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Release and diffusion of hydroxyl ion from calcium hydroxide-based medicaments

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Abstract – The release and diffusion of hydroxyl ions (OH^-) of calcium hydroxide ($Ca(OH)_2$)-based intracanal medications may be affected by the association with other substances. The aim of this study was to evaluate the diffusion of OH− ions through root dentin by the medications: G1, $Ca(OH)_2$/saline; G2, Calen; G3, Calen/camphorated p-monochlorophenol (CMCP); and G4, Calen/0.4% chlorhexidine (CHX). Root canals from bovine teeth were prepared in a standardized manner. A cavity until dentin was prepared in the middle third of the root surface of each specimen. The external surface of the root was made impermeable using a layer of adhesive, except the prepared cavity. The root canals were filled with different medications, and teeth were individually stored in flasks containing 10 ml distilled water at 37°C. The water pH was measured at 1, 3, 7, 14, 21, 30, and 60 days. Data obtained were subjected to ANOVA and Tukey's tests. Increase in pH was observed at 3 days for Calen/CHX and from 7 to 14 days for the other mixtures. Calen paste promoted pH increase up to 21 days. Calen/CMCP had the highest pH up to 21 days, and all groups had similar results at 30 days. At 60 days, the greatest pH values were observed for Calen/CMCP and Calen alone. All different formulations of $Ca(OH)_2$-based medications tested release hydroxyl ion that can diffuse through the dentin.

Key words: calcium hydroxide, endodontics, pH, vehicle

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Introduction

The success of endodontic treatment of teeth with chronic periapical lesions depends on the ability to eliminate infection in the root canal system (1). In these clinical situations, the use of intracanal medication is required to complement the disinfection of the root canal system, dentin tubules, and external surface of the root apex, which cannot be achieved by chemo-mechanical preparation (2).

Calcium hydroxide ($Ca(OH)_2$) has been widely used as an intracanal dressing for presenting antibacterial effect (3, 4), anti-exudative action (5), ability to induce mineralized tissue (6), biocompatibility (7), ability to dissolve necrotic tissues (8), CO_2 absorption (9), as well as the ability to inactivate bacterial endotoxin (LPS) both *in vitro* (10) and *in vivo* (11). Moreover, $Ca(OH)_2$ is able to inhibit resorption induced by orthodontic movement (12) or by trauma (13) and to prevent recontamination of fluids that will serve as a substrate for residual bacteria (14). The antimicrobial action of $Ca(OH)_2$ depends on its high pH (3, 15), which depends on release and diffusion of hydroxyl ions (OH^-). These ions promote antimicrobial action by direct or indirect contact, after its diffusion throughout the root canal system and dentinal tubules (16).

In spite of its excellent properties, $Ca(OH)_2$ is not effective against all bacteria present in the root canal system (17). In an attempt to increase the antimicrobial action, different substances have been associated with $CaOH_2$. Camphorated paramonochlorophenol (CPMC) associated with $Ca(OH)_2$ has demonstrated a broad spectrum of antimicrobial activity (eliminating microorganisms that are resistant to $Ca(OH)_2$), and a faster antimicrobial action than associations of $Ca(OH)_2$ with inert vehicles (water, saline, and glycerin) (18, 19).

Chlorhexidine gluconate (CHX) is a biguanide used as antibacterial agent to irrigation of root canals and intracanal medication, because of its wide antimicrobial spectrum (20) and substantivity (21). However, CHX is unable to inactivate bacterial LPS (10) and dissolve necrotic tissue (22). Therefore, the association of CHX with $Ca(OH)_2$ aims to promote a synergistic effect: the antimicrobial action of CHX complements the effectiveness of $Ca(OH)_2$ (23, 24).

