</td>
<td>&gt;5.0⁹</td>
</tr>
</tbody>
</table>

⁹Amastigotes were largely inactivated by 0.036% NCT at 37°C.¹⁰³7°C.

NCT and monochloramine

**pH effects**

The microbicidal activity against bacteria and yeasts shows a significant pH effect, with shorter killing times at pH 4–6 compared with the normal environment at pH 7,22,24 which can be explained as follows. Increasing acidity favours the formation of the protonated form NCTH⁺ where the chlorine atom bears a positive partial charge. This species is more likely to come into reaction with nucleophilic agents such as R-NH₂ than it is the case with non-protonated NCT. The entity NCTH⁺, therefore, can be regarded as the active form in chlorination reactions.70 The increase of its portion at pH 5, at any rate, makes plausible the higher chlorinating and bactericidal activity in the more acidic milieu.

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A striking effect was observed when bacteria (*S. aureus*) with a chlorine cover were incubated in saline or in 5% NH₄Cl. While in saline no decrease in cfu was observed, a decrease of >5 log₁₀
was observed within 120 min in \( \text{NH}_4\text{Cl} \). This can be explained by a transhalogenation from the bacterial surface to ammonium ions, forming the highly bactericidal monochloramine. In the presence of reducing organic material (serum, nutrient solution), the superficial c(Ox) vanishes very quickly.

Interestingly, the discovery of the viability of bacteria bearing a chlorine cover was not possible until the mild oxidant NCT was available. With other, stronger oxidants such as CAT or hypochlorite a high-level chlorine cover is formed, which obviously exerts an immediate destructive impact on the bacterial surface, whereby a rapid penetration of chlorinating molecules leads to instantaneou killing.

**Post-antibiotic effect of NCT**

Interactions with sublethal times or concentrations (e.g. 1% NCT for 1 min), which lead to measurable chlorine covers, cause a post-antibiotic effect (delay of regrowth) connected with a loss of virulence. As a consequence, eradication of bacteria by the body's own immune system can be fostered. This was impressively demonstrated by survival in mice challenged intraperitoneally with \( \text{S. aureus} \) strain Smith diffuse and \( \text{S. pyogenes} \). In line with that, a decrease of virulence factors, i.e. secretory aspartyl proteinases of \( \text{C. albicans} \) and \( \text{C. dubliniensis} \) as well as gliotoxin of \( \text{Aspergillus fumigatus} \), was found before viability of these fungi was impaired.

From these data and from earlier considerations concerning the chemistry of chloramines, there is strong evidence that penetration of oxidative activity into the pathogens is necessary for a microbicidal effect. Indeed, after incubation times in NCT leading to a significant reduction in cfu, changes within the cytosolic compartment were seen in electron microscopy of \( \text{S. aureus} \) and changes of multiple intracellular proteins of \( \text{E. coli} \) were found with proteomics.

**Microbicidal activity at physiological concentrations**

In view of the estimated in vivo NCT concentrations of 10–50 \( \mu\text{M} \), which are 2750- to 550-fold lower than the generally clinically used concentration of 55 mM (1% NCT), the question arises how the killing of invaded pathogens proceeds in vivo. In vitro experiments with these concentrations disclosed that incubation times of many hours are necessary. Given the reduction of NCT by the body’s own sulphur compounds and transhalogenation to less stable N-chloramines, a sufficient level of c(Ox) for an extended period can only be maintained if continuous production by arriving granulocytes and monocytes leads to a dynamic equilibrium between formation and loss.

**Clinical studies and case reports**

The antimicrobial and immune-modulatory features encouraged investigations of the usability of 1% (55 mM) aqueous NCT solution as a topical antiseptic for the treatment of infections in medicine.

**Ophthalmology**

The good tolerability of NCT up to 1%–2% was confirmed by tests in the healthy rabbit and human eye, and also in the inflamed human eye. Patients suffering from bacterial conjunctivitis were cured within 3–5 days in a Phase IIa open pilot study.

