DOI: 10.4067/S2452-55882020000300132

ORIGINAL ARTICLE

Determination of sodium hypochlorite concentrations in the activation of the irrigant by passive technique with ultrasonic, during the ex vivo endodontic protocol.

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Work received on 18/06/2020.
Approved for publication on 23/08/2020

ABSTRACT

Introduction: Sodium hypochlorite and ultrasonic activation have a synergistic and improving effect on canal disinfection. Some authors found irrigant decrease after activation with ultrasonic, while others described an increased concentration in later stages. The aim of this study was to determine if activation of sodium hypochlorite by passive ultrasonic irrigation reduces its concentration compared to a technique without activation. **Materials and methods:** A ex-vivo descriptive study was conducted with teeth, randomized into two groups: 10 controls and 20 experimental. The hypochlorite of groups undergoing endodontic treatment was collected, and the post-irrigation residual with saline was gathered. The activation by ultrasonic was performed in stage 4.5 with Ultrasonic Scaler NSK®, three cycles of 20 seconds each per tooth. Irrigant concentration was measured by spectrophotometry. **Results.** In the first 4 stages, there were no concentration differences between groups. Stage 4.5 demonstrated a significant difference between the treated and control group. At saline irrigation stages, there was only a significant difference in stage E5. When activation was performed, the sodium hypochlorite curve maintained concentration values close to 5% in more stages in comparison to the control group. **Conclusions:** Passive ultrasonic activation demonstrated higher significant concentration of sodium hypochlorite, compared to a technique without activation.

KEY WORDS
Ultrasonics; Sodium hypochlorite; Spectrophotometry ultraviolet; Endodontics; Root canal therapy; Root canal Irrigants.

Int. J. Inter. Dent Vol. 13(3); 132-134, 2020.

INTRODUCTION.

Treatment of pulp pathologies and periapical periodontitis involves the complete removal of inflamed and necrotic pulp tissue in stages that lead to disintegration, disinfection, and preparation of the root canal system (RCS). Root canal treatment involves chemomechanical preparation (CMP), a procedure that aims to clean and shape the RCS before obturation[1]. This strategy must demineralize dentin, dissolve pulp tissue, and neutralize microorganisms using irrigants[2].

Sodium hypochlorite (NaOCl) is used in endodontics as irrigant because of its high antimicrobial activity and capability to dissolve organic and necrotic tissues[3]. These properties are directly proportional to the concentration in the solution. For acceptable levels of cytotoxicity against bacteria, a concentration of 0.5% is recommended, but this requires at least 30 minutes of action to inhibit the growth of facultative microorganisms in vitro. In contrast, 5.25% NaOCl eliminates microorganisms in vitro in a few seconds[4, 5].

Activation of the endodontic irrigant improves its chemical and physical action in preparing the canal[6]. The activation of the irrigant involves the agitation of the fluid achieved by the oscillating instrument inside the canal[7]. Mechanical activation methods are manual or use agitation devices which include negative pressure, sonic method, and ultrasonic. The ultrasonic method uses acoustic waves with a frequency higher than the highest frequency perceptible by the human ear (approximately 20,000 Hz)[8].

The use of ultrasonic can be by ultrasonic irrigation (UI) and passive ultrasonic irrigation (PUI)[9]. The PUI is the most commonly used technique and is triggered after the mechanical preparation of the canal at diameters smaller than the master apical file[10]. It is a non-cutting technology that transmits ultrasonic wave energy to the irrigant, causing two physical effects: irrigant flow and solution cavitation[10]. There is a synergistic effect between NaOCl and ultrasonic. Better canal disinfection[8], greater efficiency in removal of pulp remnants and dentin[10], and removal of smear layer[11] are observed. The protocol indicates that the activation of NaOCl with the ultrasonic method should last between 30 seconds and 1 minute per canal, doing 3 cycles of 10-20 seconds each and constantly renewing the irrigant[12].

Activation with PUI could be associated with a faster reduction of sodium hypochlorite and consequently a higher generation of active products of hypochlorite such as hypochlorous acid and ion chlorine. However, while Macedo et al.[13] found a decrease in NaOCl after activation of the irrigant by ultrasonic, Yévenes et al.[14] found an increase in the concentration of NaOCl in the stages following activation. Observing these differences between the effects of ultrasonic on hypochlorite, this study was designed to measure if activation of NaOCl by PUI reduces its concentration compared to a technique without activation.

MATERIALS AND METHODS.

