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Stabilizing Sodium Hypochlorite at High pH: Effects on Soft Tissue and Dentin

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Abstract

Introduction When sodium hypochlorite solutions react with tissue, their pH drops and tissue sorption decreases. We studied whether stabilizing a NaOCl solution at high pH would increase its soft tissue dissolution capacity and effects on the dentin matrix compared to a standard NaOCl solution of the same concentration and similar initial pH.

Methods NaOCl solutions were prepared by mixing (1:1) a 10% stock solution with water (standard) or 2 M NaOH (stabilized). Physiological saline and 1 M NaOH served as controls. Chlorine content and alkaline capacity of NaOCl solutions were determined. Standardized porcine palatal soft tissue specimens and human root dentin bars were exposed to test and control solutions. Weight loss percentage was assessed in the soft tissue dissolution assay. Three-point bending tests were performed on the root dentin bars to determine modulus of elasticity and flexural strength. Values between groups were compared using one-way analysis of variance (ANOVA) with Bonferroni's correction for multiple testing (alpha<0.05).

Results Both solutions contained 5% NaOCl. One mL of the standard and the stabilized solution consumed 4.0 mL and 13.7 mL of a 0.1 M HCl solution before they reached pH 7.5, respectively. The stabilized NaOCl dissolved significantly more soft tissue than the standard solution, and the pH remained high. It also caused a higher loss in elastic modulus and flexure strength (p<0.05) than the control solutions, while the standard solution did not.

Conclusions NaOH-stabilized NaOCl solutions have higher alkaline capacity and are thus more proteolytic than standard counterparts.

Introduction

Sodium hypochlorite has a long history of successful usage in endodontics (1). Three key features make sodium hypochlorite solutions popular among clinicians: their non-specific antimicrobial effect and ability to dissolve biofilms (2, 3), their unique capacity to solubilize necrotic tissue (4), and their reasonable price combined with availability from many commercial sources (5, 6).

Relatively little information is published in the endodontic literature regarding the chemistry of NaOCl despite its apparent clinical importance. Sodium hypochlorite in aqueous solution can take different forms or, in chemical terms, can be present as different species, depending on temperature and pH (7). The active compound in sodium hypochlorite is the chlorine. All forms of chlorine in hypochlorite solutions are collectively termed "free available chlorine" (8). Sodium hypochlorite dissociates into Na^+ and OCl^- (the hypochlorite ion) in water. If the solution is rendered acidic, HOCl (hypochlorous acid) becomes the predominant species in the temperature range applied in endodontics (7). The pK_a value of hypochlorous acid is 7.5. This means that at pH 7.5, equal amounts of HOCl and OCl^- are in solution. It has been suspected for some time that HOCl is a stronger antiseptic than OCl^- (9). However, unaltered sodium hypochlorite solutions have a pH in the range of 11.5 to 12.5, depending on their concentration. Lowering the pH to 7.5 using hypochlorous acid makes the resulting solution unstable and more cytotoxic than a counterpart with equal amounts of available chlorine at pH 12 (10, 11). Moreover, it is questionable whether the differences in antimicrobial effect between a neutralized sodium hypochlorite solution (pH 7.5) and a counterpart at pH 12 is clinically relevant in the concentration range typically applied during root canal treatment (10). One potentially important aspect in this context is that neutralized or acidified sodium hypochlorite solutions dissolve considerably less soft tissue than unaltered counterparts (11, 12). So, rather

than adjusting the pH of sodium hypochlorite solutions to obtain a pH-neutral solution using an acid, it could make some sense to do the opposite: add alkali (usually NaOH) to stabilize the OCl⁻, as is done by some commercial producers of household bleach (5). NaOH-stabilized sodium hypochlorite could have a stronger tissue-dissolving effect compared to the standard preparation. The reason for this is that the OCl⁻/HOCl equilibrium adjusts itself exceedingly fast in non-stabilized solutions (13). In contact with microorganisms or tissues, the pH drops, so that HOCl predominates, and tissue sorption decreases (14).

In the endodontic field, there is now one company that explicitly sells an NaOH-stabilized sodium hypochlorite solution (Chlor XTRA, Vista Dental Products, Racine, WI), claiming that it should be "significantly more digestive than standard NaOCl". However, there is no data available to support this statement. Furthermore, the tissue-dissolving capacity of NaOCl preparations appear to be directly linked to their proteolytic effect, which, in turn, can damage the dentin matrix (15, 16).

