ORIGINAL ARTICLE

Antimicrobial Activity of Dexamethasone and Its Combination With *N*-Chlorotaurine

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Objective: To investigate the antimicrobial effect of dexamethasone phosphate, the endogenous antiseptic *N*-chlorotaurine (NCT), and their combination on ear, nose, and throat microorganisms.

Design: In vitro study.

Subjects: Strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus milleri*, *Aspergillus flavus*, and *Aspergillus fumigatus*.

Interventions: Bacterial and fungal strains were cultured with 0.1% dexamethasone with and without a low (0.1%) or high (1%) concentration of NCT. The killing effects of dexamethasone, NCT, and the combination were monitored.

Results: Dexamethasone killed *S milleri* and *A flavus* after incubation times of 24 to 48 hours. The low concentration of NCT caused a 90% reduction of *S aureus* and *P aeruginosa* within 30 minutes and 99.9% reduction within 50 minutes. The high concentration of NCT reduced viable counts of *S aureus* and *P aeruginosa* to the detection limit within 10 minutes. The low-concentration combination (0.1% dexamethasone and 0.1% NCT) showed significant ($P < .01$) synergistic killing of *S aureus* with 2- to 3-fold shorter killing times. The high-concentration combination (0.1% dexamethasone and 1% NCT) demonstrated more rapid killing than NCT alone in both *S aureus* and *P aeruginosa*.

Conclusions: With short and intermediate exposure times, the combination of dexamethasone and NCT showed significantly stronger antimicrobial effects than treatment with NCT alone. Significant killing of *S milleri*, *A flavus*, and *A fumigatus* was observed after extended exposure to dexamethasone. The combined application of dexamethasone and NCT might be a promising therapeutic option, producing high efficacy with low side effects.

Arch Otolaryngol Head Neck Surg. 2008;134(6):615-620

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IN THE TREATMENT OF SEVERAL EAR, nose, and throat infections, such as sinusitis and otitis externa, corticosteroids have been added to antibiotic substances because they reduce inflammation and swelling. Ear, nose, and throat infections often occur in cavities and ducts, where the inflammatory swelling induces the formation of an airtight moist chamber, which favors bacterial and fungal growth.

To break this vicious circle, it seems reasonable to combine an antimicrobial agent with an anti-inflammatory drug. In recent years we have been developing a promising antiseptic agent that might be suitable for combination with corticosteroids for topical treatment of ear, nose, and throat infections. This study was designed to investigate the antimicrobial activity of the antiseptic *N*-chlorotaurine (NCT) in the presence of a corticoid and to test the corticosteroid without additives on antimicrobial activity.

N-Chlorotaurine ($ClHN\text{-}CH_2\text{-}CH_2\text{-}SO_3H$) is a weak oxidant, produced by human granulocytes and monocytes during inflammation.[1,2] It also can be synthesized chemically as a crystalline sodium salt and is highly soluble in aqueous solution, which enables its application as an antiseptic in human medicine.[3] It is microbicidal against a broad spectrum of all classes of pathogens, including bacteria and fungi.[4-7] Killing of pathogens by NCT requires at least a few minutes because of its low activity, but it is easily sufficient for therapeutic efficacy according to clinical studies in otitis externa, purulently coated crural ulcers, and conjunctivitis.[8-10] The advantage of the mild activity is the excellent tolerability of NCT by human tissue shown in these studies. Moreover, NCT is characterized by low cellular toxicity and good nasal tolerability in humans,[8,9,11-14] accompanied by significant bactericidal and fungicidal properties.[4-7] Administration of NCT causes mi-

