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ORIGINAL ARTICLE

Effect of *N*-chlorotaurine mouth rinses on plaque regrowth and plaque vitality

K. Lorenz · D. Mayer · G. Bruhn · B. Noack · M. Brecx · C. Heumann · H. Toutenburg · L. Netuschil · M. Nagl · W. Gottardi · T. Hoffmann

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Abstract The purpose of this 4-day plaque regrowth study was to assess the effect of *N*-chlorotaurine (NCT) mouth rinses on plaque inhibition and plaque vitality. Eighty volunteers participated in this investigator-blind, randomized, clinical controlled study in parallel groups. No oral hygiene was permitted except rinsing with a 2% or 3% NCT mouth rinse, a positive or a negative control. Primary parameters were the plaque index (Silness and Löe, *Acta Odontol Scand*, 22:121–135, 1964) and plaque vitality (Netuschil et al., *J Clin Periodontol*, 16:484–488, 1989) after the final rinse. In addition, another plaque index (Turesky et al., *J Periodontol*, 41:41–43, 1970), plaque area, and bleeding on probing were recorded. All parameters were taken at baseline and day 5. *U* test was applied on a 5% error level. No differences in plaque inhibition were found between the two NCT formulations and the negative control. However, a statistically significant reduction of plaque vitality compared to the negative and positive control was observed. Discoloration of the tongue and unpleasant taste were recorded in participants in the NCT groups. NCT mouth rinses did not inhibit plaque regrowth, but they did reduce the vitality of plaque bacteria. Methods of prolonging the substantivity of the NCT mouth rinses should be investigated to enhance the antibacterial properties of these formulations.

Keywords Bacteria · Dental plaque · Dental plaque index · Mouth rinse · *N*-chlorotaurine

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K. Lorenz (✉) · D. Mayer · G. Bruhn · B. Noack · M. Brecx · L. Netuschil · T. Hoffmann
Department of Conservative Dentistry, Medical Faculty,
University of Technology Dresden,
Fetscherstr. 74,
01307 Dresden, Germany
e-mail: katrin.lorenz@tu-dresden.de

C. Heumann · H. Toutenburg
Department of Statistics, University of Munich,
Munich, Germany

M. Nagl · W. Gottardi
Department of Hygiene, Microbiology and Social Medicine
Division of Hygiene and Medical Microbiology,
Innsbruck Medical University,
Innsbruck, Austria

Introduction

Mouth rinses can be used as adjuncts to mechanical oral hygiene. Their application is aimed to influence remaining plaque on tooth surfaces in order to prevent caries and periodontal diseases. Therefore, several formulations have been developed. Different approaches on how to affect bacterial growth and dental plaque were investigated. Among the antiseptics, chlorhexidine, as the most effective antiplaque agent, has an antibacterial effect as well as a high substantivity. It also reduces plaque acidogenicity [11]. Other approaches like application of enzymes to disrupt plaque, application of agents that can affect bacterial adhesion, or addition of buffering components to increase pH levels in plaque [31] were explored.

Taurine is a free amino acid that is found in the heart and skeletal muscles, in the retina, in the brain, and in high concentrations in leucocytes of mammals [33]. As a consequence of the inflammatory respiratory burst of neutrophilic polymorphonuclear leukocytes against pathogenic agents, hypochlorous acid (HOCl) and *N*-chlortaurine (NCT) are produced. HOCl is formed from hydrogen peroxide and chlorine via the myeloperoxidase pathway. The following reaction of HOCl with taurine results in NCT. Both HOCl

and NCT have the potential to chlorinate or oxidize proteins and therefore to influence the inflammatory response. While HOCl has very strong oxidizing properties, NCT is a mild oxidant. Chlorination of antigens enhances the presentation of the proteins by antigen-presenting cells, influences the neutophile polymorphonuclear leucocyte-macrophage interactions, and selectively modulates the immune response against periodontal pathogens [18]. NCT is thought to downregulate proinflammatory cytokines like TNF-alpha, prostaglandin E-2, and interleukins [7, 16] and to be involved in termination of inflammation. When isolated fibroblast-like synoviocytes of patients suffering rheumatoid arthritis were treated with NCT, cyclooxygenase isozymes (COX-2) and PGE2 synthesis were inhibited, and therefore the inflammatory process was positively influenced [15]. The inhibitory effect of NCT on the development of cartilage and bone damage could also be shown in a septic arthritis model in mice [38].