The association of different substances with calcium hydroxide in the formulation of intracanal medications may alter its dissociation ability and, consequently, may potentially affect its antimicrobial and biological properties (25). With this in mind, it is important to verify the clinical impact of associating different substances with $Ca(OH)_2$ on its ability to promote diffusion of hydroxyl ions throughout dentin. Therefore, the aim of this study was to evaluate the ability of different $Ca(OH)_2$-based intracanal medications to release and diffuse OH^- ions throughout dentin.

Materials and methods

Fifty bovine teeth were selected for this study. The crowns were removed using a # 4258 diamond disk with an Isomet 1000 device (Buehler, Lake Bluff, IL, USA). Then, the specimens were immersed in 2.5% sodium hypochlorite at room temperature for 48 h and then transferred to distilled water. Root canals were prepared 1 mm short of the total length, up to #110 K-file (Dentsply-Maillefer, Ballaigues, Switzerland). A #5 Gates-Glidden drill (Dentsply-Maillefer, Ballaigues, Switzerland) was introduced up to 10 mm into the root canal.

Root canals were irrigated during the chemo-mechanical preparation with 5 ml of 2.5% sodium hypochlorite solution (Instituto de Química, Araraquara, UNESP – Brazil) using 2 ml at each change file. For removal of the smear layer, 17% EDTA (Odahcam Dentsply, Petrópolis, RJ, Brazil) was used in the root canals for three minutes. Then, the root canals were irrigated with 5 ml of saline.

A 4-mm-long, 2-mm-wide, and 0.5-mm-deep cavity was prepared on the proximal surface of each root between 5 and 9 mm from the root apex, using an operating microscope and a #1052 high-speed diamond bur (KG Sorensen Ind. e Com. Ltda., São Paulo, Brazil). Each cavity was radiographed and measured to ensure that all samples had similar thickness of dentin between the preparation and the root canal wall.

Before the placement of intracanal medication, teeth were stored in a flask containing distilled water for 14 days at 37°C to verify possible ionic changes from the dental structure. After this period, specimens were removed from the flasks and dried, and the external surface of each root was made impermeable with a layer of epoxy adhesive (Brascola, Joinville, SC, Brazil) complemented with nail varnish, except on the prepared cavity. Specimens in group 5 (control) were made impermeable as in the experimental groups, without filling with intracanal medication. Specimens in group 6 (control) were made completely impermeable, including the prepared surface. After the impermeable layers were allowed to dry, roots were randomly divided into 6 groups, and the root canals were filled with the following intracanal medications:

Group 1 (n = 10) – $Ca(OH)_2$ powder (Merck, Whitehouse Station, NJ, USA) mixed with saline at the ratio of 1g of powder to 1 ml of liquid;

Group 2 (n = 10) – Calen Paste (S. S. White Art. Dent. Ltda., Petrópolis, RJ, Brazil), composition: $Ca(OH)_2$, zinc oxide, colophony, polyethylene glycol 400;

Group 3 (n = 10) – Calen Paste/CMCP (S. S. White Art. Dent. Ltda., Rio de Janeiro, RJ, Brazil), composition: $Ca(OH)_2$, zinc oxide, colophony, polyethylene glycol 400, and camphorated p-monochlorophenol (CMCP);

Group 4 (n = 10) – Calen Paste associated with CHX gluconate (Arte & Ciência, Araraquara, Brazil), at a concentration of 0.4% in the mixture;

Group 5 (n = 5) – Control, no intracanal medication;

Group 6 (n = 5) – Control, completely impermeable, with root canals filled as in group 1.

The pastes composed of $Ca(OH)_2$/saline and Calen/CHX were manipulated and then inserted into the canals by using a 3-ml BD plastic syringe (Benton, Dickinson and Company, Juiz de Fora, MG, Brazil) attached to a 1.20×40 needle. Calen and Calen/CMCP pastes were inserted into the root canals using an ML endodontic syringe attached to a Septoject XL needle (Septodont Brasil Ltda., Barueri, SP, Brazil). After that, the coronal access cavities were sealed with temporary restorative material (Coltosol ZincOxide Temporary cement, Vigodent, RJ, Brazil) and made impermeable following the described procedures.