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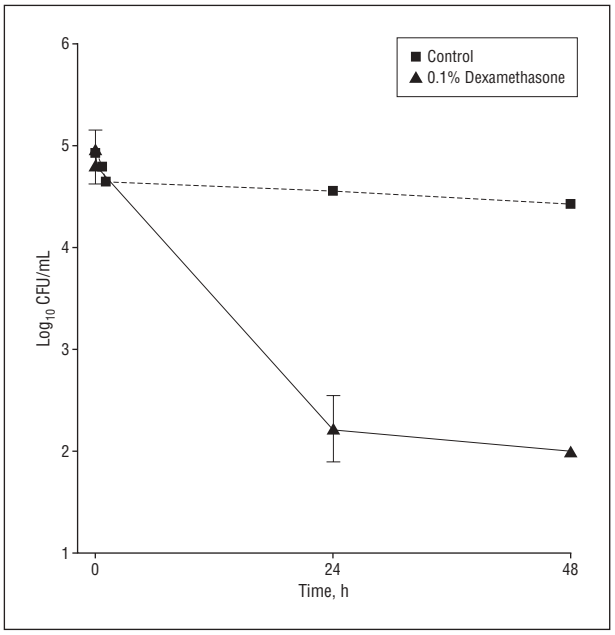

Figure 1. Bactericidal activity of 0.1% dexamethasone phosphate against *Streptococcus milleri* at 37°C and pH 7.1. Control treatment was without additives in buffer solution. Values are shown as mean ± SEM of 2 independent experiments. $P<.01$ between test and control curves. CFU indicates colony-forming units.

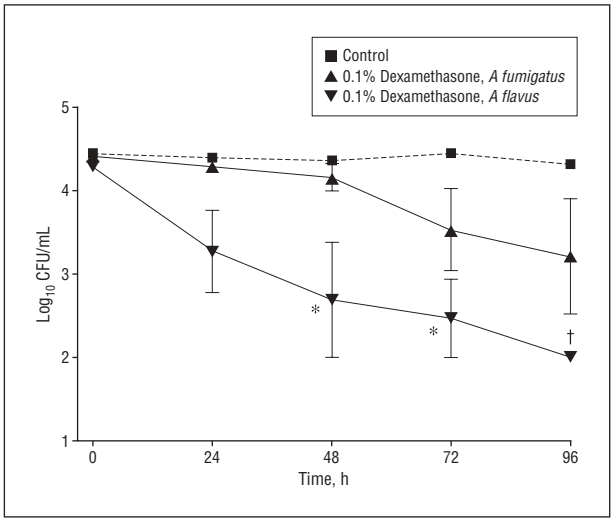

Figure 2. Fungicidal activity of 0.1% dexamethasone phosphate against *Aspergillus flavus* and *Aspergillus fumigatus* at 37°C and pH 7.1. Control treatment was without additives in buffer solution. Values are shown as mean ± SEM of 2 to 3 independent experiments. $P<.05$ (*) and $P<.01$ (†) between 0.1% dexamethasone and control curves (analysis of variance); $P<.05$ between dexamethasone against *A fumigatus* vs control (paired *t* test for overall 24-96 hours; $P>.05$ for single points calculated with analysis of variance). $P<.01$ between dexamethasone against *A flavus* vs *A fumigatus* (paired *t* test for overall 24-96 hours).

crobicidal activity without any toxic potential on cellular integrity or ciliary beat frequency.[15]

The glucocorticoid we chose for this study was dexamethasone phosphate, a synthetic, inexpensive, and well-documented drug known to have potent anti-inflammatory effects. The anti-inflammatory effects of glucocorticoids are due to specific receptor-mediated actions dependent on binding with intracellular glucocorticoid receptors.[16] Corticosteroids reduce the number of T lymphocytes, eosinophils, mast cells, basophils, and monocytes. They also diminish the effects of mediators such as histamine, tryptase, prostanoids, leukotrienes, cytokines, and chemokines.[16] Secondary to their effect on inflammation mediators, intranasal application leads to reduction of nasal congestion and rhinorrhea.

Table 1. Killing Times for Dexamethasone

	0.1% Dexamethasone Phosphate		
Microbe	K1, h	K2, h	K3, h
Streptococcus milleri	6.8	15.7	32.9
Aspergillus flavus	29.2	78.3	> 96
Aspergillus fumigatus	84.3	>96	> 96

Abbreviations: K1, time to achieve a reduction in viable counts of 1 $\log_{10}$ (90% killing); K2, time to achieve a reduction in viable counts of 2 $\log_{10}$ (99% killing); K3, time to achieve a reduction in viable counts of 3 $\log_{10}$ (99.9% killing).

