The Effects of Temperature on Sodium Hypochlorite Short-Term Stability, Pulp Dissolution Capacity, and Antimicrobial Efficacy

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Abstract

The purpose of this study was to test some effects of preheating NaOCl solutions using a commercially available syringe heating device. Irrigating solution temperatures in 10-ml syringes were measured. Stability of 5.25, 2.62, and 1% NaOCl solutions for 60 min at 20, 45, and 60°C was assessed using iodine/thiosulfate titration. Human pulp tissue dissolution capacity of a 1% NaOCl solution was gauged at the latter temperatures, and compared to corresponding values with a 5.25% solution at 20°C. Killing efficacy of diluted NaOCl solutions against 48-h incubations of *Enterococcus feacalis* ATCC 29212 was compared at 45°C and 20°C. Using the heating device, a 20°C solution reached 45°C and 60°C in 7 and 20 min, respectively. Solutions remained stable during the observation period. The 1% NaOCl solution at 45°C dissolved pulp tissues as effectively as the 5.25% solution at 20°C, while the 60°C/1% solution was significantly more effective (p < 0.05). A 100-fold increase in killing efficacy was observed between corresponding NaOCl solutions at 20°C and 45°C.

Key Words

Sodium hypochlorite, temperature, pulp dissolution, antimicrobial

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Sodium hypochlorite (NaOCl) was first recommended as an antiseptic solution by Henry Dakin to irrigate open wounds in World War I (1). Several years later, the use of chlorinated soda solutions was advocated for root canal therapy (2). Today, NaOCl solutions are still the most favored root canal irrigants, based on their antibacterial, tissue-dissolving, and lubricating properties (3). In addition, they have a good shelf life if correctly stored, are inexpensive, and easily available from many sources (4).

The choice of concentration of NaOCl is still a matter of debate. Dakin originally used an aqueous 0.5% NaOCl solution. Later, NaOCl solutions of higher concentrations were advocated for root canal debridement (5). The antibacterial efficacy of hypochlorite solutions is a function of their concentration (6), as is their tissue dissolution capacity (7), and on the other hand, their caustic potential (8). Serious incidents have been reported when concentrated hypochlorite solutions were inadvertently forced into periodontal tissues (9), or when such a solution leaked through the rubber dam onto the patient's skin (10). Simply increasing hypochlorite concentrations in irrigating solutions over 1% NaOCl to render them more effective may not be advisable.

One alternative approach to improve the effectiveness of hypochlorite irrigants in the root canal system could be to increase the temperature of low-concentration NaOCl solutions. This appears to improve their immediate tissue-dissolution capacity (11, 12). At the same time, the systemic toxicity of preheated NaOCl irrigants, once they have reached body temperature, should be lower than the one of more concentrated non-heated counterparts with similar efficacy in the root canal. However, there is only little data available on features of heated hypochlorite solutions relevant to the endodontist. Previous studies provide some insight into chemical stability, dissolution action and antimicrobial efficacy of NaOCl preparations, but the findings appear to be somewhat contradictory. In addition, available data pertaining to heated hypochlorite solutions were not always obtained using endodontically relevant tissues and/or microbiota, or at temperatures higher than 37°C. Despite this lack of knowledge, heating devices for endodontic irrigating syringes have recently been introduced to the dental market.

Consequently, the purpose of this in vitro study was threefold: (1) to evaluate the short-term chemical stability of preheated NaOCl solutions using a commercially available heating device for irrigating syringes; (2) to compare dissolution capacities of NaOCl solutions on necrotic human pulp specimens at different temperatures; and (3) to assess the efficacy of preheated NaOCl solutions on *E. faecalis*, a species associated with failed endodontic therapy (13).