Viral conjunctivitis

Adenoviral conjunctivitis (epidemic keratoconjunctivitis), which, to date, lacks a well-tolerated therapy, was investigated in a Phase Ib clinical study. The symptoms could be attenuated in severe infections, while the rate of subepithelial infiltrates was not influenced. This could be due to a late onset of therapy in some cases, but also to the low penetration of NCT into the cornea. The latter could be enhanced by the addition of ammonium chloride, with which NCT equilibrates to the more lipophilic monochloramine. Indeed, in a rabbit epidemic keratoconjunctivitis model the combination demonstrated superior activity. Fewer positive viral cultures and shorter duration of viral shedding were found. Recently, 0.1% NCT plus 0.1% \( \text{NH}_4\text{Cl} \) was well tolerated in a Phase I study, and presently this formulation is investigated in viral conjunctivitis in humans.

Otorhinolaryngology

Two animal studies dealt with the safety of NCT in the ear. When injected to the middle ear of mice via the transtympanal route, 1% and, to a higher extent, 10% NCT caused a transient increase of the auditory brainstem response threshold due to local irritation around the artificial perforation of the tympanic membrane, but no damage of the inner ear. For testing repeated dosing to the middle ear, a novel guinea pig model with an implanted catheter system was used. Injection of low volumes of 0.1% and 1% NCT, which did not lead to elevated middle ear pressure and damage of the membranes, did not cause toxic inner ear effects. The only side effect in some animals was moderate thickening of the middle ear mucosa.

External otitis

These results enabled the investigation of 1% NCT ear strips for the treatment of external otitis in a Phase Ib study. It was significantly more effective than a combination of neomycin, polymyxin B and hydrocortisone. The time needed for disappearance of the symptoms was 5.6 ± 1.6 days in the NCT group, compared with 7.4 ± 1.6 days in the control group (\( P < 0.01 \)). According to these studies, NCT seems to be a very well-tolerated and highly effective medication in external otitis.

Rhinitis and sinusitis

Regarding these diseases, application of NCT as a nasal spray and irrigation solution, respectively, is near at hand. In vivo tests with human nasal tissue demonstrated that 1% NCT had hardly an influence on the ciliary beat function of the epithelial cells in contrast to a deswelling solution used daily in practice. In a Phase IIa study, a novel catheter (YAMIK) was used to irrigate the nasal and paranasal sinuses three times a week for 1 month in patients suffering from chronic rhinosinusitis. There were no toxic side effects but some positive effects, i.e. mucosal deswelling, improved nasal breathing and olfaction, of which it is unclear up to now if they were related to the irrigations per se.
or to NCT. An immunosuppressed patient who experienced repeated maxillary bacterial sinusitis caused by *P. aeruginosa* despite antibiotic treatment could be cured by irrigations with 1% NCT.

**Dermatology**

**Crural ulcers**

Particularly, patients with leg perfusion problems develop crural ulcerations, which frequently become infected and inflamed. A Phase IIb clinical study has been performed comparing the effect of 1% NCT and 1% CAT in patients suffering from crural ulcers. CAT was chosen as it is a well-known antiseptic, which had already been used to treat infected wounds during the First World War.

Twice daily irrigations and bandages soaked with these antiseptics for ~5 days were sufficient to remove the clinical signs of infection in both groups. However, pain was significantly lower, and granulation and re-epithelialization occurred significantly earlier in the NCT group.

**Other fields**

**Urinary tract infections**

In these infections, antiseptics, in general, have been used topically with only partial success, depending on the localization of the infection. NCT (55 mM) proved to be well tolerated upon repeated irrigations via a urinary catheter. Also, in one of three cases the attempt to eradicate an omni resistant *P. aeruginosa* from the urinary bladder was sufficient, although NCT was bactericidal in vivo.

**Oral cavity**

In 2004, NCT was mentioned to inhibit the production of interleukin 6 and other proinflammatory substances, which may play a role in periodontitis. Recently, the influence of 2% and 3% NCT mouth rinse on dental plaque was assessed. Rinsing with 10 mL of the test solution two times daily for 4 days did not inhibit plaque growth, but reduced its vitality.