In numerous studies, the broad spectrum of antiseptic activity of NCT was investigated. The microbicidal effect of NCT was proven in vitro against Gram-positive and Gram-negative bacteria (e.g., [12, 19]), viruses, and fungi [22, 23]. NCT was successfully tested in animal models where it inhibited the bacterial regrowth combined with a loss of bacterial virulence [20, 21]. Furthermore, investigations have shown that NCT could be of value in tumor treatment. By concentrating in the mitochondria of human B lymphoma cells, mitochondria were damaged, and the cells underwent apoptosis [14].

In clinical studies of phase I and phase II and single cases, local rinsings of NCT appeared to be well tolerated and effective in different body sites, for instance purulent leg ulcers [24], conjunctivitis [26, 36], rhinosinusitis [3, 13, 27], and urinary tract infections [25]. NCT has shown an antiseptic and drying effect when used as an irrigation agent in the outer auditory canal in patients following tympanoplasty [28]. NCT inhibits or inactivates collagenase that is responsible for the degradation of collagen and may therefore be of value in the treatment of inflammatory arthritis [8].

Acting as an antiseptic substance as well as important regulator of inflammation in different areas of the human body, the application of NCT in the oral cavity could be of comparable value. Therefore, an aqueous NCT formulation was tested for its antimicrobial effect against plaque bacteria and its ability to inhibit plaque growth.

Materials and methods

This randomized, controlled, investigator-blinded, phase I clinical study was conducted in parallel groups. It was performed according to Good Clinical Practice/International Conference on Harmonisation regulations. Before the study was initiated, approval was received by the local Ethics Committee of the Medical Faculty, University of Technology, Dresden, Germany. The 4-day plaque regrowth model [1] was applied.

Study medication and treatment

Four mouth rinses were tested. All preparations were handed out to the test persons by a study nurse at baseline. NCT in an aqueous solution was investigated as both a 2% and a 3% formulation. The prepared NCT sodium crystals (Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Austria) were delivered in test tubes. The subjects were instructed on how to dissolve the crystals in tap water to create the rinse. Tubes were kept refrigerated in order to exclude any decrease of oxidation capacity during the study period. Chlorhexidine 0.2% (Corsodyl®, GlaxoSmithKline, Consumer Healthcare GmbH & Co. KG, Bühl, Germany) served as positive control, sodium chloride 0.9% as negative control. The positive and the negative control were ready-to-use formulations.

The participants rinsed with 10 ml of the allocated mouth rinse twice daily (in the morning and in the evening) for 1 min each. The period of study medication was 4 days. Within this period, the participants were not permitted to perform any mechanical oral hygiene.

Subjects

The study participants were healthy volunteers recruited among the students and staff of the dental school in Dresden, Germany. According to the sample size calculation based on data of a similar study [4], 80 participants were enrolled by the clinical investigator. They were randomly assigned to the test groups, i.e., 20 per test group. The study commenced with a recruitment phase to include the participants and to perform a dental prophylaxis.

Subjects to be included had to have at least 24 healthy teeth and healthy gingival tissues (Gingival index <1) [17]. The presence of systemic diseases, allergic reactions against one of treatments, pregnancy, minority, and the use of antibiotics 6 weeks prior to the beginning of the study and any concomitant medication with possible interferences with the study were exclusion criteria. All participants signed an informed consent.

Evaluation parameters

To test the influence of the test solutions on plaque bacteria and plaque growth, primary parameters were the plaque index (PlI) [34] and bacterial vitality assessed by means of the vital fluorescence technique [29]. As secondary parameters, the modified plaque index (QHI) [37], plaque area (PlA) [4], and bleeding on probing (BOP) were documented. PlA was

modified from the original method. After staining with erythrosine, digital standardized photographs were taken of the maxillary right central incisor. The stained vestibular surface was highlighted on the photograph using the drawing tool of Adobe Photoshop 7.0 software, and then the number of pixels within this area was calculated. In addition, the circumference of the whole tooth surface was highlighted with the same drawing tool, and the numbers of pixels were calculated. The relation between the plaque covered area (number of pixels) to the total vestibular tooth surface (number of pixels) gave the percentage of existing plaque.