In this in vitro study, we investigated the antimicrobial effects of NCT, dexamethasone, and the combination of these substances on ear, nose, and throat–relevant bacterial and fungal microorganisms.

METHODS

BACTERIA AND MEDIA

Bacterial strains *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Streptococcus milleri* (a clinical isolate) deep frozen for storage were grown overnight on tryptic soy agar (Merck, Darmstadt, Germany). Colonies from this agar were grown in tryptic soy broth (Merck) at 37°C overnight.

Clinical isolates of *Aspergillus flavus* and *Aspergillus fumigatus* were grown on Sabouraud agar (Merck) at 20°C for 1 week. Plates were rinsed with 0.9% sodium chloride to collect the conidia. Large particles were removed by centrifugation at 190*g* for 5 minutes, and the supernatant was removed for testing.

Both bacteria and fungi were centrifuged at 1800*g*, washed twice in 0.9% sodium chloride, and diluted to a concentration of 5×10^6 to 1×10^8 colony-forming units per milliliter before use.

REAGENTS

Pure crystalline sodium salt of NCT (molecular weight, 181.57 g/mol; METASYS, Innsbruck, Austria)[3] was dissolved in 0.1M phosphate-buffered saline (pH 7.1) at a final concentration of 1% (wt/vol) (55mM) and 0.1% (wt/vol) (5.5mM). Dexamethasone phosphate (Spectrum, Gardena, California) was added to a final concentration of 0.1% (wt/vol).

MICROBICIDAL ACTIVITY OF THE TEST SOLUTIONS

All test strains were tested separately. Bacteria and fungi were diluted 100-fold in the test solutions containing a volume of 4 mL. Subsequent to individual incubation times at 37°C, aliquots of 100 µL were removed and diluted in 900 µL of 0.6% aqueous sodium thiosulfate for inactivation of NCT. Aliquots of 50 µL were spread in duplicate onto tryptic soy agar plates with

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Table 2. Killing Times for 0.1% NCT With and Without 0.1% Dexamethasone

	0.1% NCT			0.1% NCT + 0.1% Dexamethasone Phosphate		
	K1, min	K2, min	K3, min	K1, min	K2, min	K3, min
Staphylococcus aureus	27.0	≈35[a]	≈45[a]	8.3	15.0	22.4
Pseudomonas aeruginosa	29.1	38.4	47.9	29.9	39.6	49.5

Abbreviations: K1, time to achieve a reduction in viable counts of 1 $\log_{10}$ (90% killing); K2, time to achieve a reduction in viable counts of 2 $\log_{10}$ (99% killing); K3, time to achieve a reduction in viable counts of 3 $\log_{10}$ (99.9% killing); NCT, *N*-chlorotaurine.
[a] Extrapolated from Figure 3A.

an automatic spiral plater (Don Whitley Scientific Limited, West Yorkshire, England), allowing a detection limit of 10 colony-forming units per milliliter. The plates were incubated at 37°C, and the colony-forming units were counted after 24 hours (bacteria) and 48 to 72 hours (fungi). Pathogen cultures treated without NCT or dexamethasone served as controls. As a further control, pathogens were exposed to NCT that was inactivated by thiosulfate. Those samples indicated sufficient inactivation because the growth of the pathogens was not affected. Incubation times needed to achieve a reduction in viable counts of 1, 2, and 3 $\log_{10}$ (90%, 99%, and 99.9% killing, respectively) were calculated by nonlinear regression using GraphPad software (GraphPad Software Inc, San Diego, California).

STATISTICAL ANALYSIS

We used the paired *t* test for comparison of paired means of 2 groups of measurements. One-way analysis of variance and Dunnett multiple comparison test (GraphPad software) were applied for evaluation of the significance of 3 or more groups of measurements.

RESULTS

The corticosteroid dexamethasone (0.1% wt/vol) killed *S milleri* and *A flavus* after long incubation times of 24 to 48 hours (**Figure 1**, **Figure 2**, and **Table 1**). The bacteria were killed more rapidly than the molds, and there was very slow inactivation of *A fumigatus*. The antiseptic NCT killed all bacteria and fungi used in this study as expected; the killing times depended on the concentration and pathogen.

