


# Tolerability of inhaled N-chlorotaurine in humans: a double-blind randomized phase I clinical study

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## Abstract

**Background:** N-chlorotaurine (NCT), a long-lived oxidant produced by human leukocytes, can be synthesized chemically and used topically as a well-tolerated antiseptic to different body regions including sensitive ones. The aim of this study was to test the tolerability of inhaled 1% NCT in aqueous solution upon repeated application.

**Methods:** The study was performed double-blind and randomized with a parallel test group (1% NCT) and control group (0.9% NaCl as placebo). There were two Austrian centres involved, the hospitals, Natters and Vöcklabruck. Healthy, full age volunteers were included, 12 in each centre. A total of 12 patients were treated with NCT, and 12 with placebo, exactly half of each group from each centre. The single dose was 1.2 ml inhaled over a period of 10 min using an AKITA JET nebulizer. One inhalation was done every day for five consecutive days. The primary criterion of evaluation was the forced expiratory volume in 1 second (FEV<sub>1</sub>). Secondary criteria were subjective sensations, further lung function parameters such as airway resistance, physical examination, and blood analyses (gases, electrolytes, organ function values, pharmacokinetic parameters taurine and methionine, immune parameters).

**Results:** All included 15 females and 9 males completed the treatment and the control examinations according to the study protocol. FEV<sub>1</sub> (100.83% ± 8.04% for NCT and 92.92% ± 11.35% for controls) remained unchanged and constant during the treatment and in control examinations 1 week and 3 months after the treatment (98.75% ± 7.37% for NCT and 91.17% ± 9.46% for controls,  $p > 0.082$  between time points within each group). The same was true for all other objective parameters. Subjective mild sensations with a higher frequency in the test group were chlorine taste ( $p < 0.01$ ) and occasional tickle in the throat ( $p = 0.057$ ). Taurine and methionine plasma concentrations did not change within 60 min after inhalation or later on.

**Conclusions:** Inhaled NCT is well tolerated as in other applications of different body regions. Side effects are mild, topical and transitory. The study was registered prospectively in the European Clinical Trials Database of the European Medicines Agency. The EudraCT number is 2012-003700-12.

**Keywords:** N-chlorotaurine, inhalation, clinical phase I, clinical trial, antimicrobial, antiseptic

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## Introduction

Bronchopulmonary infections are among the most frequent infections. While they usually can

be overcome rapidly in young and immune-competent patients, treatment of patients with risk factors, such as chronic obstructive pulmonary

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disease (COPD), immune suppression, cystic fibrosis, and noncystic fibrosis bronchiectasis is more complicated. Resistance against antibiotics accompanies chronic lung infections, and fungal infections in immune-suppressed people are life-threatening. In some special cases, inhalation therapy with antibiotics is performed, since the location of the infection can be reached by high concentrations (for instance against *Pneumocystis jirovecii* and in cystic fibrosis).<sup>1-3</sup> In addition, viral infections sometimes can be severe, with limited treatment options. Due to resistance problems and lack of efficacy of present therapies in a significant number of patients, new strategies and new antimicrobial agents are of high interest.<sup>4,5</sup>

One concept is to apply antimicrobials other than antibiotics to provide a different mechanism of action and to avoid cross resistance. Topical application by inhalation has the advantage of high local concentration and fewer or absent systemic adverse effects, but only a few substances are suitable because of toxicity and tolerability issues. N-chlorotaurine (NCT), the N-chloro derivative of the amino acid taurine, is an endogenous mild active chlorine compound coming into question for such use. It is produced as the main representative of the long-lived oxidants by activated human granulocytes and monocytes during the oxidative burst.<sup>6,7</sup> Due to its anti-inflammatory properties by downregulation of proinflammatory molecules such as tumor necrosis factor, prostaglandins, interleukins, nuclear factor kappa B, and others, its main function in the innate defence system has been thought to be involved in termination of inflammation.<sup>8,9</sup> The chemical synthesis of the sodium salt of NCT (Cl-HN-CH<sub>2</sub>-CH<sub>2</sub>-SO<sub>3</sub>Na) facilitated further investigations,<sup>10</sup> which disclosed its broad-spectrum microbicidal activity against Gram-positive and Gram-negative bacteria, viruses, yeasts and moulds, protozoa and worm larvae.<sup>11</sup> Due to the oxidative mechanism of action with multiple targets,<sup>11-13</sup> resistance does not occur as it is typical for active halogen compounds.<sup>14</sup> Formation of NCT *in vivo* leads to detoxification of hypochlorous acid (HOCl + taurine → NCT + H<sub>2</sub>O).<sup>6</sup> Because of its mild reactivity, NCT is very well tolerated by human cells and tissue. These properties led to the concept of using it as an endogenous antiseptic for topical therapy of infections,<sup>11</sup> and indeed NCT demonstrated tolerability and efficacy in different body sites, such as the eye,<sup>15</sup> the outer ear,<sup>16</sup> and ulcerated skin.<sup>17</sup>

In the last few years, development of NCT as an inhaled anti-infective proceeded. The ciliary beat frequency of epithelial cells of the nasal mucosa, a very sensitive parameter for toxicity, was decreased only moderately and reversibly by 1% NCT, while an anesthetic solution used in the daily routine in otorhinolaryngology caused a severe and irreversible decrease *in vitro*.<sup>18</sup> Recently, we performed two studies on the tolerability of inhaled NCT in anesthetized pigs due to the similarity of a pig's lung with a human lung.<sup>19,20</sup> In both studies, the animals inhaled 1% NCT (5 ml) *versus* 0.9% NaCl in a blind manner hourly, in total four times, on one day *via* the tracheal tube, which was connected with a nebulizer. In the second study, artificial inflammation was induced with *Streptococcus pyogenes* before the first inhalation.<sup>20</sup> In both studies, there was no difference between 1% NCT and 0.9% saline in all tested parameters, which comprised oxygenation (e.g. arterial pressure of oxygen) and hemodynamics (e.g. pulmonary artery pressure). There were no toxic signs in histology and electron microscopy, and the function of the surfactant was not impaired. Systemic absorption of NCT was undetectable. A five-fold higher concentration of NCT (5%) showed a minimally elevated pulmonary artery pressure but no further differences to saline. Only by addition of 1% ammonium chloride to 1% NCT, which leads to formation of significant amounts of monochloramine, some changes of parameters were seen, indicating the sensitivity of the model.<sup>19</sup>

To test the tolerability of inhaled NCT over a longer period, we used a mouse inhalation model.<sup>21</sup> Mice (C57BL/6N, 8 weeks old) were put into a small chamber, which was connected to a nebulizer. NCT in aqueous solution (1%, 1 ml) *versus* 0.9% NaCl was nebulized for 5 min to the mice in the chamber once or twice daily over a period of 5 days in a first study and over 15 days in a second one. There was no difference between NCT and saline in all evaluated parameters (i.e. behaviour, food and water uptake, weight increase, blood count, and histology of the lungs).