The parameters were recorded at baseline and on day 5. In addition, adverse events were documented in the case report forms. It was asked whether the participant experienced any adverse events and, if yes, what kind of adverse event it was. A dental prophylaxis was performed at the beginning and at the end of the test period.

Coding and statistics

A randomization list was created by the statistician and forwarded to the study nurse who then assigned the participants to their groups. The clinical investigator was blinded. To ensure the blinding, the study nurse distributed the test products after the collection of the clinical data was finished. The statistician had knowledge of the randomization list; the study nurse could identify whether the subjects received either the test or control rinses. Data were entered in an electronic database and were checked for discrepancies. Means and standard deviations were calculated for each parameter. A 0.05 error level was set prior to the statistical test procedures. To detect differences between the treatment groups, non-parametric *U* tests were performed based on the results of the Kolmogorov–Smirnov tests for normal distribution. Efficacy testing was based on the full analysis set that included all participants who had received at least one rinse.

Results

Eighty participants (34 men, 46 women) of a mean age of 24.8±3.9 years (range 20–43 years) were included and finished the study. No participants dropped out during the study. The gingival index at baseline (not depicted) ranged between 0.05 and 0.20 in the four groups with corresponding standard deviations between 0.10 and 0.16. This indicates that a high level of gingival health was present among the participants at the beginning of the study.

The outcome at day 5 concerning primary and secondary parameters is depicted in Tables 1 and 2. During the course of the study, plaque indices (PlI, QHI) increased. The most prominent plaque increase happened in the NCT groups. The positive control (chlorhexidine) exhibited the least plaque. Differences between the negative control group and the test groups and between the negative and the positive control group were statistically significant ($p<0.01$).

In contrast, the vitality of plaque bacteria was reduced the most by NCT 2% and 3%. Statistically significant differences between both the negative and positive control compared to both NCT formulations could be found ($p<$ 0.01). No differences in the means were detected between 2% and 3% NCT. The least vitality reduction was seen in the placebo group. A statistically significant difference compared to the positive control existed. Plaque area calculations revealed statistically significant differences between the positive control and the three other treatment groups. No differences were observed between the two NCT formulations and the negative control. Plaque area measurements did not differ between NCT 2% and NCT 3%. BOP served as a control parameter only. There was no increase compared to baseline and no difference between the groups.

All participants in the NCT groups reported an unpleasant chlorine taste during the rinsing time that was resolved a few minutes after the rinsing was finished. In addition, five participants of each NCT group observed a brownish tongue discoloration that lasted for 4 to 6 days. No severe adverse events were recorded.

Discussion

In this study, NCT was applied as an antiseptic mouth rinse in the oral cavity for the first time. Due to its bactericidal

Table 1 Primary and secondary endpoints at day 4

	Placebo (negative control)	0.2% chlorhexidine (positive control)	NCT 2% (test 1)	NCT 3% (test 2)
PlI (mean±SD)	0.61±0.34	0.19±0.27	0.90±0.27	0.90±0.28
VF (in %; mean±SD)	68.5±28.4	58.2±27.1	36.9±15.7	36.9±12.6
QHI (mean±SD)	1.10±0.45	0.46±0.27	1.91±0.49	2.04±0.48
PlA (in %; mean±SD)	16.8±10.1	5.8±9.5	17.8±23.2	13.1±10.4
BOP (mean±SD)	0.06±0.05	0.05±0.03	0.04±0.03	0.06±0.05

NCT N-chlorotaurine; *PlI* plaque index [34]; *VF* bacterial vitality [29]; *QHI* plaque index (Quigley and Hein [32] modified by Turesky et al. [37]); *PlA* plaque area [4]; *BOP* bleeding on probing [2]

Table 2 *p* values for the comparison between each treatment group and the negative (placebo) or the positive (CHX; chlorhexidine 0.2%) control; *U* test; $n=20$ per group