The low concentration of NCT (0.1% wt/vol) caused a 90% reduction in *S aureus* and *P aeruginosa* within 30 minutes and a 99.9% reduction within 50 minutes (**Table 2**, **Figure 3**). Dexamethasone (0.1% wt/vol) killed neither *S aureus* nor *P aeruginosa* (Figure 3).

The high concentration of NCT (1% wt/vol) reduced viable counts of *S aureus* and *P aeruginosa* to the detection limit within 10 minutes; 99.9% killing was achieved after 7 and 4 minutes, respectively (**Table 3**, **Figure 4**). For 90% reduction of molds, a significantly longer period of 3 to 5 hours was necessary (Table 3, **Figure 5**).

The low-concentration combination (dexamethasone, 0.1% wt/vol, and NCT, 0.1% wt/vol) showed killing of *S aureus* with 2- to 3-fold shorter killing times ($P<.01$) (Figure 3A, Table 2) compared with 0.1% NCT ($P<.01$). By contrast, for *P aeruginosa* the reduction of colony-forming units by the combination was equal to that by 0.1% NCT without dexamethasone (Figure 3B, Table 2) ($P>.05$).

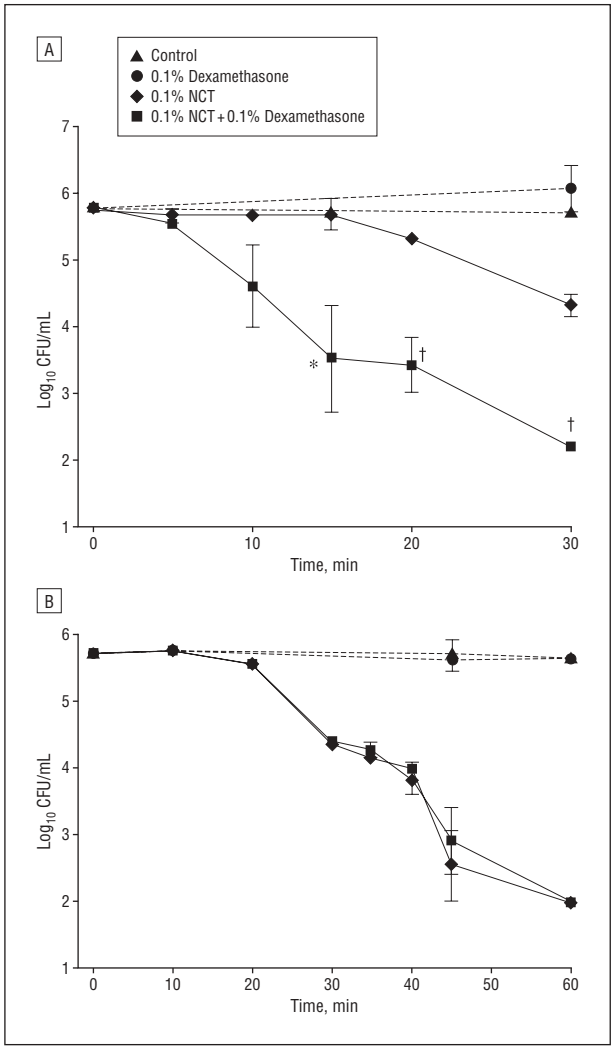

Figure 3. Bactericidal activity of 0.1% *N*-chlorotaurine (NCT) and 0.1% NCT with 0.1% dexamethasone phosphate against *Staphylococcus aureus* (A) and *Pseudomonas aeruginosa* (B) at 37°C and pH 7.1. Values are shown as mean ± SEM of 3 independent experiments. $P<.01$ between NCT with and without dexamethasone and control; $P<.05$ (*) and $P<.01$ (†) between NCT and NCT with dexamethasone. CFU indicates colony-forming units.