The aim of the present phase I clinical study was to investigate the tolerability of inhaled NCT in healthy volunteers.

## Patients and methods

### Pilot tests

Pilot tests were done by three of the involved male scientists as healthy volunteers (age 32, 42, and 45

years) and reported to the ethics committee of the Medical University of Innsbruck and to the Austrian Federal Office for Safety in Health Care. In the first test, all three inhaled once 10 ml of 1% NCT in aqueous solution over 10 min using a Multisonic LS-260<sup>®</sup> ultrasound nebulizer (MMDA 3.5  $\mu\text{m}$ , Ferdinand Menzl Medizintechnik GmbH, Vienna, Austria). Lung function measurement with the whole-body plethysmograph MasterScreen Body (Jaeger, Germany, see below) was done before and immediately afterwards. Then, one of the three persons inhaled the same dose again three hours later and again performed a lung function test. Special attention was paid to subjective sensations and adverse effects during and after inhalation.

In the second test, one of the three scientists inhaled 1% aqueous NCT solution on five consecutive days using the Pari Boy<sup>®</sup> SX nebulizer (MMDA 2.9  $\mu\text{m}$ , Pari GmbH, Starnberg, Germany). The volume inhaled once daily at 10:00 h in the morning over 10 min ranged between 2.4 and 3.2 ml. Subjective sensations were registered in detail. To obtain detailed information on the short-time pharmacokinetics, the oxidation capacity in sputum was determined daily before inhalation (background value) and 0.5, 1, 3, 5, 10, 20, 40, and 60 min after the end of inhalation. For this, approximately 1 ml of sputum was mixed with 5 ml of an aqueous solution of 1% potassium iodide and 1% acetic acid. Immediately after short vortexing, 1.5 ml were removed and centrifuged for 1 min at  $16,000 \times g$ . The supernatant was analyzed in a Beckman DU Spectrophotometer (Beckman Instruments, Fullerton, CA, USA) in a 1 cm quartz cuvette. The absorption was measured at 350 nm (triiodide-peak, absorption coefficient  $\epsilon = 22,900$ ) and the oxidation capacity (mol/l) calculated by  $\text{absorption}/\epsilon$ .<sup>22</sup> Furthermore, venous blood was taken before the first inhalation and 4 h after the last one, and taurine and cystine were measured in serum by high-performance liquid chromatography (HPLC).<sup>19</sup>

The third test was done to investigate possible absorption of NCT a short time after inhalation. Each of two scientists inhaled an aqueous solution of 1.1% NCT over 10 min with the Pari Boy<sup>®</sup> SX nebulizer (MMDA 2.9  $\mu\text{m}$ ), approximately 3 ml. Before and 5, 15, 30, and 60 min afterwards, 5 ml venous blood was taken and centrifuged at  $1800 \times g$  for 5 min. Serum was taken for

determination of taurine and cystine by HPLC, which was done soon after sampling to avoid decay of cystine.

#### *Study approval*

This university trial was in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committees of the Medical University of Innsbruck (leading ethics committee, AN4775 315/2.3 353/5.15 3647a) and of the Vöcklabruck Hospital and by the Austrian Federal Office for Safety in Health Care (717.853-0001), the responsible Department of the Austrian Ministry of Health, and it was proof-read and adjusted by the Clinical Trial Center of the Medical University of Innsbruck. The EudraCT number is 2012-003700-12.

#### *Study design*

This phase I clinical study was placebo-controlled, randomized, and double-blind. A test group inhaling 1% NCT and a control group inhaling 0.9% sodium chloride (placebo) were compared. The study was performed in two Austrian centres, the Public Hospital Natters, Department of Pneumology, belonging to the University Hospital of Innsbruck, and the District Hospital Vöcklabruck.

#### *Study population*

The total number of included persons was 24, considering 4 dropouts (see 'Statistics'). In each group, 12 healthy volunteers were treated. In each centre there were six test and six control participants. Test persons were recruited directly by the study physicians. All participants gave written informed consent.

Inclusion criteria were healthy volunteers at an age of  $\geq 18$  years. Exclusion criteria were acute disease, disease of the lung, airway hyperresponsiveness, positive history of asthma and atopy, heart or circulation disease, malign disease, medication with effects or adverse reactions on the lung function, inhalation medication, psychiatric disease, donation of semen by males during the days of treatment, minority, pregnancy, lactation period, absence of contraception of females with child-bearing potential, military service, participation in another clinical trial at the same time, and impossibility to come to the outpatient

department for inhalation on five consecutive days.

Termination criteria were the following: termination by the test person, termination by the study physician, recognition of pregnancy during the treatment period; occurrence of airway-related objective adverse events that would not allow further inhalation, in particular decrease of oxygen saturation below 90%, increase of bronchial resistance to  $>0.6 \text{ kPa} \times \text{s/l}$ , decrease of  $\text{FEV}_1$  by more than 20% compared with the baseline; occurrence of airway-related subjective adverse events that would not allow further inhalation, such as a strong tickle in the throat and dyspnoea; attacks of bronchoconstriction, cyanosis, rhonchus, abnormal sound during auscultation; allergic reaction against NCT (very improbable since it deals with an endogenous amino acid derivative); occurrence of nonairway-related subjective adverse events that moderately or severely impaired the test person, such as nausea, emesis, strong headache; occurrence of nonairway-related objective adverse events that impaired the test person, such as orthostatic reactions.

