Assessment of pH increase in the external root surface after ultrasonic agitation of different calcium hydroxide pastes: Ex vivo study

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ABSTRACT

Objective: The aim of the present study was to evaluate, ex-vivo, the pH elevation capacity on the external root surface of different calcium hydroxide (HC) pastes, utilized as intracanal medication, it was also evaluated the influence of the ultrasonic activation at the application within the root canal. **Methods:** 100 human single root superior incisors were used, which had their root canals shaped and randomly divided into 6 experimental groups (n=15) according to the following HC pastes: Calen; HC + Chlorhexidine 2% gel (CX2%); HC + distilled water, utilizing the medications and the ultrasonic activation of the paste at the time of the application as variables and 1 control group (n=10). The teeth were kept immersed in deionized water and the pH parameters were verified in 7, 14, 21 and 28 days with a pH measurement machine. **Results:** According to the data obtained, pH was elevated in the first week in all groups. Only the groups which the association of HC with CX2% was made, did show a significant increase in the pH level over the analyzed periods ($p < 0,05$). The activation of the pastes with US provided a significant increase in pH values ($p < 0,05$). **Conclusion:** According to the methodology used, we can conclude that all the HC pastes used, promote pH elevation in dentin tissue. And the activation of the pastes with US significantly influences the pH increase in dentin tissue.

Keywords: Intracanal medication. Calcium hydroxide, pH. Ultrasound.

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Introduction

Intracanal medication is widely used in cases that present symptoms of periapical origin, associated with inflammation, or due to the presence of fistulas and apical lesions.[1] As well as in traumatized teeth that have suffered injury to the vascular-nervous bundle and periodontal ligament, making the environment injured more acid favoring the action of clastic cells with a drop in pH.[1] Among the medications proposed in endodontics, calcium hydroxide pastes are the most widespread and studied, where the best associations are analyzed, time of use and more recently their activation with the help of ultrasound are analyzed.[2-4]

Studies using calcium hydroxide pastes as intracanal medication demonstrates that at a minimum intervals of 7 days a significant reduction in endodontic microbiota are presented, CO^2 consumption, alkalinization of dentinal tissue and hydrolysis of bacterial lipopolysaccharides.[5,6] Despite the fact that the calcium hydroxide's antimicrobial activity is dependent on direct contact with the bacteria, studies have shown that calcium hydroxide is not effective in eliminating bacteria that colonize the dentinal tubules in a deeper way.[7,8] Thus, it is understood that calcium hydroxide depends on other characteristics so that it can act against the present infection, such as its ability to raise the pH through the dissociation of calcium ions.[9]

Due to this limitation in antibacterial activity and its low solubility, many studies seek new methods of inserting medications such as activation with ultrasound,[4] in addition to new formulations and associations with other substances that can increase the antimicrobial action together with calcium hydroxide.[9] One of these substances is chlorhexidine, which is proven to be an excellent antiseptic agent.[3,10]

Thus, in view of the wide clinical use of intracanal medications in the control of endodontic infections, in the reduction of osteoclastic activity and in the induction of repair, the need arises to evaluate the ability to raise the pH of the external root surface of different pastes based on calcium hydroxide, in addition to evaluating the influence of ultrasonic activation of pastes inside the root canal of extracted teeth.

Materials and methods

After approval by the ethics committee (Protocol # 06-358), 100 uniradicular superior human incisors extracted for reasons unrelated to this study were collected. The teeth were stabilized around the bench and the access cavity to the pulp chamber was performed with diamond drills (# 1013, # 3081; KG Sorensen, Cotia, Brazil) in high rotation. The 2.5% sodium hypochlorite solution (NaOCl) (Asfer Indústria Química Ltda, São Caetano do Sul, SP, Brazil) was used as an irrigating agent during the preparation of the root canal. All irrigation steps were performed with the aid of a disposable Luer syringe (BD, Juiz de Fora, MG, Brazil) adapted to the specific irrigation needle (NaviTip 29G; Ultradent, South Jordan, UTAH, USA) with penetration limiter calibrated to restrict the penetration depth to 2.0 mm below the working length (WL). The WL was standardized (21.0 ± 1.0 mm) and the apical patency was performed with C-Pilot files # 20 (VDW GmbH) introduced in the root canals until they were visualized through the apical foramen (FA = 0); 25x magnification was used by means of a clinical microscope (DF Vasconcellos, São Paulo, Brazil). Also, to allow the reflux action during irrigation, the FAs were covered with utility wax (Lysanda Produtos Odontológicas, São Paulo, SP, Brazil).