The high-concentration combination (dexamethasone, 0.1% wt/vol, and NCT, 1% wt/vol) demonstrated a trend toward enhanced killing effects compared with 1% NCT without additives. This was true for both *S aureus* and *P aeruginosa* (Figure 4A and 4B, Table 3). Taking all single values of incubation times 1 to 5 together, this was significant for *P aeruginosa* ($P<.01$). A similar

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Table 3. Killing Times for 1% NCT With and Without 0.1% Dexamethasone

	1% NCT			1% NCT + 0.1% Dexamethasone Phosphate		
	K1	K2	K3	K1	K2	K3
Staphylococcus aureus, min	2.3	4.3	6.9	1.8	3.6	6.0
Pseudomonas aeruginosa, min	0.8	2.0	3.7	0.7	1.5	2.6
Aspergillus flavus, h	3.3	4.5	≈6[a]	2.4	4.2	≈6[a]
Aspergillus fumigatus, h	4.9	7.1	≈9[a]	3.3	4.8	≈7[a]

Abbreviations: K1, time to achieve a reduction in viable counts of 1 $\log_{10}$ (90% killing); K2, time to achieve a reduction in viable counts of 2 $\log_{10}$ (99% killing); K3, time to achieve a reduction in viable counts of 3 $\log_{10}$ (99.9% killing); NCT, *N*-chlorotaurine.
[a] Extrapolated from Figure 5.

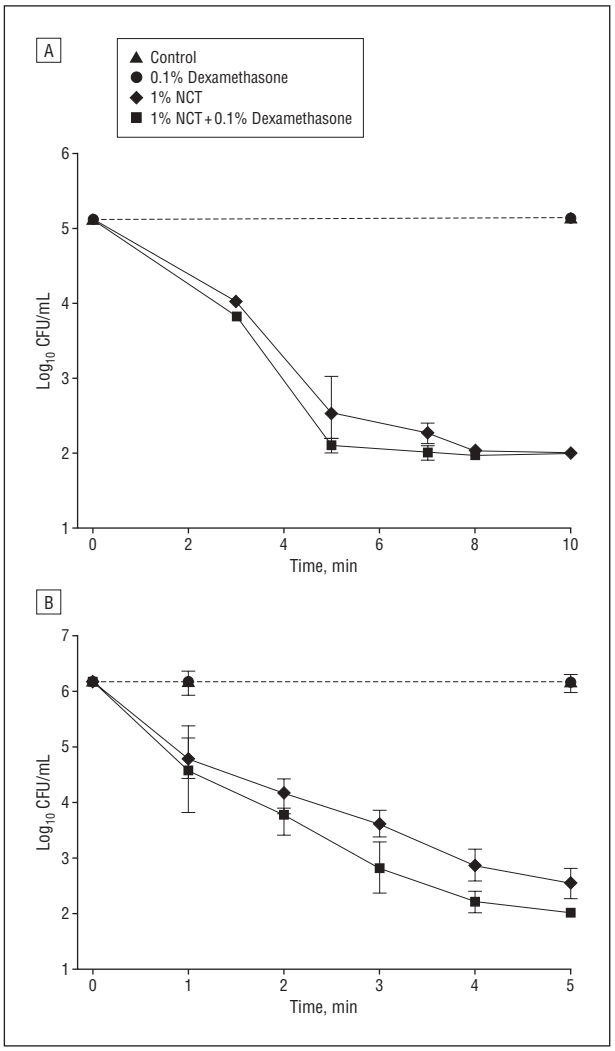

Figure 4. Bactericidal activity of 1% *N*-chlorotaurine (NCT) and 1% NCT with 0.1% dexamethasone phosphate against *Staphylococcus aureus* (A) and *Pseudomonas aeruginosa* (B) at 37°C and pH 7.1. Values are shown as mean ± SEM of 3 independent experiments. $P<.01$ between NCT with and without dexamethasone and control; $P<.01$ (in B) between NCT and NCT with dexamethasone for time points 1 to 5 minutes in total (2-sided paired *t* test). $P>.05$ for single time points (analysis of variance). CFU indicates colony-forming units.