Type of study and sample selection. The present research is a prospective, analytic, and experimental *ex-vivo* study. In this study the NaOCl concentration was measured during the application of two techniques: one without activation and the other with PUI activation, comparing the data obtained. The mathematical formula that allowed us to calculate the sample size for the comparison of two means[15], is the following: $n = 2(z\alpha+z\beta)^2 s^2 / d^2$; where zβ corresponds to the desired risk; zβ corresponds to the desired statistical power; s2, the variance of the quantitative variable; d, minimum difference value to be detected. Using the previous values obtained by Yevenes et al.[14], adding a ratio between the n of the experimental group and the control of 2, a value of n = 30 was obtained[16].

Obtaining and storing the sample. An *ex-vivo* model was designed using 30 recently extracted single-root teeth preserved in saline fluid, previously approved by the ethics committee of Universidad de Chile. Patients who underwent tooth extraction were asked to fill out and sign the informed consent form, to give their authorization to use the extracted tooth for this study.

Sample selection criteria. Healthy single-root teeth, with coronary integrity, a straight root canal or a slight curvature in the apical third, and medium to broad root canal diameters in the three-thirds of the root canal analyzed in a previous periapical radiograph. The sample was divided into two groups: the control group (endodontic protocol without NaOCl activation) and the experimental group (endodontic protocol with irrigant activation PUI).

Materials, instruments, and devices. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced and the length was recorded when the tip of the file was visible at the apical foramen. The working length was determined by subtracting 1 mm from this length. The apices of the specimens were sealed with wax to prevent the overflow of irrigating solutions. Canals were instrumented using manual K-file instruments (Dentsply Maillefer) up to master apical file (MAF) size 35 in a crown-down technique applying the following irrigation protocol: from the access cavity to finished instrumentation, the teeth were irrigated with 5% NaOCl (12 mL in total) using a Monoject Irrigation Syringe (size 27-gauge needle) between each instrument change, for a total of 4 stages recollection. The needle was placed at 1 mm from the working length with a backward and forward movement. After that, the control group was irrigated with 9 mL of saline (corresponding to stage 5, 6, and 7). The clinical irrigation protocol described above was also applied to the teeth of the experimental group, but the activation step was added after the mechanical preparation of the canal and before saline irrigation. The root canal was filled with NaOCl to the height of the access cavity and then activated by PUI in 3 cycles of 20 seconds each. Activation was carried out with NSK® Ultrasonic Scaler Various 560, in "E" mode (EndoMode), at medium power, and standardizing for samples with a # 20 file and at -1 mm from the working length. Non-refreshment of hypochlorite was incorporated between cycles. The collection of the 3 volumes (one per cycle) was carried out using a micropipette and this stage was called 4.5 (E4.5). The solutions of each root canal were collected during the different phases of the treatment using an intracanal aspiration device specially designed for this study. The 7 samples from the control group and the 8 samples from the experimental group were transferred to Eppendorf tubes (1.5 mL) and centrifuged at 10,000 rpm for 5 minutes at 4 °C, to remove residues and then we proceeded to chemical analysis. Figure 1 shows the flowchart of the stages of endodontic treatment according to the clinical irrigation protocol, with the respective collections and the indicated protocols.

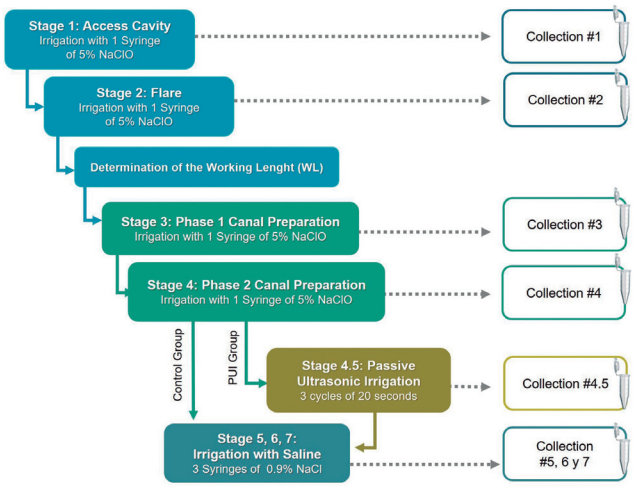

Figure 1. Flowchart of stages of endodontic treatment according to clinical protocol and sample collection per stage.

Determination of NaOCl concentration. In the samples collected during the treatment, the concentration of NaOCl was determined by spectrophotometry. For this, the wavelength at which the NaOCl has the highest absorbance was determined (λmax): 292 nm. Then the calibration curve was constructed, and the equation of the curve was obtained, which allowed to finally determine its concentration in the samples collected. Subsequently, the absorbance at λmax of the collected samples was measured on a spectrophotometer UNICAM® UV/VIS (Thermo Spectronic Unicam UV-530 UV-Visible, Rochester, NY, USA) using a quartz cuvette (1mL) against distilled white water and the absorbance values were interpolated into the equation of the calibration curve, to obtain the value of its concentration.