MATERIALS AND METHODS

Solutions

In this study 1, 2.62, and 5.25% sodium hypochlorite solutions (NaOCl in water, wt/wt) were used. The aforementioned solutions were prepared by diluting a pure 14% NaOCl solution (Thommen & Co. AG, Rüti bei Büren, Switzerland). The available chlorine (OCl⁻ and HOCl) content of NaOCl solutions was measured using a standard iodine/thiosulfate titration method (14). The solutions were protected from oxidation in tightly covered amber glass bottles and stored in a refrigerator at 5°C between experiments.

Temperature Measurements

Four 10-ml syringes (Omnifix, B. Braun AG, Melsungen, Germany) were filled with NaOCl solutions of 20°C and heated in a syringe warming device (Keydent, Vaterstetten, Germany) until they reached 45°C and 60°C, respectively. A calibrated electronic thermometer with a micro-tip (Testo AG, Lenzkirch, Germany) was used to measure irrigant temperature in the syringes. Times were recorded for the irrigants to reach the temperatures indicated on the warming device.

Short-Term NaOCl Stability

Chemical stability of heated sodium hypochlorite solutions was assessed by measuring the amount of available chlorine in solution over time, using the aforementioned iodometric titration assay. Measurements were done twice for each solution at 45°C and 60°C after a heating period of 15, 30, and 60 min. Control solutions of 20°C were cooled in a water bath to keep their temperature.

Tooth Collection

Twenty-two teeth (10 third molars and 12 premolars) were extracted for malposition or orthodontic reasons and collected at the Departments of Orthodontics and Oral Surgery, University of Zürich, School of Dental Medicine. Patients volunteered to donate their teeth, which were extracted according to their individual treatment plans, for research purposes. Informed consent was obtained from all patients. The current study protocol was approved by the institutional review board. Teeth were immediately transferred to a freezer and kept at −27°C in a tight container until further use. Because of possible interferences of disinfectants with the tissue dissolution assay (see below), experimental teeth were not treated with any chemicals. Personnel handling the specimens used universal precautions during the experiments.

Tissue Dissolution Assay

The teeth were thawed in sterile 0.9% saline. Subsequently, pilot grooves were prepared in crowns, buccal, and oral aspects without entering the pulp chamber using a diamond-coated fissure bur (Intensiv SA, Grancia, Switzerland). Teeth were then carefully split in two fragments using a micro-chisel and the entire pulp tissue was removed from the pulp chamber and the root canals using a spoon excavator. Pulp specimens were immersed in a 0.9% saline solution at room temperature for 30 min. Five human pulp specimens were randomly used per irrigating solution: 1% NaOCl at 20°C, 1% NaOCl preheated to 45°C, 1% NaOCl preheated to 60°C and finally a 5.25% NaOCl solution at 20°C (negative control). Two pulp specimens were irrigated with 0.9% saline (positive controls).

The dissolution assay was performed as follows: a round polyethylene mesh with a pore size of 0.5 mm (PE-HD, VWR International, Dietikon, Switzerland) was placed on a moistened filter paper with a pore size ≤30 μm (Schleicher & Schuell, Feldbach, Switzerland) in a Buchner filter funnel mounted on a flask connected to a vacuum pump. Before the experiments described below, the mesh had been pre-weighed in an air-tight container using a precision balance (AT 261, Mettler-Toledo AG, Greifensee, Switzerland). The moist pulp specimens were laid on this carrier; the subjacent filter paper was used to avoid small pulp remnants from being sucked into the flask. To remove excess fluid from the pulps, the vacuum pump was turned on for 60 s. Pulp specimens were then irrigated with 5 ml of test or control solutions for 60 s, using a 10-ml syringe. To stop the tissue dissolution process, pulp remnants were subsequently rinsed for 120 s with distilled water and finally the vacuum pump was left on for 60 s to remove excess fluid. The mesh containing remaining pulpal tissue was then removed from the flask and weighed. Pilot studies using the mesh only without pulp specimens showed that it did not alter its weight during the experimental process. Results are reported as per cent of initial pulp tissue weight remaining after irrigation.