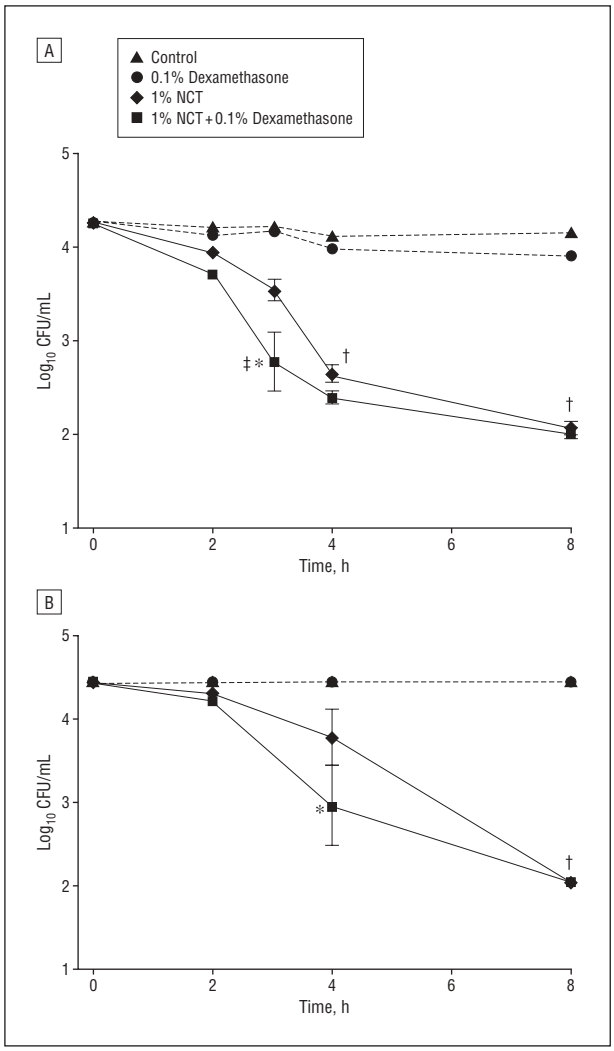

Figure 5. Fungicidal activity of 1% *N*-chlorotaurine (NCT) and 1% NCT with 0.1% dexamethasone phosphate against *Aspergillus flavus* (A) and *Aspergillus fumigatus* (B) at 37°C and pH 7.1. Values are shown as mean ± SEM of 3 to 4 independent experiments. $P<.05$ (*) and $P<.01$ (†) between NCT with and without dexamethasone and control; $P<.05$ (‡) between 1% NCT and 1% NCT with 0.1% dexamethasone after 3-hour incubation of *A flavus* (A).

trend of additive effects was found in molds (Figure 5, Table 3). Killing of *A flavus* after 3 hours was significantly stronger with the combination than with NCT alone (Figure 5A).

COMMENT

Topical corticosteroids serve as a partner in antibiotic compounds for several ear, nose, and throat infections such

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as sinusitis and otitis externa because they provide a strong anti-inflammatory and decongestive effect. Emgard et al[17,18] reported on successful treatment of bacterial otitis externa by betamethasone only. The causative mechanism was considered to be the anti-inflammatory and decongestive properties of the substance. An inherent direct microbicidal effect of corticosteroids applied in human medicine has not been reported, to our knowledge. With agar diffusion tests, inhibition of growth of some bacterial strains by steroidal glycosides and galactosides produced by green algae has been demonstrated.[19] The underlying mechanisms are unknown.

The present in vitro study demonstrates microbicidal properties of dexamethasone against *S milleri*, *A flavus*, and *A fumigatus*. It is possible that these effects contributed to the positive results of corticosteroids in otitis externa in previous studies.[17,18] The underlying mechanisms for the antimicrobial activity of dexamethasone against *S milleri* and aspergilli are unclear. Because the killing is slow, we speculate that the corticosteroid binds to a specific target like an antibiotic does. The location of such a target cannot be specified to date; it could be the cell wall or cytoplasmic membrane, as well as the transcription and translation machinery of the microorganisms.

