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Periodontal effects of 0.25% sodium hypochlorite twice-weekly oral rinse. A pilot study

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Background and Objective: The study aimed to evaluate the effect of 0.25% sodium hypochlorite twice-weekly oral rinse on plaque and gingivitis in patients with minimally treated periodontitis.

Material and Methods: The study included 30 patients with periodontitis, it lasted 3 mo, and it was performed as a randomized, controlled, single-blinded, clinical trial in parallel groups. Fifteen patients rinsed for 30 s with 15 mL of a fresh solution of 0.25% sodium hypochlorite (test) and 15 patients rinsed with 15 mL of water (control). Clorox® regular bleach was the source of the sodium hypochlorite. At baseline and at 2 wk, the study patients received professional subgingival irrigation for 5 min with either 0.25% sodium hypochlorite or water, but no subgingival or supragingival scaling. The presence or absence of supragingival plaque on facial and lingual surfaces was determined by visual inspection; each tooth was dried with air and mouth mirror rotation was used to provide light reflection to identify plaque on smooth surfaces and at the tooth line angles. Gingival bleeding within 30 s after probing to full pocket depth was assessed in six sites of each tooth. Adverse events were evaluated by questionnaire and visual examination.

Results: All 30 patients in the study completed the baseline and the 2 wk parts of the study and a subset of 12 participants completed the 3 mo part of the study. The sodium hypochlorite rinse group and the water rinse group, respectively, showed increases from baseline to 3 mo of 94% and 29% (3.2-fold difference) in plaque-free facial surfaces, of 195% and 30% (6.5-fold difference) in plaque-free lingual surfaces, and of 421% and 29% (14.5-fold difference) in number of teeth with no bleeding on probing. The differences in clinical improvement between the sodium hypochlorite rinse group and the water rinse group were statistically significant. No adverse events were identified in any of the study patients, except for minor complaints about the taste of bleach.

Conclusion: A twice-weekly oral rinse with 0.25% sodium hypochlorite produced marked decreases in dental plaque level and bleeding on probing and may constitute a promising new approach to the management of periodontal disease. Long-term controlled studies on the effectiveness of sodium hypochlorite oral rinse are needed and encouraged.

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Periodontal diseases have a global distribution and include a broad spectrum of illnesses ranging from mild gingivitis to severe periodontitis. Virtually all types of periodontal disease are infectious disorders that result from the interplay between pathogenic agents (bacteria, viruses, yeasts) and host immune responses. The major elements of causative periodontal therapy are mechanical pocket debridement, periodontal pocket irrigation with potent antiseptics, treatment of advanced disease with systemic antibiotics, and attention to proper self-care (1).

The worldwide increase of antibiotic-resistant bacteria and the high costs of new, effective antibiotics have created interest in using inexpensive antiseptics to combat surface infections, such as those of the periodontium. Antiseptics are broad-spectrum microbicidal agents that are applied topically onto living tissue to prevent or treat clinical infections by bacteria and viruses. The halogen group of antiseptics (fluorine, chlorine, bromine, iodine) possesses the highest electronegativity (i.e. ability to attract electrons to itself) of all elements (chlorine's electronegativity = 3.16), and strongly oxidizes (i.e. loss of electrons) low electronegative atoms that bond to halogens.

Sodium hypochlorite is the most commonly used chlorine-releasing agent. It is ionized in water to Na^+ and the hypochlorite ion, OCl^-, which establishes an equilibrium with hypochlorous acid, HOCl, the active moiety (2). Sodium hypochlorite destroys microorganisms by oxidation of proteins, nucleotides and lipids, and is particularly effective against biofilm infections (3). The attack of multiple components of infectious agents practically eliminates the risk of resistance development. Sodium hypochlorite occurs naturally in activated human neutrophils and macrophages, and plays an important antimicrobial role in the innate immunity system (3). It does not evoke allergic reactions, is not a mutagen, carcinogen or teratogen, and has a century-long safety record. It is used as a disinfectant in hospitals, animal facilities and in human drinking water supply, and serves as a bleaching agent and a food additive in industry. Sodium hypochlorite at 0.5% (Dakin's solution) was used with great success to control infections of combat wounded soldiers during World War I, before antibiotics (4), and was recently found effective in the treatment of wounded soldiers with potentially deadly angioinvasive fungal infections (5). The intermittent use of dilute sodium hypochlorite in "bleach baths" has shown efficacy as adjunctive therapy for atopic dermatitis (6,7).