#### Study medication

The pure crystalline sodium salt of NCT ( $\text{Cl-HN-CH}_2\text{-CH}_2\text{-SO}_3\text{Na}$ ) with a molecular weight of 181.57 g/mol was synthesized according to the method of Gottardi.<sup>10</sup> It was dissolved in water for injection (sterile, pyrogen-free) to a final concentration of 1% (55 mM) and filled in single-use flasks to 10 ml each. Sterile, pyrogen-free 0.9% NaCl was filled in similar flasks for the control group. Both solutions are colourless. To fulfil all legal requirements (good manufacturing practice), the synthesis, filling and sending of all study medication was performed by Sanochemia Pharmazeutika AG (Neufeld an der Leitha, Austria) on instruction of Gottardi and Nagl who established the manufacture in pharmaceutical quality at the Division of Hygiene and Medical Microbiology of the Medical University of Innsbruck. Additional quality checks were done by this reference laboratory.

The flasks were numbered and used consecutively according to the randomization list, which was generated by the statistics program Stata, Ralloc (Boston College Department of Economics, USA).

#### Dosing

Once on each day, 1.2 ml of 1% NCT in aqueous solution or of 0.9% sodium chloride were inhaled for approximately 10 min. This treatment was performed on five consecutive days.

Participants were continuously monitored during the inhalations.

#### Inhalation device

The AKITA JET nebulizer system was applied (Vectura Group plc, Chippenham, UK).<sup>23</sup> The nebulizer is a reusable, breath-actuated nebulizer system designed to aerosolize liquid medications. It guides the patient to inhale with a preset inspiratory flow rate and inhalation time per breath to ensure precise and targeted drug delivery to the patient's lungs. Dosimetric jet nebulizers, such as AKITA JET, are designed to deliver a specific dose of medication. In addition, this nebulizer is more efficient than conventional nebulizers as it increases deposition of medication in the small airways, a key area of infective diseases.

For each inhalation, 3 ml of the test or control solution were filled into the nebulizer and inhaled with a preset dose setting once daily. The AKITA JET inhalation system was preset *via* a smart card to a fixed delivered dose, which is preset by the number of breaths and aerosol release time during one breath. The inhalation volume was adjusted to the test person's lung function previous to the dose administration. One adjustment to the results of an initial lung function measurement is sufficient with the system.

Particle size was assessed by laser diffraction. The mass median diameter was 3.7  $\mu\text{m}$  with a geometric standard deviation of 2.0. Fine particle fraction  $<5 \mu\text{m}$  was 69.2%. Drug output was assessed by filter sampling and spectrometric and gravimetric analysis. The drug output rate of the nebulizer was 339  $\mu\text{g}/\text{min}$  during continuous nebulization. The delivered dose was 1.2 ml of the 1% NCT formulation.

#### Proceeding and time course of the study

Subsequent to consent, the participants were subjected to a detailed medical examination before inclusion and randomization. Each participant that was suitable for the study was allocated to the test or control group according to the randomization



list. Within the following 14 days, the period of treatment was started. This consisted of one inhalation per day on five consecutive days. Control examinations were done before and after each inhalation. A week (limits: 5 to 11 days) after the last inhalation, a 'final examination' followed. A 'long-term examination' was performed 8 to 14 weeks after the last inhalation.

#### *Parameters of examinations during the study*

*Examination before inclusion.* Medical history was asked in detail, particularly diseases of the bronchopulmonary and circulation system, allergy, medication, and exclusion criteria (see above). Auscultation of lung and heart, general status of the other body regions, measurement of blood pressure and pulse frequency, electrocardiogram, body temperature, lung function, methacholine inhalation test to exclude a hyperreactive bronchial system, blood gases (Astrup from the hyperemic earlobe), pulse oximetry, blood analyses (taurine as parameter for pharmacokinetics and methionine for pharmacodynamics, electrolytes Na, K, Ca, Cl, blood count, liver and renal function parameters alanine amino transferase (ALAT), aspartate amino transferase (ASAT), gamma glutamyl transferase (gGT), urea, creatinine), X-ray of the thorax to exclude lung diseases.

*Testing of bronchial hyperresponsiveness.* Persons with a hyperreactive bronchial system, who experience a decrease in FEV<sub>1</sub> of >20% or an increase of the bronchial effective resistance of >0.6 upon inhalation per se, were excluded from the study. Therefore, all test persons were subjected to a lung function test and pulse oximetry at first, followed by inhalation of 0.9% saline, followed by 15 µg, 45 µg, 180 µg and 720 µg methacholine in series, which is the standard test for airway hyperresponsiveness (for details see<sup>24-26</sup>). After each of these inhalations, the lung function was checked. In case of a decrease of FEV<sub>1</sub> >20% or of an increase of the resistance >0.6, the person was excluded from the study.

*Examinations immediately before and after inhalation of NCT.* Subjective parameters during and after the inhalation (i.e. scraping and tickle in the throat, sneezing, taste sensations, impaired breathing, dyspnoea, pain, and other sensations), were monitored on every day and graded as mild, moderate or severe. Pain was evaluated according to the Visual Analogue Scale. During

all inhalations, pulse oximetry was done after 5 and 10 min, and the test persons were monitored visually continuously.

On the first day, the following examinations were performed immediately before inhalation.

Auscultation of the lung, pulse oximetry, lung function, blood gases.

Within the period of 10 to 30 min after the inhalation (period similar on all days of treatment), auscultation, pulse oximetry, determination of lung function and determination of blood gases were done.

On days 2 to 4, before inhalation, auscultation of the lung, pulse oximetry, and determination of lung function was done. After the inhalation, auscultation, pulse oximetry, and determination of lung function were done.