The antimicrobial properties of dexamethasone are confirmed by the synergistic effect of dexamethasone in combination with the antiseptic NCT. Although it was not statistically significant with all strains used, there was a clear trend toward a weak enhancement of the activity of NCT by dexamethasone. The effect seems to be dependent on both the bacterial species and the concentration of NCT. Enhanced killing with NCT combined with dexamethasone was most pronounced in *S aureus* with 0.1% NCT; there was no significant difference between 1% NCT with and without dexamethasone. With *P aeruginosa*, the addition of dexamethasone made a difference with the higher concentration of NCT rather than with the lower one. This might indicate that there are different targets of dexamethasone in gram-positive and gram-negative bacteria.

Because the microbicidal activity of NCT as an antiseptic is much higher than that of dexamethasone, it remains unclear whether the additional activity of dexamethasone in the combination has any therapeutic effect in eradication of microorganisms in vivo. Nevertheless, it can be speculated that the combination of antimicrobial activity primarily by NCT and anti-inflammatory activity primarily by the corticosteroid are beneficial in special indications, eg, chronic rhinosinusitis or otitis externa.

The advantages of NCT for topical treatment of infections are its good tolerability, resulting from its natural occurrence in the human body and mild activity, and its broad-spectrum microbicidal activity.[4-7] Features of NCT are its availability as a crystalline sodium salt, solubility in aqueous solution, and outstanding stability compared with other *N*-chloro amino derivatives.[3] Because of the endogenous nature of NCT, an allergic reaction to this substance is generally improbable and has never been recorded in clinical trials, to our knowledge.[8,9,12,14] *N*-Chlorotaurine decomposes to the endogenous components taurine and chloride in human inflamed tissue.[1,3] Therefore, no toxic derivatives may occur. The unspecific mechanism of action, ie, oxidation of mainly thio and amino groups,[3] does not enable development of resistance during therapy. As an antiseptic, it has the advantage of use without preservatives or additives that could cause allergy.

In addition to having antimicrobial properties, NCT is considered to be involved in immune regulation. In vitro it has been reported to down-regulate proinflammatory cytokines of macrophages, dendritic cells, and T cells, namely tumor necrosis factor α, nitric oxide, prostaglandin E_2, and interleukins 1β, 2, 6, 8, 10, and 12.[20,21] Such mechanisms possibly play a role in clinical application by contributing to a decrease of edema and swelling or to a drying effect. It remains to be clarified how such effects of NCT influence the activity of corticosteroids and vice versa.

Dexamethasone is known to be resorbed in relatively large amounts when applied locally, causing systemic adverse effects in long-term and high-dosage use, eg, immunosuppression leading to infections and delayed wound healing, osteoporosis, and exogenous Cushing syndrome. These adverse effects may outweigh the therapy's benefit. For long-term treatment, dexamethasone could be replaced by modern topical corticosteroids, which have no systemic bioavailability. The investigation of a possible antimicrobial effect of these substances would be of certain interest.

In conclusion, NCT without additives killed all the bacteria and fungi used in this study, as we expected. Surprisingly, in the presence of dexamethasone, this activity was enhanced. Moreover, dexamethasone without NCT demonstrated a significant microbicidal effect against certain pathogens. A combination of dexamethasone and NCT might be a promising therapeutic option for the local treatment of microbial infections.

Submitted for Publication: January 27, 2007; final revision received June 14, 2007; accepted August 28, 2007.
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Author Contributions: Drs Neher, Gstöttner, Schäfer, and Nagl had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Neher, Schäfer, and Nagl. *Acquisition of data:* Neher, Arnitz, Kröss, and Nagl. *Analysis and interpretation of data:* Neher, Gstöttner, Schäfer, and Nagl. *Drafting of the manuscript:* Neher, Gstöttner, and Nagl. *Critical revision of the manuscript for important intellectual content:* Neher, Arnitz, Gstöttner, Schäfer, Kröss, and Nagl. *Statistical analysis:* Gstöttner, Schäfer, and Nagl. *Administrative, technical, and material support:* Neher, Arnitz, Gstöttner, and Nagl. *Study supervision:* Neher and Nagl.
Financial Disclosure: None reported.

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