

Tolerance and Bactericidal Action of N-Chlorotaurine in a Urinary Tract Infection by an Omniresistant *Pseudomonas aeruginosa*

Markus Nagl¹, Bettina Pfausler², Erich Schmutzhard², Manfred Fille³,
and Waldemar Gottardi¹

¹ Institute for Hygiene, Leopold-Franzens-Universität Innsbruck, Fritz-Pregl-Straße 3,
A-6010 Innsbruck, Austria

² University Hospital of Innsbruck, Department of Neurology, Anichstraße 35, A-6020
Innsbruck, Austria

³ Bundesstaatliche Bakteriologisch-Serologische Untersuchungsanstalt, Institute for
Hygiene, Leopold-Franzens-Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Inns-
bruck, Austria

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Summary

N-chlorotaurine, a weak antimicrobial oxidant produced by stimulated human leukocytes, was used to treat cystitis caused by an omniresistant *Pseudomonas aeruginosa*. A 21-year-old male patient was treated by repeated daily lavages of the urinary bladder with an aqueous solution of 1% N-chlorotaurine for one month. N-chlorotaurine was well tolerated; no local or systemic side effects could be detected. Despite killing of $> 10^6$ cfu/ml of bacteria within ten minutes *in vitro* and *in vivo*, it was not possible to eradicate the *Pseudomonas* infection obviously caused by inflammation of the upper urinary tract and perpetuated by intravesical concretions. Nevertheless, in actually localized infection, treatment with N-chlorotaurine might be successful because of its sufficient bactericidal action.

Introduction

NCT demonstrated significant bactericidal (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*), fungicidal (*Candida albicans*) and vermifugal (*Schistosoma mansoni*) efficacy (10, 11, 17).

On the other hand, cytotoxicity of NCT against leukocytes and erythrocytes (5), lung epithelial cells (1) and murine macrophages RAW 264.7 (13) proved to be low compared to the more powerful oxidant hypochlorite. Hypochlorite and monochloramine, but not NCT, increased microvascular permeability in isolated perfused rat lungs (15). Moreover, in a recent clinical study, 1% NCT solution was well tolerated in the human and rabbit eye (9).

Because of these positive in-vitro and in-vivo data, local application of NCT in a patient suffering from an infection of the urinary bladder by an omniresistant *P. aeruginosa* was considered to be appropriate and ethically warranted. Especially tolerance, pharmacokinetics and bactericidal efficacy were monitored.

Patient and Methods

Patient

We report on a 21-year-old male patient who suffered from a traumatic brain injury, a complete paraplegia subsequent to a luxation-fracture of C6/7, various fractures and pulmonary as well as intestinal lesions. Eleven weeks after the accident and systemic treatment with different antibiotics because of pulmonary and urological infections, a cystitis caused by *P. aeruginosa* occurred. Attempts to influence this infection by systemic therapy with ciprofloxacin, gentamicin and nitrofurantoin for two weeks were not successful because the strain proved to be resistant against all available antibiotics (see below). No signs of urosepsis were found (no elevated body temperature, normal renal function, no alterations of other organs except a peritonitis), but some signs of infection in peripheral blood (leukocytes 10 000–14 000/ μ l, C-reactive protein 3–11 mg/dl) and significant signs in urine (leukocytes > 500/ μ l, erythrocytes 50–> 250/ μ l, pH 7–9, protein 25–150 mg/dl, positive nitrite; sporadic granulocyte cylinders, plate and circular epithelium in urine sediment). The capacity of the bladder determined during cystoscopy was diminished, to 100–150 ml. Having almost fully recovered from the traumatic brain injury the patient was able to comprehend his medical situation and gave his consent to the following kind of therapy.

Preparation of NCT

NCT was prepared as the crystalline sodium salt (MW = 181.52 g/mol) (4) and its purity proved by iodometric titration (calculated 19.53% Cl⁻, found 19.3% Cl⁻, which corresponds to a 99% purity). Solutions of 1% NCT in sterile double-distilled water (pH \approx 8) were prepared freshly at least every three days and stored at 4°C without loss of stability (11). Before usage, the solutions were allowed to reach room temperature.

Application of NCT

50–100 ml of NCT solution were instilled through a suprapubic catheter, which was clamped subsequently for 20–60 min as well as the urethral catheter. During the last

seven days of treatment, the urethral catheter was removed and no clamping performed. Temporary attempts to rinse the entire bladder wall were done by turning the patient to the back, the left and right side and to the back with elevated legs, for 15 minutes each. Detailed information about the periods of treatment is given in Table 1.

Stability of NCT in urine

Oxidation capacity measured by titration of iodine released at addition of excess iodide (NCT + 2I⁻ + H⁺ \rightarrow taurine + I₂ + Cl⁻) (14) was expressed as % NCT, although it is known that also other compounds equilibrating with NCT contribute to oxidation capacity (11).

Bacteria and resistance testing

Bacteria were cultivated from the patient's urine and identified biochemically as *P. aeruginosa*. MIC values determined by a standard agar diffusion assay (12) and by E-test (7) (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar (Oxoid) exceeded 256 μ g/ml for all available groups of antibiotics (monobactams, β -lactams, cephalosporines, quinolones, aminoglycosides, carbapenems, tetracyclines, trimethoprim, sulfonamides, fosfomycin, macrolides) except for amikacin (32 μ g/ml).