Antimicrobial Test

Stationary phase *E. faecalis* ATCC 29212 cells were cultured in Tryptic Soy Broth (TSB, Difco, Detroit, MI) for 48 h at 37°C from stocks (TSB + 10% glycerol vol/vol) stored at −70°C. The cells were washed once with phosphate-buffered saline (PBS) and harvested by centrifugation at $10,000 \times g$ for 10 min at +4°C. Subsequently, bacteria were suspended in PBS to an optical density $A_{660} = 0.1$, corresponding to a cell concentration of approximately 4×10^6 colony forming units (CFU) per milliliter. Test solutions were: NaOCl 0.001, 0.0001, and 0.00001% (wt/vol); control solutions were: 0.1 M sodium thiosulfate, 0.1 M sodium thiosulfate + 0.001% NaOCl (1:10, vol/vol), and PBS. Solutions were either preheated to 45°C or cooled to 20°C in a water bath. Ten microliters of bacteria in PBS was added to 890 μl of these solutions, and incubated for 10 min at the respective temperatures.

Subsequently, 100 μl of a 0.1 M sodium thiosulfate solution was added to stop antimicrobial NaOCl activity (15). A 10-fold dilution series was made in PBS. Droplets of 20 μl from these series were cultured on tryptic soy agar (Difco) for 48 h at 37°C, and colonies were counted.

Data Analysis

Data obtained in the pulp dissolution experiments were compared using one-way ANOVA. Post hoc comparisons were performed using *t* tests followed by Bonferroni's correction. The level of the alpha-type error was set at <0.05.

RESULTS

Using the syringe irrigant warming device under investigation, the hypochlorite solutions reached 45°C and 60°C in 7 min and 20 min, respectively. The temperature scale on the device was found to be accurate. Aqueous hypochlorite solutions of 1, 2.62, and 5.25% kept 100% of their available chlorine at 20, 45, and 60°C during the whole experimental period (60 min).

A comparison of original tissue weights (mean = 41.1 ± 20.8 mg) showed no significant difference between groups (p > 0.25). The two control specimens irrigated with saline only kept their weight throughout the experimental process. When percent values of dissolved tissue after hypochlorite irrigation were compared, significant differences (p < 0.05) were recorded between all experimental groups, i.e. a 1% NaOCl solution at 60°C was significantly more efficient than a corresponding solution at 45°C, which, in turn, was more efficient than a 1% solution at 20°C (Fig. 1). A 1% solution at 45°C was equally effective as the negative control solution, 5.25% NaOCl, at 20°C. A 1% solution at 60°C dissolved 96.0 ± 3.7% of the original pulp weight in 60 s, thus, was significantly (p < 0.05) more effective than the 5.25% solution at 20°C.

Microbiological experiments using *E. faecalis* showed that incubation at 60°C in an inert PBS solution killed all bacteria, while they tolerated a 10-min incubation at 45°C in PBS without reduction in viability counts (Table 1). Equal results were obtained when bacteria were incubated in 0.1 M thiosulfate or 0.001% NaOCl blocked with the latter solution. With the pure 0.001% and 0.0001% NaOCl solutions, the antimicrobial efficacy was roughly two orders of magnitude higher at 45°C as compared to 20°C. A 0.00001% NaOCl solution was ineffective at both 20°C and 45°C.