Samples were immersed in lidded flasks (JProlab, São José dos Pinhais, PR, Brazil) containing 10 ml distilled water, which were sealed and kept at 37°C. After 1, 3, 7, 14, 21, 30, and 60 days, the pH of the water in each flask was measured with a Digimed DM-21 pH meter (Digicrom Analítica Ltda., São Paulo, Brazil), previously calibrated at 25°C using buffer solutions. Data were recorded, analyzed, and subjected to statistical analysis using ANOVA and Tukey's test at 5% significance level.

Results

All $Ca(OH)_2$-based pastes tested had alkalinizing action, increasing the pH in comparison with the control groups. Statistical differences were observed, starting at 3 days in group 4 (Calen/CHX) and from 7 up to 14 days in the other groups. Group 2 (Calen) showed significant increase up to 21 days (Fig. 1). $Ca(OH)_2$ mixed with saline (group 1) showed progressively higher pH values

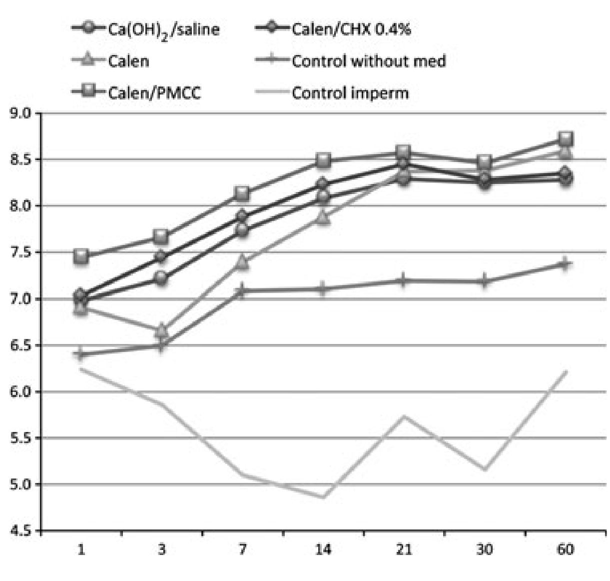

Fig. 1. Mean pH values of the groups in the different experimental periods.

Table 1. Comparison of the pH values of each medication in different experimental periods (means and standard deviations)

Days	$Ca(OH)_2$/saline	Calen	Calen/CPCM	Calen/0.4% CHX
1	6.98 (0.30)^{B,c}	6.91 (0.10)^{B,d}	7.44 (0.13)^{A,c}	7.03 (0.21)^{B,d}
3	7.20 (0.18)^{B,c}	6.66 (0.48)^{C,d}	7.66 (0.29)^{A,c}	7.44 (0.22)^{AB,c}
7	7.73 (0.18)^{AB,b}	7.39 (0.37)^{B,c}	8.13 (0.34)^{A,b}	7.88 (0.30)^{A,b}
14	8.08 (0.38)^{AB,ab}	7.88 (0.47)^{B,b}	8.48 (0.14)^{A,a}	8.23 (0.32)^{AB,ab}
21	8.29 (0.20)^{B,a}	8.37 (0.20)^{AB,a}	8.57 (0.12)^{A,a}	8.45 (0.22)^{AB,a}
30	8.25 (0.20)^{A,a}	8.38 (0.29)^{A,a}	8.46 (0.21)^{A,a}	8.28 (0.26)^{A,a}
60	8.28 (0.39)^{B,a}	8.59 (0.20)^{AB,a}	8.72 (0.16)^{A,a}	8.35 (0.32)^{B,a}

Equal letters denote lack of statistically significant difference. Uppercase letters indicate comparison between groups, and lowercase letters indicate comparison between periods.

up to 14 days and stable values at 21, 30, and 60 days (Fig. 1).