Sodium hypochlorite in concentrations of 1.0–6.0% has been employed in root canal disinfection for a century, and remains the favored antiseptic irrigant in endodontics (8). However, use of sodium hypochlorite for treating other types of oral infection has been surprisingly rare. Only two periodontal studies have been published on the clinical efficacy of dilute sodium hypochlorite. Lobene *et al.* (9) studied dental hygiene students with a generally healthy periodontium; De Nardo *et al.* (10) studied prisoners who had undergone professional periodontal therapy. The study individuals, in the framework of the experimental gingivitis model, received sodium hypochlorite rinse or water rinse and abstained from other types of oral hygiene. The sodium hypochlorite group showed 47% (9) and 48% (10) reduction in dental plaque and a significant improvement in gingival inflammation compared to the water rinse group. The American Dental Association Council on Dental Therapeutics has designated 0.1% sodium hypochlorite a "mild antiseptic mouth rinse" and suggested its use for direct application to mucous membranes (11). The present study was undertaken to confirm and expand the usefulness of sodium hypochlorite rinse in periodontal therapy. The clinical improvement was assessed in patients with periodontitis, who received initial subgingival irrigation with 0.25% sodium hypochlorite (half-strength Dakin's solution) or water but no subgingival or supragingival scaling, and who were instructed to rinse orally with 0.25% sodium hypochlorite twice-weekly for 3 mo.

Material and methods

Study participants

A total of 17 males and 13 females, with a mean age of 41 years, who were patients at the graduate periodontology clinic at the Ostrow School of Dentistry of USC, were enrolled in the study. Patients had an average of 27 teeth. Each patient exhibited at least four separate teeth with a pocket depth of ≥ 6 mm. None of the participants required emergency dental care or revealed systemic conditions that would affect their periodontal status or contraindicate participation in the study. Excluded from the study were patients who were diabetic, pregnant, immunocompromised, unable to comply with the research protocol, smoked > 10 cigarettes daily, or had received periodontal therapy or systemic antibiotics during the 6 mo before entering the study. The study was approved by the University of Southern California Health Sciences Campus Institutional Review Board (no. HS-10-00509). All patients understood and signed informed consent and HIPAA documents before enrolling in the study.

Study design and clinical measurements

The patients were divided into two groups and the study was performed as a randomized (using a random number table), controlled, single-blinded, clinical trial in parallel groups according to established criteria (12). The test group of 15 patients received sodium hypochlorite rinse, and control group of 15 patients received water rinse. The clinical examination was performed by a single-blinded examiner, who was calibrated to accomplish more than 90% reproducibility in repeated measurements of the clinical variables studied.

All study patients received a comprehensive clinical examination at baseline (visit 1), at day 14 (visit 2) and at month 3 (visit 3). The

following clinical variables were assessed in each participant, according to the standard protocol for patients with periodontitis: medical questionnaire and general oral examination, full-mouth dental radiographs, number of teeth, presence of dental plaque, gingival bleeding on probing, periodontal pocket depth (in mm), gingival recession (in mm), furcation involvement and tooth mobility.

The main clinical variables of the study were assessed as follows. The presence or absence of supragingival plaque on the facial and lingual surfaces of each tooth was determined by visual inspection with no use of disclosing solution. The tooth surface was dried with air for enhanced visibility, and indirect vision and light reflection with mouth mirror rotation were used to assess presence of plaque on smooth surfaces and at the tooth line angles. A value of "0" was assigned to surfaces with absence of plaque and a value of "1" was assigned to surfaces with any presence of plaque. Bleeding on probing was assessed within 30 s after probing to the full pocket depth, and was recorded on the facial, lingual, mesiofacial, distofacial, mesiolingual and distolingual surfaces of each tooth. A value of "0" was assigned to teeth showing absence of gingival bleeding in all six study sites, and a value of "1" was assigned to teeth revealing gingival bleeding in any of the six tooth sites. Probing depths were measured in millimeters using a Marquis CP-12 probe (Hu-Friedy Mfg. Co., Chicago, IL, USA) with a probing force of approximately 0.75 N. Pocket depth and gingival recession were measured on the facial, lingual, mesiofacial, distofacial, mesiolingual and distolingual surfaces of each tooth.