On day 5, the last day of treatment, before inhalation, auscultation, pulse oximetry, determination of lung function, and determination of blood gases were done. After the inhalation, auscultation, pulse oximetry, lung function, and blood gases were done. Blood was taken to measure taurine, methionine, blood count, and electrolytes 10 min after the end of the inhalation. Taurine and methionine were investigated again after 30 and 60 min to monitor pharmacokinetics. A peak flow meter (Vitalograph asma-1, Vitalograph GmbH, Hamburg, Germany) was explained and handed out to the volunteers for measurements of peak flow at home 2, 4, 6, and 8 h after the inhalation. A mean value was calculated from three measurements at the outpatient department, and an individual threshold value, which was 20% lower than the mean value. Participants were asked to immediately call the study physician if their values at home fell below the threshold value.

The final examination 1 week after the inhalation phase included auscultation, pulse oximetry, lung function, the methacholine test on bronchial hyperresponsiveness, blood gases, and blood parameters taurine, methionine, electrolytes Na, K, Ca, Cl, blood count, liver and renal function parameters ALAT, ASAT, gGT, urea, and creatinine.

The long-term examination 8–14 weeks after inhalation included auscultation, pulse oximetry,

lung function, and the methacholine test on bronchial hyperresponsiveness.

*Lung function.* For measurement of the lung function, the whole-body plethysmograph MasterScreen Body (Company Jaeger, D-97204 Hoechberg, Germany) was used. The apparatus is in accordance with the directives of the European Union (Medical Device Directive) and the United States Food and Drug Administration (US FDA). The measurements were done according to the ERS/ATS 2005 standards. One adequate test required a minimum of three acceptable forced vital capacity manoeuvres.

*Taurine, methionine, and cystine in plasma.* If NCT in amounts used for inhalation comes into contact with blood, it is immediately inactivated by thio groups and degrades into taurine and chloride. Therefore, taurine is an ideal measure for systemic uptake of topically applied NCT.<sup>19</sup> Whole blood was taken and centrifuged at  $1950 \times g$  for 10 min at 4°C, and the plasma was pipetted into another vial, which was frozen at minus 18°C. All transfers to the laboratory were done on dry ice to warrant permanent freezing. Taurine was analyzed by ion-exchange chromatography with ninhydrin detection (Biochrom 30plus, Biochrom, Cambridge, UK).

Before analysis, lithium heparin plasma was deproteinized by mixing 500 µl of the sample with 50 µl lithium loading buffer (pH 2.2; Biochrom, Cambridge, UK) containing S-2-amino-ethyl-L-cysteine internal standard (2.5 µmol/ml) and 50 µl of 50% sulfosalicylic acid. The mixture was then centrifuged for 5 min at  $10,880 \times g$ . The supernatant (300 µl) was mixed with 300 µl of lithium loading buffer and 30 µl 1 N NaOH. Then, 50 µl of this mixture was used for amino acid analysis. The external standard contained taurine and methionine among other amino acids, 100 µmol/l of each (Laborservice Onken, Grundau, Germany). Taurine, methionine, and cystine concentration was determined by ion-exchange chromatography with ninhydrin detection on a Biochrom 30plus system. Elution was conducted using five lithium buffers (pH 2.80 to 3.55, Biochrom, Cambridge, UK) successively.

Quality of amino acid measurements was assured by participating in the ERNDIM (European Research Network of Inherited Disorders of

Metabolism) quantitative amino acid scheme and proficiency testing program.

Methionine and cysteine would be oxidized by significant amounts of NCT in the blood, which would lead to a decrease of methionine and an increase of cystine (oxidation product of cysteine). Therefore, these parameters were used for pharmacodynamics. Cystine was abandoned in the main study due to its instability upon longer storage.

*Neopterin, tryptophan, and kynurenine in plasma.* Aliquots from the frozen plasma samples were used for these investigations. Time points zero, 30 min after the last inhalation and 1 week after the last inhalation were taken. Neopterin concentrations were determined by enzyme-linked immunosorbent assay with a detection limit of 2 nmol/l (Brahms, Berlin, Germany).

Serum tryptophan and kynurenine concentrations were assessed by reversed-phase HPLC on a ProStar Varian system (USA) according to the protocol described earlier.<sup>27</sup> In brief, serum specimens were deproteinized with trichloroacetic acid and separated on reversed-phase C18 material using 15 mmol/l acetic acid-sodium acetate buffer (pH 4.0) and a flow rate of 0.9 ml/min. Tryptophan was monitored by means of its native fluorescence at 286 nm excitation and 366 nm emission wavelengths (ProStar 360 detector, Varian, USA). Kynurenine was detected by ultraviolet-absorption at 360 nm wavelength (Shimadzu SPD-6A UV detector, Austria) in the same chromatographic run. Finally, kynurenine to tryptophan (Kyn/Trp) ratio was calculated as an indirect estimate of indoleamine 2,3-dioxygenase activity by dividing kynurenine concentrations (µmol/l) by tryptophan concentrations (mmol/l).

*Statistics.* Primary criterion of evaluation for this randomized, double-blind study was the FEV<sub>1</sub>, which should not be reduced by 10% compared with the baseline and by 20% compared with 0.9% NaCl. The sample size was prespecified with a number of 20 participants (10 per group) to detect an intra-individual difference of 10% in FEV<sub>1</sub> before *versus* after treatment with a statistical power of 80% on a two-sided level of significance of 0.05, assuming a standard deviation of 10% (a realistic value according to literature).<sup>28,29</sup> The number of test persons was determined with 24 in total, considering a dropout rate of 4.

Secondary criteria were the airway resistance and other lung function parameters, subjective parameters, blood gases and oxygenation of the blood, auscultation results, taurine and further blood parameters. Depending on the distribution of the variables, the paired *t* test or Wilcoxon test was applied for the before–after comparison, and the unpaired *t* test and Mann–Whitney *U* test, respectively, for comparison between the groups. For evaluations regarding the time course, an analysis of variance (ANOVA) for repeated measurements was additionally used in normally distributed variables. Categorical variables (e.g. subjective sensations, adverse events) were compared between treatment groups using Fisher's exact test. A *p*-value <0.05 was considered significant.