Group 3 (Calen/CMCP) presented the highest pH throughout the experimental period. However, at different periods, these specimens presented similar pH values to other groups, as shown in Table 1. At 60 days, the greatest pH values were observed for Calen/CPCM and Calen, followed by Calen/0.4% CHX (Table 1).

Discussion

Elevation in the pH induced by release and diffusion of hydroxyl ions from $Ca(OH)_2$-based pastes is essential for their performance as intracanal dressings (13, 26). Haapasalo et al. (27) demonstrated that the pH increase promoted by $Ca(OH)_2$ can become difficult because of the buffering capacity of the dentin. In the present study, all evaluated $Ca(OH)_2$-based pastes presented alkalinizing action, demonstrated by pH increase in the external solution, similarly to previous studies (25, 28–31).

For Pacios et al. (31), vehicles and other substances added to $Ca(OH)_2$ may affect its ability to release ions. In this study, significant pH increase was observed from 3 up to 14 days, except for group 2 (Calen), which showed significant increase up to 21 days. The association $Ca(OH)_2$/saline (group 1) showed progressive increase in pH values up to 14 days, but at 21, 30, and 60 days, the pH was similar. This may be explained by reduction in the amount of OH^- ions available, because of the fast ionic dissociation that occurs with use of aqueous vehicle (25).

Group 3 (Calen/CMCP) maintained the highest pH throughout the entire experimental period, despite its pH values being similar to other groups in distinct periods. According to Camargo et al. (25), ion release from this paste is influenced by formation of calcium paramonochlorophenolate, which promotes gradual and continuous ionic release. Simon et al. (32) studied the effects of association of $Ca(OH)_2$ with four different vehicles on the pH and calcium ion release. These authors also reported higher pH values for the mixture containing CMCP. Calen/CMCP paste includes propylene glycol in its formulation, along with a small amount of CMCP to enhance the antimicrobial action of the dressing. Esberard et al. (30) compared the pH changes when $Ca(OH)_2$/CMCP and $Ca(OH)_2$/aqueous vehicle (Pulpdent) were used as intracanal dressings, observing favorable results for the mixture containing CMCP.

In the present study, the highest pH values at 60 days were observed for Calen/CMCP and Calen, followed by Calen/0.4% CHX. These observations may be related to the effect of using a viscous vehicle (polyethylene glycol 400), which promotes prolonged ionic release (30) and enhanced antibacterial action (2).

The rationale for associating $Ca(OH)_2$ with CHX is the achievement of synergistic antimicrobial effect (23, 24). The concentration 0.4% CHX in Calen paste was based on the study by Silva et al. (33), who showed that association of 0.4% chlorhexidine with a calcium hydroxide paste did not affect osteogenic cell cultures, allowing *in vitro* formation of mineralized nodules.

Few studies have focused on the effects of CHX on the pH of $Ca(OH)_2$. Basrani et al. (34) observed that the presence of CHX did not alter the pH of $Ca(OH)_2$, similarly to the findings of Souza Filho et al. (20), Yücel et al. (35), and Freire et al. (36). However, these studies only assessed the pH over a short period of time. In the present study, Calen/0.4% CHX promoted progressive increase in pH values starting at day 3, with higher pH values than Calen at 7 days, and no statistical difference in the subsequent periods.

This study was carried out using bovine teeth. These teeth have been used in several studies, showing similar properties between human and bovine teeth (37–39). Camargo et al. (25) demonstrated that this is a viable methodology for evaluation of pH changes through the dentin. Schmalz et al. (40) analyzed the permeability of human and bovine teeth *in vitro* and verified that both tissues have similar characteristics.

Considering the evaluation periods, all evaluated $Ca(OH)_2$-based pastes were able to maintain ionic dissociation. The different formulations of $Ca(OH)_2$-based pastes promoted diffusion of OH^- ions throughout the root dentin. It was possible to conclude that CPCM and CHX do not interfere with the ability of $Ca(OH)_2$ to release and diffuse hydroxyl ions throughout dentin.

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