Statistical analysis. Through the analysis of the results we sought to establish the differences of the measured concentrations of NaOCl between both groups, "without activation" and "with activation by PUI". The data were tabulated in terms of absorbance and NaOCl concentration. The data obtained were subjected to the Shapiro-Wilk statistical test to determine the type of distribution. If the samples did not present a normal distribution, the data were subjected to the Mann-Whitney test to establish significant differences using the IBM SPSS statistical software. A 95% confidence interval was set accepting statistically significant differences when the p-value was <0.05.

RESULTS

Using the spectrophotometric method, the concentration of sodium hypochlorite was measured in the volumes collected in stages E1 to E4, which included the stages from the access cavity to the step back which is collection No. 4. Also, the residual NaOCl concentration after washing the root canal with the intermediate irrigant (E5 to E7) was determined. Concentrations during the activation corresponding to step E4.5 were also measured. The results obtained are shown in Table 1. In the first 4 stages, there were not concentration differences between groups. In stage 4.5 (PUI activation) there was a significant difference between treated and control groups (p <0.05). At saline irrigation stages, there were only significant differences in E5 between the groups. In both groups, it was possible to quantify NaOCl in small concentrations. For the other stages, there was no statistical difference between the two groups.

Table 1: Comparison of sodium hypochlorite concentrations in the stages of endodontic protocol between control group (without activation) and experimental group (passive ultrasonic activation).Bottom legend.S1 to S4 include stages from the access cavity to the second phase of canal preparation (Step Back technique) which is sample number 4. S5 to S7 corresponds to the residual NaOCl from the saline washing steps. S4.5 corresponds to activation of NaOCl with PUI in experimental group. While the values of control group in S4.5 are equal to the stage S4. * Significant difference SD= standard deviation. PUI= Passive Ultrasonic Irrigation.

STAGE	S1	S2	S3	S4	S4.5	S5	S6	S7
Mean Control Group (%)	4.507	4.635	4.439	4.702	4.702	0.133	0.044	0.024
SD	0.333	0.226	0.406	0.321	0.321	0.109	0.067	0.036
Mean Experimental Group (%)	4.565	4.734	4.811	4.801	5.580	0.298	0.060	0.021
SD	0.182	0.207	0.243	0.172	1.333	0.159	0.014	0.025
P Value	>0.05	>0.05	>0.05	>0.05	**<0.05***	**<0.05***	>0.05	>0.05

In Figure 2, the linear representation of the concentration of sodium hypochlorite obtained in the stages of the endodontic protocols is shown. It is observed that the graph of the activated irrigant reaches higher concentrations of NaOCl in a greater number of stages, for the protocol without activation, highlighting stage 5.

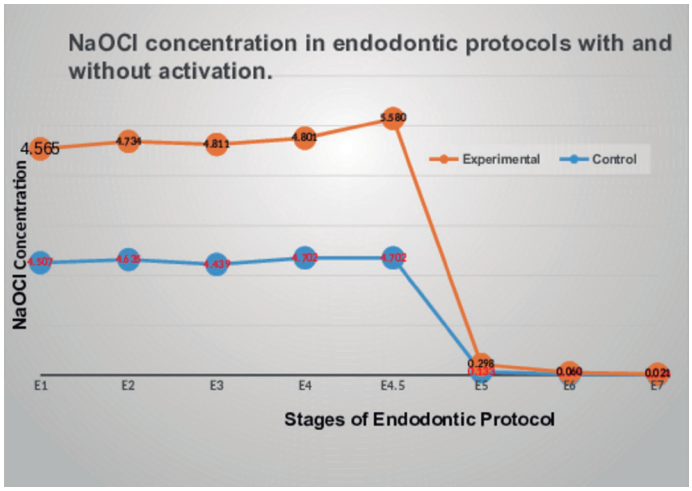

Figure 2. Variations in NaOCl concentration in the stages of endontic processes with or without activation by passive ultrasonic irrigation (PUI).

DISCUSSION

In endodontic treatments, the penetration of the irrigant into dentinal tubules of the root canal improves the disinfection of the RCS and the prognosis of the treatment[17,18], even more so considering that at least 40% of the root canals are not instrumented, even in circumferential root canals[19].

To remove bacteria from the canal walls, following the preparation the irrigant must reach most of the RCS, since there are uninstrumented

areas [20]. The use of ultrasonic in endodontics has optimized the treatment by improving access and cleaning of secondary canals, leading to less intracanal obstruction, better conformation, and obturation of the root canal[21]. However, the chemical influence that ultrasonic can have over the irrigant, which allows it to be associated with greater effectiveness in endodontic treatment, is unknown.