A pooled microbiological sample from two deep periodontitis lesions of each study patient was obtained at all three study visits before the clinical examination and was analyzed by established anaerobic culture methods (13). The microorganisms studied included *Aggregatibacter actinomycetemcomitans*, *Prophyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Dialister pneumosintes*, *Fusobacterium* species, *Campylobacter rectus*, *Parvimonas micra*, *Eubacterium* species, beta-hemolytic streptococci, staphylococci, gram-negative enteric rods and *Candida* species.

Patient treatment and instruction

Clorox® regular bleach (The Clorox Company, Oakland, CA, USA) diluted with tap water served as the source of 0.25% sodium hypochlorite. According to the manufacturer, Clorox regular bleach contains (in order of declining concentration) water, 6% sodium hypochlorite, sodium chloride (stabilize formula), sodium carbonate (alkalinity builder), sodium hydroxide (< 1%; pH adjuster), and a small amount of sodium polyacrylate (assist cleaning). It has a pH of about 11 and forms no dioxins nor causes buildup of by-products over time. The retail cost of 1 US gallon (3.8 L) of Clorox regular bleach is 2.0–2.5 US$. Diluted bleach loses activity over time and may be discarded after 1–2 d.

At visit 1 and at visit 2, the study participants received oral hygiene instruction followed by professional subgingival irrigation with either 0.25% sodium hypochlorite (test) or water (control). No subgingival or supragingival scaling was carried out. The subgingival irrigation was performed using a 3 mL Monoject® Endodontic Syringe with a 23-gauge cannula (metal with a blunt end and side ports) (Covidien, Mansfield, MA, USA). The syringe tip was placed at the bottom of the pockets of each tooth, and sodium hypochlorite or water rinse was repeatedly applied in a circular manner around all teeth of the dentition for a total of 5 min. Participants were also given a manual Oral-B® toothbrush and dental floss samples and instructed to brush twice a day, using the modified Bass technique, and to floss once a day.

The instructions for oral rinsing at home were as follows. Participants were asked to rinse their mouth every Wednesday and Sunday for 30 s with either 15 mL of a fresh solution of 0.25% sodium hypochlorite or 15 mL of water. Participants in the test group were provided with Clorox bleach (6%) and instructed to mix 5 mL (one teaspoonful) of Clorox bleach with 120 mL (one-half glass) of tap water to yield a sodium hypochlorite concentration of 0.25%. A fresh bleach solution should be made up at each time of rinsing. Following rinsing with the diluted bleach solution, the test group participants were to expectorate and refrain from rinsing with water for at least 10 min. The procedure for mixing the bleach with water and the rinsing method were provided in writing, and explained and practiced under the supervision of the investigator. The control group (the participants believed that the supplied water contained an active ingredient) was instructed in the same manner, but used water rinse only. In an effort to ensure compliance, all study participants were provided with a rinse log to record the exact date and time of rinsing, and they were requested to bring the rinse log with them to the final visit. They were also telephoned regularly to remind them to rinse and to inquire about any problems with the rinsing protocol. In addition, participants received an end-of-study written questionnaire to gain evaluative feedback about their satisfaction or concerns with the study.

At the conclusion of the study, each study participant received standard therapy for periodontitis, including further explanation about the cause of the disease, instruction in oral hygiene, scaling and root planing, supragingival polishing, and recall schedule to prevent disease recurrence.