The procedures of chemical-mechanical preparation (CMP) of the specimens were performed by a single operator previously trained, using the crown-apex technique. The surgical diameter was standardized at # 80, and irrigation with 2.0 mL of 2.5% NaOCl at each instrument change. At the end of the instrumentation, the chelating agent EDTA at 17% (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil) was used. Shaking of the chelating solution with an Irrisonic tip (Helse, Santa Rosa de Veterbo, SP, Brazil) activated by EMS PIEZON ultrasound (PM200; EMS, São Bernardo do Campo, SP, Brazil) at power 3 (30%) was used. It was given a special attention to maintaining the tip centered in the root canal so it did not touch the dentinal walls and positioned 2.0 mm below the WL. Irrigations were performed with 1.0 mL of chelating agent for 1 min. Within this 1 min. 30 seconds of ultrasonic agitation were performed; this procedure was repeated 2 more times, making a total time of 3 min. and 3.0 ml of EDTA. After the PQM and EDTA agitation / activation procedures were completed, the specimens received final irrigation with 25.0 mL of 0.9% saline (Farmence, Barbalha, CE, Brazil) to be subsequently dried with absorbent paper cones (Endopoints, Paraíba do Sul, RJ, Brazil).

The specimens were autoclaved in moist heat at 121° C for 20 minutes and then randomly divided into 6 experimental groups (n = 15), with the medications used and the ultrasonic activation (AUS) of the paste at the time of application as variables. In the control group (n = 10) the teeth remained empty, with no intracanal medication. Prior to filling the channels with calcium hydroxide pastes, all teeth had their apical foramina sealed with cyanoacrylate, preventing the exit of intracanal medication during the evaluation period.

Calcium hydroxide (HC) pastes used in the study:

» Calen® Paste - Product sold in tubes and with the following composition: HC, Polyethylene Glycol 800 and Colophony.
» Calcium hydroxide with 2% chlorhexidine gel - (HCX) - the paste was prepared by combining HC PA with 2% chlorhexidine gel (Endogel), in the proportion of 1 ml vehicle / 1 g powder, obtaining paste consistency.
» Calcium hydroxide with distilled water - (HCA) - the paste was made by joining the HC PA with distilled water, in the proportion of 1 ml vehicle / 1 g powder, obtaining paste consistency.

Study groups

Each group had their specimens filled with a different HC paste, as shown in Table 1.

The filling of the root canal in the specimens of the HCX and HCA groups was performed with the aid of disposable syringes (BD, Juiz de Fora, MG, Brazil) adapted to the specific irrigation needle (NaviTip 29G; Ultradent, South Jordan, UTAH, USA) with penetration limiter calibrated to restrict the penetration depth to 2.0 mm below the WL. For the Calen paste groups, the Mario Leonardo endodontic syringe (SS White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil) and 27G long needle (Septoject XL; Sptodont, Saint Maur DES Fosse´s, Cedex, France) for the application of this medication, as indicated by the manufacturer. A calibrated penetration limiter was used to restrict the penetration depth to 2.0 mm below the WL.

For the groups that received AUS, the Irrisonic tip (Helse, Santa Rosa de Veterbo, SP, Brazil) used in ultrasound EMS PIEZON (PM200; EMS, São Bernardo do Campo, SP, Brazil) was used at power 3 (30%). Special attention was given to maintaining the tip centered in the root canal so as not to touch the dentinal walls. Three cycles of 30 seconds were used with the 2.0 mm instrument tip below the WL. After filling the pastes, a sterile cotton swab was placed at the entrance to the root canals and the coronary sealing was performed with glass ionomer cement (Vidrion R; S.S. White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil). In order to prove the complete filling of the channels, a radiographic capture of the specimens was performed.

To check the pH, the specimens were suspended in individual supports in a Becker flask containing 10.0 mL of deionized water. The evaluations were carried out in the periods of 7, 14, 21 and 28 days. To determine the pH, a pH meter previously calibrated with solutions of known pH (04, 07 and 10) was used. At each period, the specimens were removed from the test tubes and the tubes transported to an agitator where they remained for 5.0 seconds and then placed in contact with the pH meter electrode (model Ba74;

Table 1. Averages of pH values for the different groups and times analyzed.