## Results

### *Pilot tests*

Subjective sensations were similar in all three test persons. Using the Multisonic LS-260® ultrasound nebulizer, upon forced inspiration a scraping and tickle in the throat or on the epiglottis was experienced and led to occasional coughing. When the inspiration was performed less forced, but still deep, the inhalation was well tolerated over 10 min with a consumption of 10 ml of 1% NCT. A chlorine taste, which was milder than the one upon gargling of 1% NCT, was noticed during inhalation and a few min up to 10 min further on.

The experience with Pari Boy® SX was similar. The sensations mentioned above did not increase during the once daily inhalation over 5 days, and sport exercise and speaking over 2 hours as a lecturer were not influenced by the treatment in the test person during this period.

Objective parameters did not change upon inhalations. Saturation with oxygen was constant between 97% and 99% (pulse oximetry) and no differences in lung function before and after inhalation were recorded, particularly no changes in lung volumes, FEV<sub>1</sub> and airway resistance. Serum levels of taurine (57.6 to 55.5 µmol/l) and cystine (45.1 to 46.9 µmol/l) remained constant after the 5 days treatment in the test person. The same was true in the last pilot test with two test persons, with the exception that there was a slight increase within the normal range of taurine (32–138 µmol/l) from 75 and 78 µmol/l to 92 and 102

µmol/l after 5 min. After 15 min, however, the values decreased again to 74 and 65 µmol/l and remained between 73 and 82 µmol/l at time points 30 and 60 min. Cystine remained constant between 27 and 34 µmol/l. The results from the oxidation capacity measured in the sputum are detailed below under pharmacokinetics.

### *Phase I study*

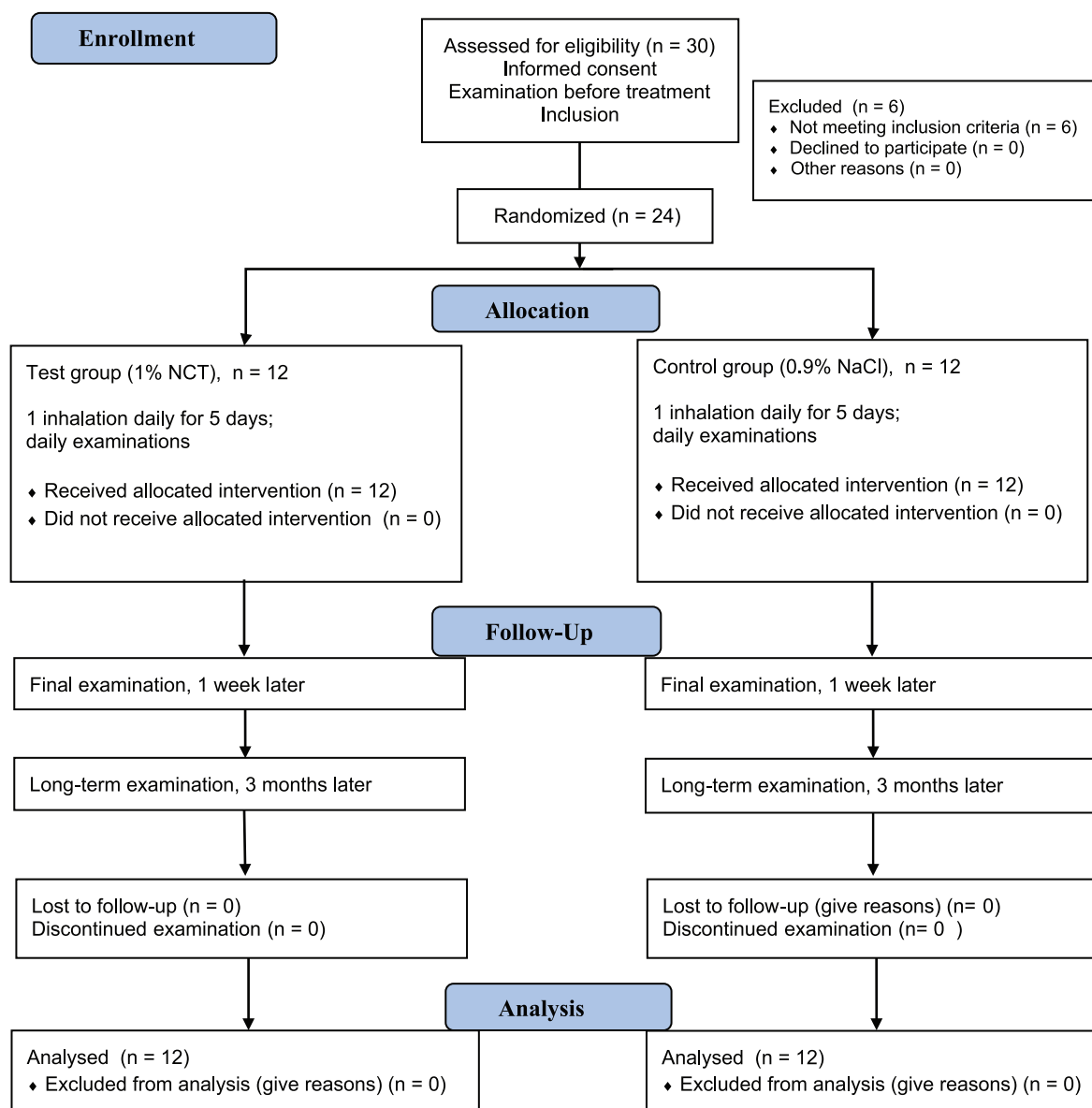
*Inclusion and characteristics of test persons.* From 30 screened persons, 6 had to be excluded since they did not meet the inclusion criteria because of bronchial hyperresponsiveness, bronchitis, or obstructive lung disease with inadequate lung function (Figure 1). All remaining 24 persons were included and completed the study according to the protocol. Thus, the intention-to-treat population matched the per-protocol population for statistical analysis. Distribution of test persons and demographics are listed in Table 1. There were no statistical differences between both centres and between the test and control group regarding sex, age, body weight, body height, body temperature, pulse frequency, and blood pressure. There were two participants in the test group who were smokers (10 cigarettes/day for 14 years and 0.4 cigarettes/day for 2 years) and one had smoked formerly (10 cigarettes/day for 15 years), and one smoked in the control group (15 cigarettes/day for 11 years).