Statistics

Individual patients served as the unit for statistical analysis. The mean value of each study variable was obtained for each patient at each visit and used in the statistical analysis. For each of the dependent variables, a Wilcoxon signed-rank test was used to test for difference within each study group. The Wilcoxon–Mann–Whitney test was used to compare improvement between study groups by calculating the score difference between

visits. The Friedman test was used to assess differences in the dependent variables within each study group over time. Spearman's correlation test determined the correlation coefficient between the difference in absence of plaque from visit 1 to visit 3 and the difference in absence of bleeding on probing from visit 1 to visit 3. The significance level was set at $\alpha = 0.05$. The statistical analysis was performed using the STATA statistical software release 12 (StataCorp, College Station, TX, USA).

Results

Of the 30 study participants, 18 adhered to the rinsing regimen for 2 wk and then exited the study. Concerns expressed were unwillingness to delay periodontal treatment for the additional 10 wk required for visit 3, and difficulty with transportation due to residing in locations distant from the dental school. Of the 12 participants completing the 3 mo study, seven were in the test group and five in the control group. The participants who left the study prematurely were clinically similar to those who completed the study.

Evaluation of the questionnaire of compliance revealed that 25 of the 30 (83%) participants fully understood the instructions for mixing the rinsing solution and complied with the twice-weekly rinsing guidelines. Of the five participants who did not completely follow the rinsing protocol, three forgot to rinse at least once (one was test and two were control group participants) and two missed some rinses due to a perception of a "bad taste." Thirteen participants complained of a bad taste after using the solution, which lasted from 5 min to 1 h, but was not by itself severe enough for them to request discontinuance from the study; all of the individuals claiming bad taste were in the test group. Two of the participants swallowed part of the bleach solution, but experienced no adverse effects. Twelve of the 30 participants noticed less gingival inflammation and bleeding; 10 were test group and two were control group participants. Eleven perceived whitening of their teeth; all of these participants were in the test group. No study participants reported stain or discoloration of their teeth or dental restorations. None of the participants experienced abrasion or tingling sensation of the buccal mucosa or the tongue.

Table 1 shows that the initial therapy in the sodium hypochlorite group resulted in a statistically significant increase in facial tooth surfaces free of dental plaque. No significant change was found in any of the clinical study variables in the control group. Table 2 reveals that the clinical improvement continued in the sodium hypochlorite group until the end of the study, with significant increases in plaque-free facial and lingual tooth surfaces and in number of teeth associated with absence of bleeding on probing. Again, no significant clinical improvement was identified in the control group during the 3 mo study period.

Table 3 depicts the level of clinical improvement within and between the test and control group of those individuals who completed the study. From baseline to third visit, the sodium hypochlorite group showed statistically significant multifold increases in plaque-free tooth surfaces, and as much as a 4.2-fold increase in bleeding on probing-free teeth. The control group exhibited only non-significant Hawthorne-like effect improvements of 0.3-fold (Table 3). The sodium hypochlorite group demonstrated clinical changes beyond that shown by the control group for every study variable, including a 3.2-fold increase in plaque-free facial surfaces ($p = 0.04$), a 6.5-fold increase in plaque-free lingual surfaces ($p = 0.04$), and a 14.5-fold increase in bleeding on probing-free teeth ($p = 0.01$) (Table 3).

The study found a statistically significant correlation (Spearman's correlation coefficient of 0.62; $p = 0.03$) between the difference in absence of plaque and the difference in absence of bleeding on probing from visit 1 to visit 3. No statistically significant changes were found in periodontal pocket depth, gingival recession, furcation involvement or tooth mobility, within or between the test and control groups, from visit 1 to visit 2 or visit 3.

The microbiological study of the sodium hypochlorite group revealed statistically significant decreases in the subgingival proportion of fusobacteria at visit 2 and in gram-negative enteric rods at visit 3. The control group demonstrated no significant decrease in any of the microorganisms studied. None of the test microorganisms showed overgrowth to indicate superinfection.