Bactericidal effect of NCT

In-vitro assay. Bacteria were grown in tryptic soy broth at 37°C to 10⁸ cfu/ml and ten-fold diluted in the patient's urine. NCT was added to a concentration of 1% (55 mM) and inactivated by addition of 3% sodium thiosulfate after different incubation times at 37°C. Quantitative cultures were done on tryptic soy agar and reduction factors were computed as log (cfu_{original}) – log (cfu_{NCT}).

In-vitro assay. Sodium thiosulfate was added immediately after gaining the urine samples to inactivate NCT. Bacterial counts were determined as described above.

Table 1. Mode of treatment with NCT (1% aqueous solution)

Day of treatment	Application interval (h)	Volume (ml)	Clamping (min)	Turning of patient
1	6	50	60	no
2–4	6	100	60	no
5–6	6	75	60	yes
7–10	3	75	60	yes
11–13	3	75	20	no
14–18*	3	100	30	no
19–25**	3	100	no	no

* External condom catheter instead of urethral catheter.

** External condom catheter removed, excretion into a urinal.

Results

Pharmacokinetics of NCT

After one hour of clamping, 5–19% percent of the original oxidation capacity was detected (Table 2). Subsequent to each removal of the clamps (Table 2) and during the period without clamping, millimolar levels of NCT were found for another 10–30 minutes, depending on the varying production of urine and washout of NCT. There was no decrease of systemic haemoglobin values indicating the failure of significant penetration of the hydrophilic substance NCT into the blood.

Tolerance of NCT

NCT was well tolerated; no local or systemic side effects could be observed. Repeated cystoscopy revealed suppurative, but non-haemorrhagic cystitis with cystolithiasis. No signs of toxicity appeared in urine sediment.

Bactericidal effect of NCT

In vitro, mean reduction factors and standard deviations of four replicates (incubation at 37 °C) each were

2.5 ± 0.5 at 3 min,
4.6 ± 0.3 at 7 min,
> 7.0 at 10 min.

The in-vivo time course of bacterial counts in relation to application of NCT is illustrated in Table 2. All samples exhibiting oxidative activity were sterile.

Therapeutical result

Subsequent to disappearance of the oxidative efficacy, bacterial counts rose within a few minutes, even at short application intervals of three hours. Therefore it was not possible to eradicate the pathogen. Nevertheless, septic signs could not be observed during therapy.

Discussion

In this study, the bactericidal action of NCT has been proved *in vivo* for the first time. Killing times were similar to those against gram-negative bacteria in different inflammation samples and therefore even superior to the ones in aqueous solution (11). This phenomenon is probably caused by equilibration between NCT and amine compounds available in body fluids, above all ammonium, which is highly concentrated in urine (2). As a consequence, more

Table 2. Absolute urinary concentration of NCT (%) and bacterial counts after clamping the suprapubic and urethral catheter for 20–60 minutes

Point of time*	NCT (%)	cfu/ml	n
0 min	0.11 ± 0.06**	< 10	9
10 min	> 0, not quantified	< 10	3
	0	3–6 × 10 ⁵	3
30 min	0.02; 0.10	< 10	2
	0	1 × 10 ⁶	1
60 min	0.03	< 10	1
	0	2 × 10 ³ –5 × 10 ⁴	6

* Immediately (= 0 min), 10 min, 30 min and 60 min after the removal of the clamp the concentration of NCT and the bacterial count were determined by iodometric titration and quantitative cultures, respectively.

** mean value ± SD

Bacterial counts just before each instillation of NCT were 2×10^2 – 2×10^6 cfu/ml (n = 30).

bactericidal monochloramine (NH₂Cl) and other corresponding N-chloro derivatives will be formed by transhalogenation.

Nevertheless, NCT will retain the main part of oxidation capacity after instillation, as it is the most stable N-chloro amino acid (18). Actually, a bactericidal level could be detected for more than one hour after clamping the catheters.

The good tolerance of NCT in spite of the intensive treatment for one month confirms its low cytotoxicity (1, 5, 9, 13, 15). It should be mentioned that in our patient more invasive diagnostic procedures like biopsy and ureteroscopy were not performed to avoid a potentially harmful spread of the pathogen. However, cytotoxicity of NCT against the epithelium is improbable, as temporary irritations caused by catheters and external condom catheter (a flexible urine catheter with the upper end fixed like a condom) disappeared again during treatment. NCT was well tolerated at a molar concentration about 80 times higher than the one of the much more reactive sodium hypochlorite used in a former study (3).

Despite a sufficient bactericidal action of the therapy, eradication of the *P. aeruginosa* was not achieved. The most favorable reason, an origin of the inflammation in the upper urinary tract, was confirmed in the last analysis by pyelocaliceal concretions and temporary dilatation of the ureter detected by computer tomography and urogram. Continuous flow of infected urine would explain the immediate reoccurrence of high bacterial counts after each decay of the NCT level. These findings are in agreement with the fact that urinary tract infections frequently cannot be cured by local instillations of antibiotics and disinfectants except for actually localized cystitis (3, 6, 8).

To summarize, NCT as an endogenous mild oxidant seems to be a well tolerated antimicrobial agent suitable for application to body cavities. Further studies on lavages with NCT to cure localized infections should be performed to evaluate its clinical usability.

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Corresponding author: Dr. Markus Nagl, Institut für Hygiene, Leopold-Franzens-Universität Innsbruck, A-6010 Innsbruck, Austria