DISCUSSION

Using endodontically relevant tissues and microbiota, this in vitro study corroborated three desirable effects of preheated NaOCl solu-
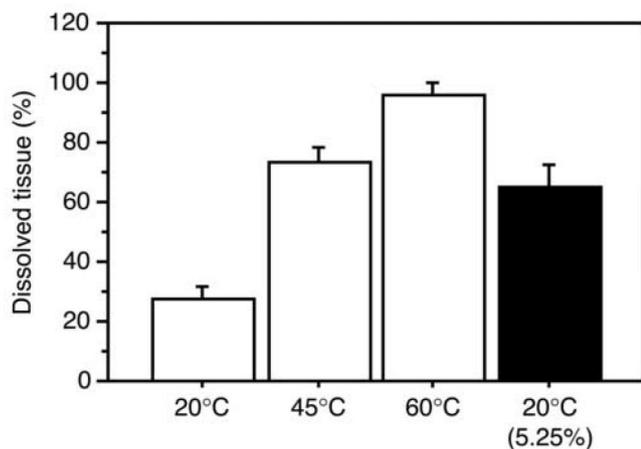
Figure 1. Bar chart depicting relative amounts (percent of original weight) of remaining human dental pulp tissue ($n = 5$ per group) after a 60-s irrigation with 5 ml of aqueous NaOCl. Differences between all groups were statistically significant at the 0.05 level (ANOVA/Bonferroni), except for the 1% NaOCl solution at 45°C and the 5.25% solution at 20°C.

TABLE 1. Mean $\log_{10}$ CFUs (48 h) of surviving stationary-phase *E. faecalis* cells exposed for 10 min to test and control solutions

NaOCl concentration (%, wt/vol)	20°C	45°C
0.001	2.0	(no CFUs)
0.0001	4.9	3.1
0.00001	5.4	5.2
PBS (positive control)	5.3	5.3

tions, namely their stability for a clinically relevant short period of time, improved tissue dissolving capacity and antimicrobial efficacy.

It should be noted that the current investigation was performed in an ideal in vitro environment. Results may therefore not be directly extrapolated to the clinical situation. Furthermore, it remains unclear whether preheated solutions would be less toxic than more concentrated solutions of similar efficacy administered at room temperature. Moreover, heat transfer may occur during rinsing of the root canal system with preheated solutions through dentin and jeopardize adjacent periodontal tissues (16). However, it may deduced from existing literature that irrigating solutions used clinically would reach a temperature equilibrium relatively quick (11). Consequently, more concentrated solutions most probably will, if administered to the periodontal tissues, be more toxic than preheated counterparts of lower concentrations (8). Furthermore, human dentin demonstrates low thermal conductivity (17). An intact vasculature in the area and the thermal conductivity of the periodontal membrane and the alveolar bone may also help dissipate the heat rise on the root surface. Nevertheless, further studies are necessary to find the ideal temperature/concentration combination for hypochlorite solutions to be used in vivo.

The current results pertaining to heat stability of NaOCl solutions are in agreement with earlier reports on the topic (18). The found an increased amount of tissue dissolution by increasing the concentration, contact time, and volume of sodium hypochlorite and by maintaining the temperature of the solution at 36°C (19). Results pertaining to an increased tissue dissolution capacity of heated NaOCl solutions were later confirmed by other authors (11, 12), and were corroborated in the current study using human pulp specimens. Furthermore, heated hypochlorite solutions remove organic debris from dentin shavings more efficiently than unheated counterparts (20). The antimicrobial properties of heated NaOCl solutions have also been discussed in the

past. As early as 1936, the effect of NaOCl temperature on *Mycobacterium tuberculosis* survival was demonstrated (21). A 50 ppm (0.005%) NaOCl solution, obtained complete kill in 30 s at 60°C, in 60 s at 55°C, and in 150 s at 50°C. With the taxa tested so far, bactericidal rates for sodium hypochlorite solutions are more than doubled for each 5°C rise in temperature in the range of 5 to 60°C (22). This was corroborated in the current study using *E. faecalis* cells; a temperature raise of 25°C increased NaOCl efficacy by a factor 100. An increased killing effect of heated solutions against endodontic microbiota appears to be present not only with NaOCl but also with other antiseptic irrigants such as chlorhexidine gluconate (23).

In conclusion it may be stated that preheating sodium hypochlorite solutions appears to improve their necrotic pulp tissue dissolution capacity and efficacy against stationary phase *E. faecalis* cells. Heating NaOCl solutions chair-side using a heating device bears the advantage that the desired irrigant temperatures can be reached within a relatively short period of time from stock solutions stored at low temperatures.

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