### *Duration of inhalation*

The nebulizers were adjusted to the lung function of the test persons and to provide inhalation of a volume of 1 ml to the lower airways. Accordingly, five persons in each group inhaled for 7–9 min, six persons for 9–13 min, and one each for 10–17 min. The duration of inhalation was significantly longer in Natters than in Vöcklabruck ( $10.70 \pm 1.38$  min *versus*  $7.92 \pm 1.05$  min, *p* < 0.01). However, there were no differences between the test and control group (*p* > 0.79), both overall and within each centre.

### *Subjective adverse effects*

During the inhalation period, only slight subjective sensations occurred so that all test persons performed all inhalations according to the protocol. There was no dyspnoea, no pain, and no severe graded event. A detailed list of symptoms and their severity and duration is provided in



**Figure 1.** Overview of the study according to Consolidated Standards of Reporting Trials guidelines. NCT, N-chlorotaurine.

Table 2. At least one slight subjective adverse sensation was noted in all 12 participants of the test group and in 5 of 12 participants in the control group. As expected from the pilot tests, the only highly significantly more frequent sensation caused by NCT compared with NaCl was a mild to maximally moderate chlorine taste during inhalation and up to 180 min after inhalation ( $p < 0.01$ ). This was also the by far most frequent adverse effect, occurring in nine (75%) of the test persons treated with NCT. A participant experienced it as ‘mildly bitter’. Another

participant in the control group experienced a ‘chlorine taste’, and another one the expected ‘saline taste’. None of the participants were impaired significantly by these sensations and no one terminated the inhalations. The taste did not increase during the 5 days of treatment but decreased with the number of inhalations. All three participants that felt it as moderate in the first days rated it mild from day 2–5, mild on day 5, or absent on days 4 and 5 (1 participant each). In addition, there was a trend to experience a tickle in the throat during inhalation in the test



**Table 1.** Demographics.

	Centre	Female	Male	Age (median; range)
overall		15	9	34; 21–74
	NA	7	5	47; 21–74
	VB	8	4	34; 24–67
NCT		9	3	34; 24–74
	NA	5	1	40; 24–74
	VB	4	2	34; 25–40
Placebo		6	6	43.5; 21–67
	NA	2	4	48.5; 21–56
	VB	4	2	33.5; 24–67

NA, Hospital Natters; NCT, N-chlorotaurine; VB, Hospital Vöcklabruck.

group (Table 2). All other sensations were less frequent (below 20%) without a difference between the test and control group (Table 2).

Immediately after inhalation, the chlorine or saline taste were the only sensations noticed for a further 1–30 min (up to 180 min in one test person) in the test group ( $p < 0.01$  versus control group). In the control group, different symptoms were noticed, but only occasionally and not statistically different from the test group (Table 2).

The sensations noticed later, not immediately after the inhalations, were the following:

In the test group, mild scraping in the throat occurred 3 h later for 1 h on day 1 and 2 (one participant). In the control group, mild sneezing was perceived 3.5 h later on day 2 (one participant), scraping in the throat 1 h later on day 1 for approximately 1.5 days (one participant).

### Objective results

We could not detect any statistically significant changes of objective parameters measured before, during and after the inhalation period, that persisted over the study period, neither in the test nor in the control group.

The primary evaluation criterion, the FEV<sub>1</sub> as percentage of the norm is depicted in Figure 2. As

can be seen, the mean initial value before inhalations was by 7.91% higher in the test than in the control group ( $p = 0.061$  by an unpaired  $t$  test). However, this difference remained stable during the whole study period and at the long-term examination 8–12 weeks after the inhalation period with 7.58%. Accordingly, when the initial values (100.83%  $\pm$  8.04% for the test group, 92.92%  $\pm$  11.35% for controls) were compared with the values after 10 days (98.83%  $\pm$  8.14% and 92.25%  $\pm$  11.46%, respectively) and 8–14 weeks (98.75%  $\pm$  7.37% and 91.17%  $\pm$  9.46%, respectively), there was no difference within each group ( $p > 0.082$  between the time points by paired  $t$  test, Figure 2). Comparing the FEV<sub>1</sub> within each group before and after inhalation, there was a small significant decrease on the first day in both groups, by 1.75% in the test ( $p = 0.0089$ ) and by 3.00% in the control group ( $p = 0.0055$ ). Such a decrease by 0.91% reached significance in the NCT group on day 2 ( $p = 0.0137$ ), too. There were no further differences ( $p > 0.1$ ). When FEV<sub>1</sub> was used for the calculations as values in liters, there was no statistically significant difference between the test and control group, too, while the intra-group results were similar to FEV<sub>1</sub>%.

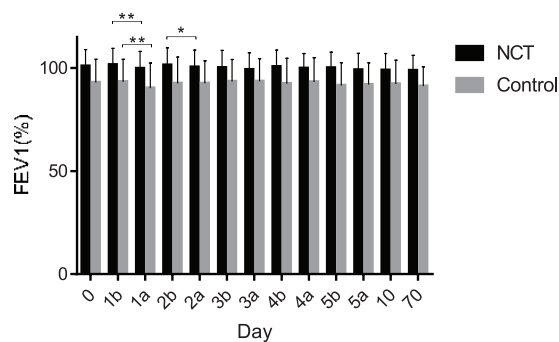
Airway resistance (R<sub>eff</sub>) did not change during the study and was not different between the groups. There were also no other values of lung function measurement out of the norm.