**Bovine mammary gland**

Naturally, not only human but also veterinary medicine may be of field of interest. Irrigation of the bovine mammary gland for 5 days with 0.1%–2.0% NCT caused a transient increase of leukocytes in the milk, similar to 0.9% saline, but no severe side effects. The absence of both systemic resorption and c(Ox) in the milk for >5 h after lavage can be conceived as an advantage of NCT compared with other antimicrobial agents.

**HIV**

A further interesting approach has arisen recently. According to its properties as an antiseptic, NCT kills also HIV (also H. Stoiber and M. Nagl, unpublished results). The viruses became inactivated but remained immunogenic in a recent study. In a mouse model, HIV treated with 2–5 mM NCT acted as a whole-killed highly active vaccine against murine AIDS. The authors stressed that no harsh inactivation or purification steps of the viruses were necessary, which may be a decisive advantage regarding preparation and efficiency.

**Arthritis**

Due to the down-regulation of proinflammatory cytokines in synovocytes *in vitro*, NCT has been considered an interesting medication in arthritis. In a collagen-induced arthritis mouse model, administration of NCT (2 mM, 0.5 mL) for 21 days after the first immunization resulted in a delay of the onset of symptoms, while similar application after the booster immunization diminished the incidence of the disease. Since NCT was applied subcutaneously in this study and did not achieve a sufficient concentration in the joints, the mechanism for the seen effects remains unclear. In a septic arthritis mouse model, locally (1–100 mM, 20 μL) but not intraperitoneally applied NCT diminished the number of arthritic lesions.

**Pharmacokinetics**

Activity in clinical studies is in good correlation with the pharmacokinetics of NCT. In human inflammation samples, c(Ox) could be detected for a few hours *in vitro*, if started with 1% (55 mM). In vivo, c(Ox) was present for 15 min after the application of one drop of a 1% solution into the eye. After irrigation of the maxillary sinus with the 1% solution, c(Ox) could be detected for 3–4 h. In the urinary bladder it has been found for >1 h (90% decomposition within 1 h) after the instillation of a 1% solution, if the catheter was clamped.

**General reflections on active halogen compounds and the position of NCT as an antiseptic**

The rate of microbial killing by an active halogen compound (–O-Cl, >N-Cl) correlates with its oxidative power. Accordingly, hypochlorous acid, the strongest active chlorine compound that is stable in an aqueous system, will surpass all weaker oxidants (CAT, NCT and others) in this view, provided that tests are done with washed bacteria. However, this approach does not consider the conditions prevailing in practice, most notably the presence of proteinaceous material (body fluids, corpuscular material such as living and dead bacteria) and the contact with body surfaces (skin, mucous membranes). The result of these interactions is unwanted side reactions that lead to: (i) consumption of c(Ox); (ii) irritation of tissue. Since the (wanted) microbicidal activity and the quoted (unwanted) side reactions are based on the same fundamental reactions (oxidation and transhalogenation), a compromise has to be made between sufficient microbicidal activity and a minimum of side reactions. Depending on the sensitivity of the treated body site, the adequate agent has to be chosen. A rather strong oxidant such as CAT might be qualified for the disinfection of intact skin, while a mild agent like NCT should be preferred for mucous membranes and other delicate sites (for instance eyes, ears, vagina, wounds). NCT can be regarded as an agent of choice for irritated body sites because of the following reasons.
NCT represents a native endogenous agent, which is produced in vivo by leucocytes upon human host defence

The production of N-chloramines by the immune system is not restricted to NCT, but encompasses the N-chloro derivatives of all amine compounds (amino acids, peptides, ammonia) present in the intra- and extracellular environment of white blood cells capable of the respiratory burst.\(^3,4,9,15,16,96\) The relevance of NCT originates both from its high in vivo concentration—nearly 50% of these amines is made up of taurine\(^4\)—and the superior stability of NCT.\(^4,15,16,58\) Its creation by the immune system suggests that the body has developed tolerance not only towards NCT, but also to its possible conversion products (e.g. NDCT) or metabolites (e.g. sulphoacetaldehyde).