Discussion

There exists an urgent need to develop effective and affordable self-care techniques for the prevention and treatment of periodontal disease. As

Table 1. Clinical variables after initial subgingival irrigation and oral rinse with 0.25% sodium hypochlorite or water

	Sodium hypochlorite (test) group			Water (control) group		
Items	Baseline (visit 1)	Week 2 (visit 2)	*p*-values*	Baseline (visit 1)	Week 2 (visit 2)	*p*-values*
Total number of teeth (patients)	394 (15)	394 (15)	–	404 (15)	404 (15)	–
Plaque-free facial surfaces	33%[a] (25%)	52% (26%)	0.009	40%[a] (25%)	47% (23%)	0.19
Plaque-free lingual surfaces	19% (21%)	29% (18%)	0.09	21% 24%)	23% (23%)	0.84
Bleeding on probing-free teeth	32% (29%)	37% (29%)	0.22	42% (30%)	46% (31%)	0.09

*The Wilcoxon signed-ranked test for comparison between visit 1 and visit 2.
[a]Percentage of total surfaces/teeth (standard deviation among patients).

Table 2. Clinical variables after 3 mo rinse with 0.25% sodium hypochlorite or water for individuals completing the study

	Sodium hypochlorite (test) group				Water (control) group			
Items	Baseline (visit 1)	Week 2 (visit 2)	Month 3 (visit 3)	*p*-values*	Baseline (visit 1)	Week 2 (visit 2)	Month 3 (visit 3)	*p*-values*
Total number of teeth (patients)	205 (7)	205 (7)	205 (7)	–	138 (5)	138 (5)	138 (5)	–
Plaque-free facial surfaces	38%[a] (29%)	62% (20%)	74% (17%)	0.02	34%[a] (30%)	49% (26%)	44% (30%)	0.63
Plaque-free lingual surfaces	18% (23%)	37% (20%)	53% (13%)	0.07	30% (33%)	38% (27%)	39% (23%)	0.95
Bleeding on probing-free teeth	12% (18%)	18% (25%)	62% (29%)	0.002	31% (32%)	34% (33%)	40% (45%)	0.84

*Friedman's non-parametric repeated measures analysis over the three study visits.
[a]Percentage of total surfaces/teeth (standard deviation among patients).

Table 3. Comparison in clinical improvement between 0.25% sodium hypochlorite rinse and water rinse for individuals completing the study

				p-values*	
Items	Fold improvement between visit 1 and visit 3 for the sodium hypochlorite rinse group	Fold improvement between visit 1 and visit 3 for the water rinse group	Additional fold improvement in the sodium hypochlorite rinse group vs. the water rinse group	Between visit 1 and visit 2	Between visit 1 and visit 3
Plaque-free facial surfaces	0.94	0.29	3.2	0.17	0.042
Plaque-free lingual surfaces	1.95	0.30	6.5	0.17	0.042
Bleeding on probing-free teeth	4.21	0.29	14.5	0.72	0.012

*The Wilcoxon–Mann–Whitney test to compare the clinical improvement between the sodium hypochlorite rinse group and the water rinse group.

pointed out elsewhere, the current methods for removing dental plaque are cumbersome, provide limited benefits and perform poorly for many individuals (1). Toothbrushing reduces the average plaque scores only by about half and plaque is left behind on 85% of interdental surfaces; interdental brushes, toothpicks and dental floss fail to eliminate all interdental plaque and are only used by 5–10% of the population; most toothpastes are expensive and do not significantly enhance plaque removal by toothbrushing alone; and commercial mouthwashes are expensive and, with the exception of chlorhexidine, essentially only serve a cosmetic purpose (1). High prices and low performance of current self-care devices and therapies are some of the biggest issues in periodontal healthcare.