It was the aim of the current study to compare a standard 5% NaOCl solution with a counterpart of the same NaOCl concentration that was stabilized at high pH. The outcomes studied here were soft tissue dissolving efficacy and negative impact on human root dentin stability.

Materials and Methods

Solutions and their characterization

A standard NaOCl solution of 14% (Kantonslabor, Zürich, Switzerland) was used. Its content of available chlorine was iodomterically titrated. The solution was then diluted to 10% NaOCl using ultrapure water. A 2 M NaOH solution was obtained from a commercial source

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(Titrisol, Merck, Darmstadt, Germany). Its molarity was determined using a standardized HCl solution (Merck). Four solutions prepared/ used for the current study:

- a 1:1 mixture of 10% NaOCl with water (standard NaOCl)

- a 1:1 mixture of 10% NaOCl with 2 M NaOH (NaOH-stabilized NaOCl)

- a 1:1 mixture of 2 M NaOH with water

- unbuffered physiological saline (0.9% NaCl, B.Braun, Melsungen, Switzerland)

The hypochlorite solutions were iodometrically titrated again to test their concentration immediately after preparation and 25 days (at termination of the experiments) subsequently. To test the alkaline capacity of the solutions, they were titrated using 0.1 M HCl in an automated titration device (Titroprocessor 686, Metrohm, Zofingen, Switzerland). The pH was determined using a calibrated pH electrode (Model 6.0210.100, Metrohm). The drift was 1 mV/min, titration volume 0.5 mL per step, stop pH 1.5. The volume of 0.1 M HCl necessary to reach pH 7.5 in the solution, i.e the pK_a value of HOCl, was measured. All chemical assessments were done in triplicate and mean values are reported.

Tissue dissolution assay

A standard soft tissue dissolution assay using porcine palatal mucosa was used in the current study (17). Forty-eight frozen tissue specimens of defined surface area and similar weight (205 ± 5 mg) were obtained with a rectangular punch applied at the crest of the rugae. These specimens were thawed at room temperature in a humid environment. They were blotted dry and then weighed using a precision balance (AT 261; Mettler Instrumente AG, Nänikon-Uster, Switzerland). If the weight was too high, it was adjusted to approximately 205 mg using a

scalpel. Tissue specimens (N = 12 per group) were immersed in individual microcentrifugation tubes containing 1.5 mL of test or control solutions for 8 min at room temperature. The tubes were agitated (IKA-Vibrax-VXR, Janke und Kunkel GmbH, Staufen, Germany) at 400 rotations/min. Subsequently, specimens were removed from their tubes, rinsed with ultrapure water, blotted dry, and weighed again. The weight loss was calculated in % of the original tissue weight.

The pH values of test and control solutions before and after exposure to the soft tissue were determined in each tube using a calibrated pH-electrode (713 pH Meter, Metrohm).

Assay on mechanical dentin properties

Forty-eight human root dentin bars were prepared from 12 extracted maxillary third molars as described earlier (18). As the teeth were taken from the department's pooled collection of extracted teeth, the age and gender of the donors was not known. The teeth had been stored in a 0.2% thymol solution at 5°C for a maximum of one year. Dentin bars were prepared using a saw microtome (SP1600, Leica Microsystems, Glattbrugg, Switzerland) to a profile of 0.8 mm x 1.2 mm; their length was adjusted to 8-10 mm. The orientation of the dentinal tubules was perpendicular to the greater bearing surface of the bars and was similar for all specimens. The dentin bars were pooled and stored in sterile saline solution (B.Braun) until further usage.