NCT fulfils the requirement of sufficient microbicidal activity combined with good tissue tolerability

Microbicidal activity was assessed in vitro for a broad spectrum of pathogens, and in vivo for bacteria and viruses (Tables 1 and 2). Clinical studies revealed the applicability of NCT for the therapy of several diseases and confirmed its tissue tolerability.

The final products of NCT after reaction with reducing substrates are the non-toxic compounds taurine and chloride

Taurine and chloride are present in the human body at relatively high concentrations (the human body is made up of 0.1% taurine,\(^97\) human serum contains 96–110 mM chloride), so the therapeutic application of NCT does not lead to measurable changes. This is a unique feature in disinfection and antiseptic practice. For instance, for iodine-containing preparations, which were promoted as possible substitutes for NCT, has been reported.\(^98\) Recently, the in situ expression of some cytokines was measured in healthy pigs that inhaled NCT, NCT plus ammonium, or saline as placebo. No differences in the cytokine levels were found between the groups, but standard deviations were high.\(^102\) Therefore, the question of whether anti-inflammatory effects of NCT play a role remains open to date, although the signs from previous in vitro and in vivo studies are rather supportive.

N-chloro derivatives of 2,2-dimethyltaurine

Recently, the synthesis of C-methylated derivatives of NCT, which were promoted as possible substitutes for NCT, has been reported.\(^103\)–\(^105\) Mainly, the monochloro-dimethyltaurine (MCDMT) and dichloro-dimethyltaurine (DCDMT) were suggested as suitable candidates, because they show microbicidal properties and, at the same time, higher stability than NCT. The latter assertion is quite plausible because no hydrogen atom exists adjacent to the N-Cl function, which is necessary for splitting off HCl (see the ‘Stability’ section above).

This advantage, however, is counteracted by the fact that MCDMT and DCDMT cannot be designated as the body’s own compounds. As a consequence, the premise about the developed tolerance of the body against NCT and its transformation products cannot be similarly assigned to the new C-methylated derivatives of NCT. This drawback has to be considered, since in delicate antiseptic applications (inhalation,\(^102\) application in mammary glands, disinfection of mucosa and large-area wounds), a resorption of the compound or its reaction products is conceivable by all means.

Conclusions

Hitherto published literature reveals the man-made sodium salt of the endogenous compound NCT as a very valuable antiseptic.

In vitro anti-inflammatory properties of NCT might play a role in clinical application

As mentioned in the ‘Historical survey’ section, there exists a substantial number of studies in which a down-regulation of proinflammatory cytokines produced mainly by stimulated leukocytes was found in the presence of NCT (for review see Koprowski and Marcinkiewicz,\(^4\) and Marcinkiewicz\(^6\)). It may be that such properties are responsible for the therapeutic success, besides the antimicrobial activity in treatment of infections. This is very difficult to differentiate clinically and has never been investigated in detail. In a small pilot study, NCT was applied to the outer ear canal for post-operative care after tympanoplasty. The operation wound and the canal dried earlier in the NCT group than in the control group, although there were no signs of infection.\(^101\) Recently, the in situ expression of some cytokines was measured in healthy pigs that inhaled NCT, NCT plus ammonium, or saline as placebo. No differences in the cytokine levels were found between the groups, but standard deviations were high.\(^102\) Therefore, the question of whether anti-inflammatory effects of NCT play a role remains open to date, although the signs from previous in vitro and in vivo studies are rather supportive.
in human and veterinary medicine. It offers a well-balanced compromise between sufficient microbialic activity and excellent tissue tolerability. In combination with ammonium chloride, very potent preparations containing monochloramine, as well as an endogenous compound, are available, which kill even mycobacteria and moulds after very short incubation times. Not only NCT but also its reaction products taurine and chloride are the body’s own compounds, a fact that is unique in disinfection practice since it points to an unrivalled tolerability.

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### Transparency declarations

None to declare.

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