	PII		VF		QHI		PlA		BOP	
	Placebo	CHX	Placebo	CHX	Placebo	CHX	Placebo	CHX	Placebo	CHX
CHX	0.001*	–	0.301	–	0.000*	–	0.000*	–	0.409	–
NCT 2%	0.008*	0.000*	0.001*	0.004*	0.000*	0.000*	0.308	0.001*	0.150	0.284
NCT 3%	0.006*	0.000*	0.001*	0.004*	0.000*	0.000*	0.354	0.023*	0.754	0.409

NCT N-chlorotaurine; *PlI* plaque index [34]; *VF* bacterial vitality [29]; *QHI* plaque index (Quigley and Hein [32] modified by Turesky et al. [37]); *PlA* plaque area [4]; *BOP* bleeding on probing [2]
*Statistical significance $p<0.05$

effect, NCT might be useful for prevention and treatment of dental plaque.

The 4-day plaque regrowth model was chosen because it is one of the early short-term tests on new antiseptic substances. Originally, it was intended to investigate the ability of a substance to inhibit plaque [1]. In the present study, this effect was planned to be examined in combination with the bactericidal action of NCT. Therefore, the vital fluorescence technique [29] was applied to detect bacterial vitality after the last rinse.

The study revealed that neither of the two NCT formulations inhibited plaque growth. However, NCT reduced the plaque vitality to very low levels of about 30% and even exceeded the values of the chlorhexidine group. To serve as an antiplaque agent, a formulation should either reduce the total mass or the total number of oral pathogens [9]. The results of the present study seem to support the idea that NCT inactivates bacteria in existing plaque rather than it prevents their colonization on the tooth surface.

The participants in this study performed the last rinse within 2 h of the time that the parameters were recorded. The bactericidal effect was still in evidence when the plaque was examined. It was not investigated how long this effect persisted after the last rinse. However, it could be shown that NCT had a bactericidal effect on a 4-day-old biofilm.

The finding of a strongly reduced bacterial vitality accompanied by higher plaque scores after NCT rinses seems paradoxical because usually an effective substance would reduce both vitality of the microorganisms and plaque [4, 5]. Other clinical studies also failed to show effects on the amount and composition of plaque when promising plant extracts were used as adjunctive gels [35]. A combination of different effects may explain these unexpected findings of the present investigation:

1. It was depicted and discussed by Netuschil et al. [30] that young plaque consists mainly of dead microorganisms and that new, vital plaque will reproduce on this "underground". If substances with a high substantivity such as chlorhexidine (CHX) are used to kill parts of the plaque biofilm, the regrowth of remaining plaque is strongly hampered by their long-lasting antibacterial action. However, NCT seems not to possess any substantivity. Thus, it is assumed that at any NCT rinse, a quite high portion of plaque bacteria was immediately killed, but these dead organisms could act after a very short time as a welcome source of food for the still vital and viable part of the biofilm. Please note that a generation time of about 1 h was documented for the first periods of plaque regrowth [39] leading to a theoretically 2000-fold increase in bacterial numbers between one rinse and the other.

2. From Table 1, it is obvious that the strongest index discrepancy between placebo and NCT rinses was found after disclosing, i.e., when the modified QHI was recorded. A similar effect was also described by Binney et al. [6]. After a single rinse of CHX on 72 h plaque, they documented an increase in their plaque index (QHI) and plaque area recordings compared to the control (water). They stated that this "surprising…negative performance" may be due to a binding of the disclosing agent to the still remaining plaque, leading thus to a "scoring error."

3. This effect of an overestimation of disclosed, albeit dead plaque was also described by Gehlen et al. [10]. After a 2-day rinsing period with CHX, performed with adolescents wearing brackets, these authors even recommended a stronger tooth brushing only due to the usage of CHX—which contradicts the idea to administer CHX when tooth brushing is not possible.

Addition of substances that can establish substantivity and therefore create a plaque inhibitory effect over a few hours might be a future strategy for NCT formulations. Further studies are necessary to investigate the potential value of NCT to be used as an oral antiseptic.

This study on the first application of NCT mouth rinses has proven that NCT has a strong bactericidal effect.

However, this bactericidal effect was not combined with a plaque inhibition over the 4-day test period. Before NCT formulations can be used as antiseptics in the oral cavity, their substantivity has to be increased.

Conflict of Interest Statement The authors declare that they have no conflict of interest.

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