Paste	Ultrassound
Calen	No
Calen	Yes
HCX	No
HCX	Yes
HCA	No
HCA	Yes
Control	-

Micronal, São Paulo, SP, Brazil). The collected data were tabulated and analyzed in relation to the normality curve (Kolmogorov-Smirnov test) and showed non-parametric behavior. Values were expressed as means and compared using the Kruskal-Wallis test followed by the Dunn post-test, with a significance level of 5%.

Results

The results showed that, for all groups, there was a significant increase in pH just after 7 days of using intracanal medication in relation to the control group ($p < 0.05$).

Among the groups that did not use ultrasound, at 7 days, HCA showed superior results ($p < 0.05$) in relation to the HCX and CALEN groups. This behavior was maintained at 14 days, with superiority of the group.

For the CALEN and HCX groups, where the vehicles are viscous, the behavior of the pH values peaked at 21 days, and were the same ($p > 0.05$) and higher than the HCA group ($p < 0, 05$). This behavior was maintained at 28 days for the HCX group, being higher than the other groups ($p < 0.05$).

Among the groups in which the pastes were activated with the use of ultrasound, a different behavior can be noted, with anticipation of pH peaks at 7 days (group HCA / US) and 14 days (groups CALEN and HCX). At 7 days, the HCA / US group was higher ($p < 0.05$) than the other groups with agitation, which were the same ($p > 0.05$). At 14 and 21 days, all groups with agitation were equal ($p > 0.05$). And at 28 days there was an inversion with the groups Calen / US and HCX / US higher than HCA / US ($p < 0.05$).

In the intra-group analyzes, comparing the pH values with and without activation of intracanal medication with ultrasound, it can be noted that there was a significant increase in pH values ($p < 0.05$) in all groups for the periods of 7 and 14 days. In the 21-day period, ultrasound agitation showed no difference in the HCX group. And in 28 days, the values declined and showed no differences between them ($p > 0.05$).

All pH values obtained for the different groups and time periods analyzed are shown in Table 2 and Figure 1.

Table 2. Average pH values for the different groups and times analyzed.

Groups	Negative control	Calen	Calen / US	HCX	HCX / US	HCA	HCA / US
pH 7 days	7.01^{c}	$8.03^{B.b}$	$8.32^{A.b}$	$8.01^{B.b}$	$8.39^{A.b}$	$8.54^{B.a}$	$9.18^{A.a}$
pH 14 days	7.05^{c}	$8.12^{B.b}$	$8.68^{A.a}$	$8.18^{B.b}$	$8.85^{A.a}$	$8.46^{B.a}$	$9.07^{A.a}$
pH 21 days	7.07^{c}	$8.33^{B.a}$	$8.51^{A.a}$	$8.57^{A.a}$	$8.53^{A.a}$	$8.01^{B.b}$	$8.97^{A.a}$
pH 28 days	7.09^{c}	$7.84^{A.b}$	$8.14^{A.a}$	$8.00^{A.a}$	$8.08^{A.a}$	$7.87^{A.b}$	$7.95^{A.b}$

^{a,b,c} Different lower case letters represent significant differences between groups according to the materials, according to the Kruskal-Wallis tests followed by the Dunn post-test ($P < 0,05$).

$^{A, B}$ Different capitals represent significant differences considering the effect of ultrasonic agitation on each material according to the Kruskal-Wallis test followed by the Dunn post-test ($P < 0,05$).

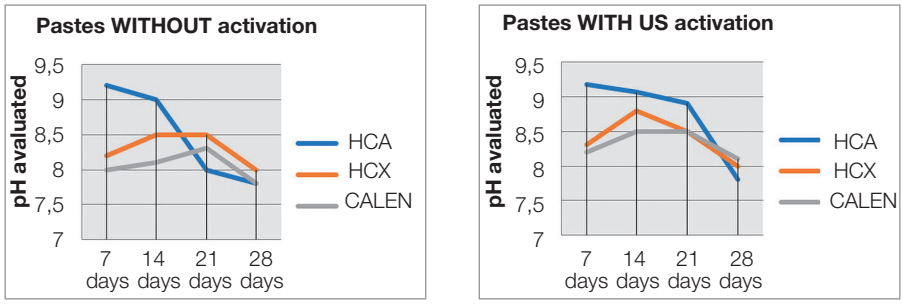

Figure 1. Study groups according to the type of HC paste used and activation with ultrasound.

