In Vitro Study on the Influence of *N*-Chlorotaurine on the Ciliary Beat Frequency of Nasal Mucosa

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ABSTRACT

Background: *The aim of this study was to investigate the* in vitro *effects of* N-*chlorotaurine (NCT), a new endogenous antimicrobial agent, on the nasal ciliary beat frequency (CBF) in nasal mucosa samples of 10 adult patients who underwent conchotomy.*

Methods: *CBF was measured by a photometric technique, combining a light microscope, a photometer, a photograph multiplier, and a computed analyzing unit.*

Results: *CBF decreased ~10% after 20 minutes of incubation in aqueous 1% NCT solution compared with 3% in 0.9% saline, and 0.1 and 0.01% NCT had no effect. However, a solution of 7% cocaine, which is used routinely for shrinking the nasal mucosa before rhinoscopy lowered the CBF to 50% after the same incubation time.*

Conclusion: *These results confirm the very low toxic potential of NCT, and they are fundamental for clinical investigations regarding its topical application in the nasal and paranasal sinuses.* (American Journal of Rhinology 17, 149–152, 2003)

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The respiratory tract and the middle ear are covered mainly with ciliated epithelium. The mucociliary clearance system protects the respiratory tract against inhaled particles, including pathogens, by transporting them toward the pharynx. Primary, for instance in innate primary ciliary dyskinesia,[1] and secondary disorders of the ciliary movement are frequently associated with inflammation of the respiratory system.[1,2] Therefore, the ciliary beat frequency (CBF) proved to be a good measure for testing toxic effects of substances coming into contact with mucous membranes in otorhinolaryngology.[3–5]

It was the aim of this study to test by this sensitive method the influence of *N*-chlorotaurine (NCT; Cl-HN-CH_2-CH_2-SO_3^-), a long-lived oxidant produced by human granulocytes and monocytes,[6,7] on the CBF of nasal ciliary epithelium. For a few years NCT has been available as a pure crystalline sodium salt well soluble in water,[8] and its bactericidal, fungicidal, and virucidal activity has been investigated.[9–12] Moreover, its bactericidal activity has been shown to increase in the presence of single amine compounds (ammonium, alanine, and glycine) and abscess material of different body regions *in vitro*.[8] This can be explained by chlorine transfer to these compounds and formation of the corresponding *N*-chlorine derivatives, which show higher activity against bacteria and fungi.[8] Accordingly, fungi are killed by NCT more rapidly within nasal mucus than in buffer solution.[13] Therefore, NCT is potentially useful for treatment of bacterial as well as eosinophilic fungal rhinosinusitis.[13] This is supported by the excellent tolerability of 1% aqueous NCT solution by mucous membranes *in vivo*, for instance, in the eye, urinary bladder, and the middle ear.[12,14,15] Ensuring that NCT does not show significant toxicity against ciliated epithelium is important before clin-

ical evaluation of its tolerability in the nasal and paranasal sinuses.

MATERIALS AND METHODS

Chemicals

Pure NCT as a crystalline sodium salt (molecular weight, 181.57 g/mol) was kindly prepared by Waldemar Gottardi (Institute of Hygiene and Social Medicine, Innsbruck, Austria) and was dissolved in distilled water (pH 8.1) to concentrations of 1% (55 mM), 0.1% (5.5 mM), and 0.01% (0.55 mM).

For comparison with NCT, 0.9% saline and an aqueous solution containing 7% cocaine hydrochloride, 0.18% potassium sulfate, and 20% adrenaline bitartrate were used. This solution is routinely applied in our hospital for shrinkage before rhinoscopy.

Ciliated Epithelium Samples and Course of Toxicity Testing

Epithelium mucosa samples of ~10 mm^2 were excised after conchotomy from removed tissue and put into 0.9% saline. Each sample was from a different patient. All tests were done at a constant temperature of 22°C. After 20 minutes in saline, CBF was determined. Subsequently, saline was replaced by 1, 0.1, or 0.01% NCT solution for 20 minutes. CBF was measured again, and the samples were rinsed with saline. After an additional 20 minutes in saline, CBF was measured for the last time. In control experiments, the samples were exposed to 0.9% saline and cocaine/adrenaline solution, respectively, instead of NCT.

Determination of the CBF

The determination of CBF is based on the following principle. The effective ciliary beat is straight, while the return movement is deflected. The resulting rhythmic motion causes a deflection of light, which is measured photometrically. We used a detection method similar to that previously described[4] with equipment consisting of a Laborvert FS light microscope and a Type MPV Combi photometer (Leitz, Jena, Germany) placed on a pneumatic vibration-absorbing table (Barry Controls, Ltd., Hersham, UK). CBF was measured at 500× magnification, and the diameter of the photosensitive field on the sample was 5 μm. The signal was photograph multiplied, digitalized at a sample frequency of 400 Hz, and transformed into a time-amplitude signal. A fast Fourier transformation analysis was performed every 1.6 seconds. For each sample, CBF values were detected at five different sites. Results are presented as the mean values of these single measurements. Student's paired or unpaired *t*-test was used to test for statistical significance, and values of $p < 0.05$ were considered significant.