The aim of this investigation was to measure NaOCl concentrations in stages of endodontic treatment by comparing two irrigation techniques, one passive and one with activation, to describe the effect generated using ultrasonic on the irrigant. Studies have contradicted each other in the action generated by this activation method, while in this study a controlled methodology was developed, standardized instruments were used, and current guidelines for endodontic treatment with PUI were implemented. The sample was randomly divided into two groups: without activation and with activation. The irrigation protocol applied is from the Endodontic Clinic of the University of Chile, until stage 7. In the experimental group, ultrasonic was incorporated after standardized CMP.

According to the described results, the hypothesis was not met, since there was an increase in the concentration of NaOCl when using the ultrasonic method, in two stages. In steps 5, 6, and 7, saline was used as the irrigant to dilute the residual NaOCl. In the experimental group, the residual NaOCl concentration was higher for the fifth ($P < 0,05$) and the sixth stage. One explanation of this is the saturation of the irrigant inside the RCS, an effect caused by the absence of bacterial proteins or available collagen, leading to more NaOCl molecules available in the canal to elute with saline, explaining the increasing hypochlorite concentrations in comparison to the control group, and the fact that ultrasonic favors an infiltrating action of the irrigant and for a long time. This agrees with Yevenes et al.[14] in their study, where after activating the irrigant, NaOCl concentrations proved to be higher than the control group. In figure 2, the experimental group maintains higher concentrations of NaOCl for a greater number of stages, so this greater amount of NaOCl available is due to greater penetration into the RCS.

Macedo et al.[13], found that the chlorine available decreased during the use of ultrasonic due to the decomposition of the original molecule, thus explaining its biological effect. The results obtained in this ex-vivo study showed that ultrasonic generates significantly higher concentrations of NaOCl in the first irrigation stage (E4.5). Another explanation for the data obtained could be the NaOCl evaluation method by spectrophotometry. Spectrophotometry is used to measure the amount of light absorbed by a solution. Certain solutions can have an equal absorption under the same light spectrum; this situation is known as an isosbestic point and corresponds to an absorbance at which several wave spectra intersect[22]. Under this theory, it could be assumed that the sodium hypochlorite and its reaction products (HClO and ClO-) have coincident absorbance values and the reading suggests a higher concentration of NaOCl than the real one. Previous studies have found isosbestic points between HClO and chlorine dioxide[23]. This fact would make it possible to clarify why the values obtained in stage 4.5 were different between the two protocols, but to verify this, more studies should be conducted such as using mass spectrometry, which is a methodology that allows not only to quantify but also to identify substances present in a sample.

Finally, our results suggest that using PUI contributes to improving irrigation by not only turbulence and penetration of the irrigant inside the dentinal tubules but also maintaining NaOCl concentration for a longer time than a conventional technique without activation. This could be useful to consider in clinical practice, when choosing an activation method or in clinical situations of difficult anatomic access.

Conclusions. This study and its results prove that the activation of NaOCl by passive ultrasonic irrigation increases its concentration compared to a technique without activation.

CLINICAL RELEVANCE.

Irrigant activation methods should be preferred by dentists. In PUI, the energy is transmitted from the oscillating file to the irrigant improving the penetration inside the canal. Activation by ultrasonic increase effectiveness because it would act on the irrigant producing a greater release of active by-products such as hypochlorous acid and hypochlorous ion capable of reaching non-instrumentable canals. This activation device should be implemented in clinics of teaching-healthcare practice as it provides a better disinfection of the root canal. Can be used in various clinical situations such as retreatments, in cases of complex endodontic access or persistent apical periodontitis.

ACKNOWLEDGMENTS

Thanks to Mr. Juan Fernández de los Ríos, from the Language and Translation services, Direction of Academic Affairs, Faculty of Dentistry, Universidad de Chile, for kindly proofreading and checking the spelling and grammar of this article.

DECLARATION OF CONFLICT OF INTEREST AND SOURCE OF FINANCING.

1. Identification data. Dra. Carmen María Pirela, Dra Silvana Maggiolo. Prof. Ismael Yevenes.

2. Information on the funding received during the entire process of carrying out the work, directly or indirectly (through the Institution). The publication was made in the chemistry area of the Faculty of Dentistry of the University of Chile. Its financing was through Grant FIOUCH 13-15 and by the institution.

3. Other sources of financing. There is not.

4. Conflicts of interest. The undersigned indicate that they do not present any type of conflict of interest that affects the publication.

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