The 48 specimens were randomly divided into 4 groups of 12 specimens each. Specimens were immersed individually in polypropylene containers containing 5 mL of test or control solutions at room temperature and agitated for 30 min as described above. Subsequently, specimens were washed in ultrapure water and three-point bending tests were performed using a universal testing machine (Zwick, Ulm, Germany). The dentin bars were kept moist with physiological saline solution during all manipulations. Before they were transferred to the testing

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apparatus, their width and depth were measured using a sliding calliper. A specimen holder with 2 cylindrical supports with a radius of 1 mm and a span of 6 mm was used. Specimens were placed with the greater bearing surface centered on the support (i.e. with the tubules parallel to the cross-head). The cross-head speed of the testing machine was set to 0.5 mm min^{-1} and the bars were tested until failure. The elastic modulus was calculated from the slope m of the load-displacement curves within the linear elastic region using the formula

$$E = \frac{l^3 m}{4bh^3}$$

with the support span l, and the width b and the height h of the specimen. The flexure strength (σ) was calculated according to the formula

$$\sigma = \frac{3Fl}{2bh^2}$$

with F representing the load at fracture. Registration of the load at fracture and calculation of modulus of elasticity as well as flexure strength were performed using a commercially available computer program (testXpert, Zwick).

Data presentation analysis

As viewed on box plots, values pertaining to tissue weight loss in %, flexural strength and modulus of elasticity were evenly distributed. Consequently, parametric statistics were applied to compare mean values between groups: one-way analysis of variance (ANOVA) followed by the Bonferroni correction for multiple testing. The alpha-type error was set at 0.05.

Because of their logarithmic nature, pH values are presented as median values and ranges.

Results

Both hypochlorite solutions used in this study contained 5.0% NaOCl as determined iodometrically. This content remained stable over one month. One mL of the standard solution consumed 4.0 mL of a 0.1 M HCl solution before it reached pH 7.5. The stabilized counterpart consumed 13.7 mL of 0.1 M HCl per mL before it reached that pH level.

The NaOH-stabilized sodium hypochlorite solution dissolved significantly ($p < 0.05$) more soft tissue than the standard counterpart (Table 1). The pH in the NaOH-stabilized solution remained constant, while it dropped to ~ 7.9 in the standard NaOCl solution. Saline caused no tissue weight loss, the 1 M NaOH solution a slight increase in tissue weight (Table 1).

The NaOH-stabilized sodium hypochlorite solution caused a higher decrease in elastic modulus and flexure strength values than the standard NaOCl solution (Table 2). This difference was not statistically significant ($p > 0.05$) under the current conditions. However, the NaOH-stabilized NaOCl solution caused a significant ($p < 0.05$) drop in modulus of elasticity and flexural strength compared to the NaOH and saline control treatments, while the standard NaOCl solution did not.

Discussion

All the NaOCl solutions that are available to the clinician are alkaline. The reason for this is two-fold: unaltered sodium hypochlorite solutions are alkaline *per se*, and neutralized or acidified solutions are unstable and thus cannot be stored or marketed that way (10). This study tested the hypothesis that adding more alkali to a standard (and thus already alkaline) solution would increase its proteolytic effect in contact with tissues. The results showed that a NaOH-stabilized sodium hypochlorite solution indeed dissolved soft tissue more efficiently than a

standard counterpart with the same content of available chlorine. However, the negative effects on dentin stability appeared to be increased as well. The rationale behind this study was that in non-stabilized NaOCl solutions, the pH drops while active chlorine is consumed by its interaction with tissues. In that course, there is a shift from OCl⁻ as the predominant species towards HOCl, which could be counteracted by adding alkali to the solution. To the best of our knowledge, this has never been specifically investigated.

This being a laboratory study, there are obvious limitations and extrapolations to the clinical situation are not warranted. Porcine palatal tissue was chosen for the soft tissue dissolution assay because tissue specimens can be better standardized than human or bovine pulps. Available surface area and mass of tissue are confounding factors (14) that can easily be controlled in this setup. On the other hand, palatal mucosa is epithelialized and more fibrous than pulp, and thus it takes longer for a sodium hypochlorite solution to digest this tissue compared to pulp. However, the differences are quantitative, not qualitative (unpublished observations). A further limitation of the current study is the fact that the experiments were done at room temperature, not body temperature. It is well-known that raising temperature increases the solvent action of sodium hypochlorite (19, 20). It is likely that the proteolytic properties of the stabilized and the standard NaOCl solutions used here should be affected similarly by temperature, i.e. the difference in proteolytic affect should exist also at body temperature. However, this remains unknown until properly investigated. The dentin bar assay to test for effects of the solutions under investigation on mechanical dentin properties also has its limitations. The solutions could affect the dentin from four sides, a situation not encountered clinically. In addition, the relation between dentin and solution was inversed in the experimental setup compared to the clinical situation: small dentin specimens were immersed in the test and

control solutions. In a clinical situation, the volume of the irrigant in a root canal is small compared to surrounding root dentin.