Discussion

Since the 1980s, the diffusion of hydroxyl ions of calcium hydroxide through the dentinal tubules has already been proven, resulting in an increase in the pH of dental tissues, making the environment inhospitable for the vast majority of bacterial species that colonize the pulp cavity.[5]

The time intervals selected for the pH measurement were based on technical reasons and related to the routine in the dental office, which are determined by studies present in the literature.[10,11]

The methodology used to read the pH of the solution is valid and published in the literature, capable of proving the arrival of hydroxyl ions on the external root surface, which immediately sensitize the pH meter's electrodes. Other studies have evaluated the direct pH on the external surface of the root simulating external resorption through cavities made with drills.[12,13] Despite different methodologies for measuring pH, studies have found similar pH values. The association of NaOCl with CHX generates the formation of a brown precipitate that can have harmful effects on the dental element, such as obliterating the entry of dentinal tubules and preventing the action of endodontic drugs.[14] However, to avoid the formation of this precipitate was used at the end of the PQM procedures 3.0 mL of EDTA under ultrasonic agitation / activation, and finally irrigation with 25.0 mL of 0.9% saline.

The sealing of the apical foramen served to avoid direct contact of the medication with the solution (deionized water) in which the specimens were immersed, avoiding influences of the results. This, because what causes the change in the pH of the samples would be, according to the literature, the diffusion of intracanal medication, via dentinal tubules, through the dentinal tissue, reaching the periodontal tissues externally, represented in the study by the solution in which the specimens, with a consequent increase in the pH of the external root surface.[4]

Our results differ from studies in the literature,[2,4] which demonstrate that the vehicle is not a relevant factor in relation to alkalinization. However, in our study in the periods of 7 and 14 days, the paste with aqueous vehicle promoted a better alkalinity than the paste with viscous vehicles. And that at 21 days this alkalinity was reversed in relation to vehicles. This fact can be justified by the dissociation of calcium hydroxide that occurs directly proportional to the vehicles used, and aqueous vehicles allow reaching a pH close to 12.6, due to a faster dissociation and diffusion rate of hydroxyl ions. The viscous vehicles, because they present a lower dissociation speed and diffusion of hydroxyl ions, need longer times to reach high pH levels. However, they remain at high pH for a longer period[15].

The results of the measurements reveal a better stability in the pH levels over time when the association of calcium hydroxide with chlorhexidine 2% gel. However, when the paste was activated with ultrasound, the association of calcium hydroxide with water was better until 21 days. The Calen® paste showed adequate initial results, however it did not show maintenance of the pH rise, as is expected in pastes with viscous vehicles. Probably the fact that the paste is ready for use, that is, it has been manipulated for some time, the calcium and hydroxyl ions react internally and promote the formation of Calcium Carbonate.[4]

The evaluations included groups submitted to ultrasonic agitation. Its use is based on the transmission of acoustic micrometric energy through the use of ultrasonic activation. Such mechanical vibration energy is spent from the insert and propagated to the material, favoring a greater interaction between the constituents, potentiating the reaction and making it more homogeneous. In addition to providing greater penetration in the dentinal tubules and a better adaptation of the paste / dentin interface.[4,16-18] The better embedding of the pastes in the dentinal tubules justifies the elevation of the pH on the external surface of the dentin, since the pH level is dependent on the distance that the hydroxyl ions are from the external surface of the root, confirmed by the first Fick's diffusion law.[19]

In this way, ultrasonic agitation, in addition to promoting an earlier pH rise, allowed a significant increase in pH values for the HCX and CALEN groups in the periods of 7 and 14 days, in addition to keeping the high pH constant in the HCA group by up to 21 days. In the 21-day period, ultrasonic agitation showed no difference in the HCX group, which remained with constant alkalinity. After this period, a decrease in alkalinity was noted for all groups studied. As a result, the use of ultrasound to activate calcium hydroxide pastes used as intracanal medication allows the potentiation of the effect of these medications and leads to the possibility of reducing the time between sessions. It is worth mentioning that after 28 days, all

pastes, regardless of activation with ultrasound, showed a decrease in alkalinity, indicating the need to change the medication, if there is a clinical indication.

The literature demonstrates that there is no ideal intracanal medication, however, research must be carried out so that we can target the best choices in relation to existing drugs, as well as their associations. Studies analyzing the profile of medications over time, verifying their antimicrobial activities and the formation of compounds should be carried out in order to seek the ideal substances and associations for dental and endodontic use.

Conclusion

According to the methodology used, it can be concluded that: (i) all the pastes used promoted an increase in pH in the dentinal tissue; (ii) the ultrasonic activation of the pastes significantly influenced the increase in pH.

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