RESULTS

The effect of NCT on CBF proved to be dependent on the concentration used (Table I). Although 0.01 and 0.1% NCT had no influence compared with saline, 1% NCT caused a significantly higher reduction of CBF in 4 of 10 samples (samples 1 and 5–7, $p = 0.028$, unpaired *t*-test of NCT versus saline). Compared with the start values in saline, this decrease was significant also ($p = 0.006$, paired *t*-test). A tendency of partial recovery of CBF could be observed in three of the mentioned four samples when NCT was replaced by saline again.

It must be stated that there was also a small but significant CBF reduction in the saline control experiments ($p = 0.016$ after 40 minutes; $p = 0.0007$ after 60 minutes; paired *t*-test). To estimate the meaning of these results, we investigated a cocaine/adrenaline solution (Table I). In the presence of these agents, CBF decreased ~50% after 20 minutes, and no signs of recovery could be observed subsequent to washing off with saline.

DISCUSSION

As a mild long-lived oxidant produced by activated human leukocytes, NCT is known to be markedly less cytotoxic than highly reactive oxidants like hypochlorite or oxygen radicals.[7,16] It is thought not to be involved in tissue damage *in vivo* but in down-regulation of proinflammatory cytokines and termination of inflammation.[17] The good tolerability of NCT could be proved in recent clinical trials in the human eye and urinary bladder as well as the middle ear of the mouse at a concentration of 1%, which exceeds the physiological one by ~1000-fold and shows markedly increased antimicrobial activity.[12,14,15]

The small influence of NCT on the ciliated epithelium confirms these findings. Although CBF decreased in the presence of NCT at the highest tested concentration in 4 of 10 samples, the extent of this effect was significantly lower than that caused by a cocaine preparation, which is applied frequently in otorhinolaryngology.[5] Moreover, the mucociliary alteration by NCT seems to be reversible at least in part, indicating only a functional and temporary change of epithelium cells.

Cocaine has been reported to produce *in vitro* a significant decrease of CBF at concentrations of 1.75% and a complete depletion of ciliary beating at 7%[5]; 5% decreased the mucociliary transport *in vivo*, and 20% caused a complete ciliary paralysis *in vitro*.[18] In accordance with our study, this effect was not fully reversible.[5] Using an apparatus with a perfusion chamber, even 0.9% saline has been shown to reduce CBF,[3] which is confirmed by our findings (Table I).

In a chicken model, Ukai *et al.* showed that CBF and mucociliary transport may be independent of each other.[18] In a previous study with budesonide there was no correlation between CBF and rhinoscopic appearance.[19] These findings make evident that despite the high sensitivity of

TABLE I

CBF in NCT, Saline, and Cocaine/Adrenaline

	Sample Nos. Single Values										Mean	SD	Percent Reduction from the Start Value*		
	1	2	3	4	5	6	7	8	9	10			Mean	Minimum	Maximum
NaCl	5.9	6.3	6.1	5.9	6.0	5.6	6.0	6.5	6.6	5.5	6.04	0.35			
NCT 1%	5.1	6.1	5.9	5.7	4.9	4.1	5.1	6.3	6.4	5.3	5.49	0.72	9.1#	3.0	26.8
NaCl	5.4	6.1	5.9	5.8	5.2	4.1	5.6	6.3	6.4	5.3	5.61	0.67	7.1	3.0	26.8
NaCl	6.0	6.1	5.9	6.0	5.6	5.8	5.5	6.0	6.2	5.6	5.87	0.24			
NaCl	5.9	6.1	5.8	5.9	5.4	5.8	5.3	5.5	6.2	5.4	5.73	0.31	2.4	0.0	8.3
NaCl	5.7	6.0	5.8	5.8	5.4	5.7	5.4	5.6	6.1	5.5	5.7	0.24	2.9	1.6	6.7
NaCl			5.8	6.4	6.1						6.1	0.3			
Cocaine/adrenaline			2.9	3.2	3.0						3.0	0.2	50.3§	50.0	50.8
NaCl			2.7	3.1	3.0						2.9	0.2	51.9§	50.8	53.4
NaCl			6.4	5.5	5.9						5.7	0.5			
0.1% *NCT*			6.3	5.3	5.9						5.6	0.5	1.8	1.6	3.6
NaCl			6.3	5.4	5.8						5.6	0.5	1.8	1.6	1.8
NaCl			6.2	5.5	6.2						5.85	0.4			
0.01% *NCT*			6.2	5.4	6.1						5.8	0.4	1.7	0.0	1.8
NaCl			6.1	5.5	6.1						5.8	0.3	0.9	1.6	1.6

Values are CBFs (Hz) at the end of each of these periods.
**All mucous membrane samples were treated for 20 min with saline (start value), followed by 20 min with test solution (italic) and by 20 min with saline.*
#$p < 0.05$ vs saline
§$p < 0.01$ vs saline and 1% NCT

CBF measurement and the low influence of NCT on CBF, clinical trials will be necessary to evaluate the *in vivo* tolerability of this compound. The *in vitro* results using ciliated epithelium provide additional support for the very low toxic potential of NCT on mucous membranes, and they are a basis for its topical application to treat infections of the nasal and paranasal sinuses.

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