The current findings support earlier observations on the synergistic effect between calcium hydroxide and sodium hypochlorite regarding soft tissue dissolution (21, 22). NaOH and $Ca(OH)_2$ are both strong bases. The higher proteolytic effect of sodium hypochlorite solutions in the presence of a strong base may be explained by several factors. In unaltered NaOCl solutions, the pH drops as the hypochlorite gets consumed (14). It was shown in the current study that with the NaOH-stabilized NaOCl in the tissue dissolution assay, pH levels remained high, while they dropped in the NaOCl solution that was not spiked with alkali. It is conceivable that in non-stabilized hypochlorite solutions, pH levels can drop locally and HOCl can be present in conjunction with OCl^-. Presumptively, HOCl penetrates membranes better than OCl^- and causes intracellular effects, while OCl^- destroys cellular surfaces (13). Sodium hydroxide (or any other strong base) keeps the pH levels high despite the consumption of the available chlorine, so that all the available chlorine remains in the form of OCl^-. A second factor relates to the direct effect of a strong base on tissues. As was observed in this study with NaOH, the tissue was affected, but not dissolved. This is reflected in the increase of tissue weight in the assay, a finding most likely explained by water sorption due to a more complex surface area in the tissue specimens exposed to 1 M NaOH. A higher surface area can be attacked more readily by the active chlorine is solution. Thirdly, NaOCl spiked with NaOH has a higher osmotic pressure than unaltered NaOCl at the same concentration of available chlorine, and thus may add to the tissue-disruptive effect by osmosis.

As indicated above, this was a study to gain some basic insight into the effect of adding alkali to sodium hypochlorite solutions. The resulting higher proteolytic effect can be clinically advantageous yet at the same time have untoward consequences. There are two major concerns

with the clinical use of concentrated sodium hypochlorite solutions. The first relates to the caustic effect of these solutions if inadvertently extruded over the root apex (23). The second relates to the destructive effect of concentrated sodium hypochlorite on the dentin matrix (16), possibly rendering teeth more prone to fracture (24). Adding alkali to sodium hypochlorite may aggravate both these unwanted effects.

In conclusion, the stronger proteolytic effect of NaOH-stabilized hypochlorite suggests that such solutions, if applied carefully, could abbreviate the chemical soft tissue debridement during endodontic treatment. On the other hand, NaOH-stabilized NaOCl solutions can also inflict more harm to dentin than standard counterparts, and could potentially be more caustic.

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TABLE 1. Tissue weight loss (means ± standard deviations) after 8 min immersion in test and control solutions (N = 12) and pH values (medians, ranges) of these solutions before and after the experiment

Solution	Weight loss (%)	pH before	pH after
Saline	0.3 ± 0.9^A	5.9 (5.9, 5.9)	7.5 (7.2, 7.9)
1 M NaOH	- 5.0 ± 1.8^B	13.3 (13.2, 13.3)	13.1 (13.0, 13.2)
Standard NaOCl	44.7 ± 5.2^C	12.5 (12.5, 12.5)	7.9 (7.4, 8.4)
Alkalized NaOCl	63.3 ± 5.1^D	13.4 (13.4, 13.4)	13.1 (12.7, 13.3)

Differing superscript letters indicate that there was a significant difference between data sets pertaining to tissue weight loss at the 0.05 level (ANOVA, Bonferroni).

TABLE 2. Modulus of elasticity and flexure strength values (means ± standard deviations) of human root dentin bars (N = 12) after 30 min exposure to 5 mL of solutions at room temperature

Solution	Modulus of elasticity (GPa)	Flexure strength (MPa)
Saline	7.4 ± 0.1^{AB}	180.8 ± 32.9^{a}
1 M NaOH	8.2 ± 0.8^{B}	183.4 ± 36.5^{a}
Standard NaOCl	7.1 ± 0.9^{AB}	155.2 ± 42.0^{ab}
Alkalized NaOCl	6.5 ± 1.6^{A}	124.0 ± 42.3^{b}

Shared superscript letters indicate that there was no significant difference between data sets at the 0.05 level (ANOVA, Bonferroni).