**Table 2.** Subjective sensations during and immediately after inhalation.

		Scraping in the throat			Tickle in the throat		
		Frequency	Duration	Grade	Frequency	Duration	Grade
<i>During inhalation</i>							
Sum per 12 participants	NCT	1 (8.3%)	6–7 min	mild	3 (25%)	1–7 min	mild
		$p = 1.0$			$p = 0.217$		
Sum per 12 participants	Placebo	1 (8.3%)	5 min	mild	0 (0%)		
Sum per 60 days of treatment	NCT	2			5		
		$p = 1.0$			$p = 0.057$		
Sum per 60 days of treatment	Placebo	1			0		
<i>After inhalation</i>							
Sum per 12 participants	NCT	0 (0%)			0 (0%)		
		$p = 1.0$			$p = 1.0$		
Sum per 12 participants	Placebo	1 (8.3%)	10 min	mild	1 (8.3%)	10–540 min	mild
Sum per 60 days of treatment	NCT	0			0		
		$p = 1.0$			$p = 0.50$		
Sum per 60 days of treatment	Placebo	1			2		
		Abnormal taste			Other sensations		
		Frequency	Duration	Grade	Frequency	Duration	Grade
<i>During inhalation</i>							
Sum per 12 participants	NCT	9 (75%)	4–10 min	mild (6)	2 (17%)	5–10 min	mild (1) <sup>a</sup>
		$p = 0.0123$		moderate (3)	$p = 1.0$		moderate (1) <sup>a</sup>
Sum per 12 participants	Placebo	2 (17%)	6–10 min	mild	3 (25%)	2–7 min	mild (3) <sup>b</sup>
Sum per 60 days of treatment	NCT	34		mild (26)	4		mild (3)
		$p < 0.001$		moderate (8)	$p = 1.0$		moderate (1)
Sum per 60 days of treatment	Placebo	5		mild (5)	4		mild (4)
<i>After inhalation</i>							
Sum per 12 participants	NCT	9 (75%)	1–180 min	mild (8)	0 (0%)		
		$p = 0.0123$		moderate (1)	$p = 0.217$		
Sum per 12 participants	Placebo	2 (17%)	3–30 min	mild (2)	3 (25%)	10–540 min	mild (3) <sup>c</sup>
Sum per 60 days of treatment	NCT	22		mild (20)	0		
		$p < 0.001$		moderate (2)	$p = 0.119$		
Sum per 60 days of treatment	Placebo	4		mild (4)	4		mild (4)

<sup>a</sup>dry mouth.<sup>b</sup>prickling tongue (2–4 min), dry mouth (7 min), hoarseness (540 min).<sup>c</sup>prickling tongue (10–20 min), dyscrinism (540 min), hoarseness (540 min).

NCT, N-chlorotaurine.



**Figure 2.** Course of FEV<sub>1</sub>% during the study.

1b before inhalation, day 1.

1a after inhalation, day 1.

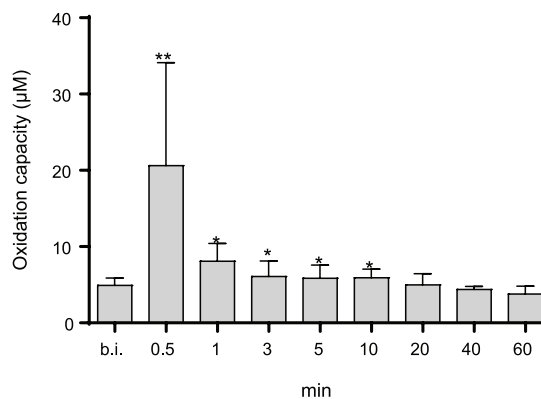
10 1 week after inhalation.

70 8–14 weeks after inhalation.

Mean values  $\pm$  SD of 12 participants.

\* $p = 0.014$  and \*\* $p < 0.01$  by paired  $t$  test, evaluating within persons differences (before–after) separately for treatment groups.

FEV<sub>1</sub>, forced expiratory volume in 1 second; NCT, N-chlorotaurine.



**Figure 3.** Kinetics of inhaled NCT in sputum.

Oxidation capacity was measured after 10 min inhalation of 1% NCT. Mean values  $\pm$  SD,  $n = 5$  ( $n = 4$  for 1 min,  $n = 3$  for 40 min).

\*\* $p < 0.01$  versus all other time points (one-way ANOVA with Dunnett's multiple comparison test);

\* $p < 0.05$  versus 60 min (paired  $t$  test).

ANOVA, analysis of variance; b.i. before inhalation; NCT, N-chlorotaurine.

Measurements of peak flow by the test persons at home on day 5 every 2 h up to 8 h after inhalation remained constant and revealed no hint for a delayed impaired lung function. Values for NCT were  $525 \pm 115$  l/min when the test person left the outpatient department and  $522 \pm 111$  l/min 8 h later ( $p = 0.686$ ), and  $571 \pm 173$  l/min upon leaving and  $559 \pm 177$  l/min 8 h later for saline ( $p = 0.066$ ).

There was no induction of a bronchial hyper-responsiveness in any of the participants. Oxygen saturation by pulse oximetry showed no changes before, during, and after each inhalation and in the long-term examination and ranged between 95% and 100% except for four single values in total (once 91% and 93% in the control group, and 94% once each in both groups).

### Pharmacokinetics

Repeated laborious immediate measurements of the oxidation capacity in the sputum in the test person that inhaled for 1 week during the pilot tests showed clear positive values for 0.5 and 1 min after the end of inhalation, followed by a period of 10–20 min with very low values close to the detection limit (Figure 3). This indicates reduction of the oxidation capacity of NCT by organic matter of the mucus. Because of unfeasibility of such experiments in the phase I study, we

decided to use serum values of taurine, the decay product of NCT after reduction, to measure systemic absorption and pharmacokinetics. The initial values of  $54.8 \pm 9.8$   $\mu\text{mol/l}$  taurine in the test and  $49.1 \pm 11.1$   $\mu\text{mol/l}$  in the control group ( $p > 0.1$  between groups, mean values  $\pm$  SD) did neither change significantly 10, 30, and 60 min after inhalation on day 5 nor 1 week later ( $52.8 \pm 8.8$  and  $49.4 \pm 14.1$   $\mu\text{mol/l}$ , respectively;  $p > 0.1$ ). Similarly, methionine values did not change at the same time points of evaluation [ $23.7 \pm 6.2$   $\mu\text{mol/l}$  in the test and  $24.4 \pm 7.7$   $\mu\text{mol/l}$  in the control group upon screening,  $23.2 \pm 7.0$  and  $28.4 \pm 14.3$   $\mu\text{mol/l}$  60 min after inhalation, and  $23.5 \pm 5.4$  and  $22.2 \pm 4.4$   $\mu\text{mol/l}$  ( $p > 0.1$  between groups)].