Sodium hypochlorite represents potentially an efficacious, safe, inexpensive and readily available complement or alternative to current periodontal self-care techniques. A highly diluted sodium hypochlorite solution (0.05%), used twice-daily as an oral rinse in an experimental gingivitis design study, was able to reduce dental plaque index scores by 48%, gingival inflammation index score by 52%, and bleeding on probing sites by 39% compared with water rinse (10). However, as the twice-daily use of a freshly made sodium hypochlorite solution may be impractical for most individuals, we sought to examine if a five times more concentrated sodium hypochlorite oral rinse solution (0.25%), used only twice in a week, would provide similar clinical improvements. The present findings are consistent with those of previous studies (9,10). The sodium hypochlorite oral rinse group showed marked improvements from baseline to 3 mo in plaque-free facial surfaces ($p = 0.02$), plaque-free lingual surfaces ($p = 0.07$) and bleeding on probing-free teeth ($p = 0.002$). The clinical changes in the control group were statistically non-significant. The sodium hypochlorite rinse group and the water rinse group, respectively, showed increases of 94% and 29% (3.2-fold difference) in plaque-free facial surfaces, of 195% and 30% (6.5-fold difference) in plaque-free lingual surfaces, and of 421% and 29% (14.5-fold difference) in number of teeth with no bleeding on probing. The sodium hypochlorite rinse group, but not the water rinse group, demonstrated continued clinical improvements up to the 3 mo endpoint of the study, suggesting that the difference between the sodium hypochlorite test group and the water control group would widen even further with a longer study period. The biochemical basis for the remarkable decrease in dental plaque after only a twice-weekly sodium hypochlorite rinse is unknown, but substantivity to the tooth surface is an intriguing possibility.

Oral rinses penetrate only 0.1–0.2 mm into periodontal pockets (14,15), but this study found marked clinical improvements in periodontitis

lesions as well. The most likely explanation relates to the sustained absence of supragingival plaque, which is known to affect the subgingival ecology and reduce periodontopathogen counts in pockets up to 5 mm in depth (16–18). Another possibility is that the initial subgingival bleach irrigation suppressed periodontal herpesviruses, which are highly susceptible to sodium hypochlorite. As herpesvirus infections support periodontopathic bacteria (19), a reduced herpesvirus level may decrease subgingival bacterial counts and clinical signs of disease. The significant reduction observed for fusobacteria, a numerically important group of periodontal pathogens (20), after irrigation with sodium hypochlorite might be due to herpesvirus suppression or a direct antibacterial effect.

Bleeding on probing can serve as an indicator of future periodontal breakdown. Lang *et al.* (21) showed that periodontal sites demonstrating bleeding on probing at four consecutive maintenance visits had a 30% risk of losing attachment, whereas sites exhibiting bleeding on probing in one of four recall visits had only a 3% risk of further breakdown. In addition, patients with periodontitis with more than 25% of sites demonstrating bleeding on probing were at higher risk of additional breakdown than patients with periodontitis revealing fewer than 10% of sites with bleeding on probing (21). That bleeding scores decreased by 4.2-fold in the sodium hypochlorite group points to the possibility that oral rinse with diluted bleach can prevent the development of periodontitis in at-risk sites. However, no statistical decrease in pocket depth was observed, perhaps due to the short follow-up period, the limited number of study individuals and the absence of scaling.

This study did not examine the potential caries preventive effect of oral rinsing with sodium hypochlorite. However, it is reasonable to assume that the strong antiplaque effect of the sodium hypochlorite rinse will reduce the dental load of *Streptococcus mutans* and other cariogenic bacteria. It may also be important that the sodium hypochlorite oral rinse can interfere, at least for 24 h, with the ability of dental plaque to produce an acidic environment (9) and that dilute sodium hypochlorite can penetrate 0.1 mm into exposed dentinal tubules and potentially kill invading cariogenic bacteria (22).

In conclusion, the increasing costs of periodontal healthcare combined with a growing number of underserved populations worldwide underscores the need for finding alternative ways of managing periodontal disease. Patients ought to be able to prevent or control mild and moderate types of periodontal disease, and perhaps extend the interval between maintenance recall appointments, by using an effective antiseptic agent capable of reaching the disease-producing plaque. Sodium hypochlorite seems to constitute an ideal periodontal antiseptic in terms of effectiveness, safety, affordability, accessibility and convenience. However, in spite of the encouraging findings on the sodium hypochlorite oral rinse, controlled studies are still needed to determine its periodontal benefits with long-term use. In addition, although the concept of antimicrobial periodontal therapy is simple, studies may be needed to identify the most successful way of introducing sodium hypochlorite treatment to the public.

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