### Other laboratory findings

Blood values of electrolytes Na, K, Ca, Cl, blood count, liver and renal function parameters ALAT, ASAT, gGT, urea, creatinine did not show any significant changes. The same was true for neopterin, tryptophan, kynurenine, and the kynurenine/tryptophan ratio in plasma.

### Clinical examinations

Auscultation of lung and heart revealed no pathological findings and never changed before and after inhalation.

### Sex aspects

Due to the limited size of the study, the number of participants was too low to provide a reliable evaluation according to sex. The most frequent subjective symptom, chlorine taste in the test group occurred in women and men with no statistical difference. There was no parameter that changed specifically for sex.

### Discussion

Infections of the bronchopulmonary system by different pathogens are among the most frequent in human medicine. Bacteria and fungi multiresistance against antibiotics and viruses is a permanent problem, mainly in critically and chronically ill patients.<sup>30</sup> The availability of a well-tolerated and effective inhaled antiseptic with anti-inflammatory properties would be a significant progress to counteract resistance problems, in particular for chronic pulmonary diseases, such as COPD or cystic fibrosis.

The mild antiseptic NCT is a candidate of interest following this concept. As an endogenous mild oxidant assumed to be involved in termination of inflammation *in vivo*, it has demonstrated good tolerability, but also anti-infective efficacy in different body regions.<sup>11,31</sup> In the last few years, tolerability of the clinically applicable concentration of 1% was shown upon inhalation of NCT in pigs and mice, which paved the way for the phase I study in humans.<sup>19–21</sup> Because of the good tolerability in the pilot tests in man and considering efficacy for the future, we decided to use 1% NCT in the main phase I trial, too. This turned out to be all right since the safety and tolerability was confirmed. We wanted to test repeated inhalations mimicking a treatment period. Concurrently, it should be feasible for the participants and the hospital personnel. Therefore, we decided on a repeat protocol of five consecutive working days, which was practicable for all involved persons.

There were no systemic adverse effects, obviously due to the absence of systemic absorption of NCT in amounts that affect the physiological serum concentration of taurine. This was confirmed by the absence of elevation of electrolytes or other blood parameters. The rapid small increase of taurine after inhalation in the pilot test could not be verified in the larger collective and appears to be rather a laboratory standard deviation than a real effect. In any case, the taurine level did not

become elevated above the normal range. The volunteers were told not to use energy drinks during the study, and they obviously followed this recommendation.

Moreover, methionine and cystine did not change at all, indicating the absence of absorption of detectable amounts of active chlorine to the blood. The oxidative activity of NCT was measurable topically in sputum at significant concentrations only for 1 min after the end of the inhalation. The low level for further 10–20 min may indicate a chlorine cover as it can be seen on the human skin or on bacteria and fungi after treatment with chloramines.<sup>32,33</sup> Inactivation of the active chlorine occurs by reactions with mainly thio groups of the protein matter in mucus.<sup>10,31</sup>

The presence of chlorine was experienced as chlorine taste in the majority of the test persons, the only adverse effect with high statistical significance compared with placebo. The taste was not considerably unpleasant, but it indicates activity of NCT for some time, which may be important for efficacy. A scraping and tickling sensation in the throat are more important adverse effects for tolerability and acceptance of NCT inhalation. They can be kept mild or absent by avoiding too forced an inspiration and disappear again within seconds. Further subjective sensations can either not be related specifically to NCT because of a similar frequency of occurrence in the control group (dry mouth) or occasionally occurred only in the control group without significance (prickling tongue, hoarseness, dyscrinism). Inhalations were tolerated with three different nebulizers, AKITA JET, Multisonic LS-260®, and Pari Boy® SX during our studies. AKITA JET was applied for the main study since it delivers the best-defined amount of test substance to the lower airways.<sup>23</sup>

The primary evaluation criterion, FEV<sub>1</sub>, did not change over time by the treatment. It was by chance on average higher in the test than in the control group at the initial examination before inhalations, but this difference remained constant, indicating the same course in both groups. The minimal, but significant decrease of FEV<sub>1</sub> before and after inhalation in the first 2 days was similar in both groups, and obviously it was related to some degree of stress by the study situation itself with the repeated lung function tests. In any case, all single deviations were minimal and far distant from a 10% difference and clinical

significance.<sup>28,29</sup> This is confirmed by the absence of significant deviations of other lung function parameters, particularly airway resistance (R<sub>eff</sub>), and of blood oxygenation.

Furthermore, there were no hints of subacute or long-term adverse effects on the bronchopulmonary system or systemic effects, as indicated by the peak flow self-measurements and by the results of the respective examinations up to 3 months after the treatment period. The absence of an increase of leukocytes or elevated neopterin and kynurenine/tryptophan concentrations confirms the absence of an inflammatory reaction caused by NCT inhalations. In agreement, NCT has shown downregulation of these parameters in activated mononuclear cells *in vitro*.<sup>34</sup> Hyperresponsiveness of the bronchi was not induced.

Persons with bronchial hyperresponsiveness were excluded from the study to avoid uncertainties in the evaluation. A test person suffering from such a disorder had no problems with 10 min inhalation of 1% NCT in an additional pilot test on testing the AKITA JET system with NCT. Additional studies are necessary to obtain reliable data on the tolerability of NCT in such disorders. Further limitations of the study are the small size, which, however, warranted its feasibility at academic institutions within reasonable time, and the small number of different nebulizers used in tests up to now.

### Conclusion

We conclude that inhaled 1% NCT has demonstrated high tolerability and absence of considerable adverse effects in this phase I study. Mild subjective sensations (i.e. mainly chlorine taste and tickle in the throat), are not limiting or can be avoided by a correct inhalation technique. The good tolerability of NCT for the first time renders an antiseptic interesting for inhalation.

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### Conflict of interest statement

M. Nagl is co-inventor of a patent on the application of NCT for inhalation. B. Müllinger is an employee at Vectura GmbH, which is manufacturing the AKITA JET inhalation system. He is an inventor of patents covering the AKITA JET device technology. All other authors declare no conflict of interest.

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