DETERMINATION OF pH AND CALCIUM ION RELEASE PROVIDED BY DIFFERENT CALCIUM HYDROXIDE PASTES

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ABSTRACT

Background and Aim: To evaluate the pH and calcium ion (Ca\(^{2+}\)) release of calcium hydroxide (Ca(OH)\(_2\)) powder mixed with 3 different vehicles and one Ca(OH)\(_2\)-based commercial paste.

Materials and Methods: Forty-five polyethylene tubes with simulated root canals were used and divided into four groups (n=10); Group 1: Ca(OH)\(_2\) based-paste (SURE-Paste), Group 2: Ca(OH)\(_2\) powder mixed with articainHCl+epinephrine (Maxicaine 2ml), Group 3: Ca(OH)\(_2\) powder mixed with clindamycin phosphate (Clinda 600 mg), Group 4: Ca(OH)\(_2\) powder mixed with metranidazole (Flagyl 0.5mg/100 ml). The forty tubes with pastes and five empty tubes (negative control) sealed one of the ends were then immersed in 10 ml deionized water. After 1 and 24 hours, 7 and 30 days, the Ca\(^{2+}\) level and pH of groups was measured. Data were analyzed statistically by two-way ANOVA.

Results: pH values and released amount of Ca\(^{2+}\) were gradually increased for groups over time, except negative control group. Regarding the pH and released Ca\(^{2+}\), there were significant differences among experimental groups (p<0.05). In all periods, higher Ca\(^{2+}\) release was observed in Group 2. On day 7, Group 4 had the lowest pH value, while Group 1 had the highest. On day 30, Group 2 showed the highest pH value and Ca\(^{2+}\) release.

Conclusions: The pH values of the experimental groups were rather alkaline, nevertheless additional microbiologic culture studies can be done for more conclusive results. Antibiotic solutions can be used as vehicles to be mixed with Ca(OH)\(_2\) powder in terms of pH and Ca\(^{2+}\) release.

Key words: Antibiotics, Calcium Hydroxide, Hydrogen-Ion Concentration, Local Anesthetics

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INTRODUCTION

Ca(OH)₂ has been recommended for use as intracanal medication based on its antimicrobial, antiresorptive and tissue-dissolving properties.¹⁻⁴ It has low solubility in water, an inherently high pH (12.5–12.8), and is insoluble in alcohol. When used as intracanal medication, Ca(OH)₂ has been shown to be effective in eliminating bacteria from the root canal space. Its high pH has a destructive effect on bacterial cell membranes and protein structures.³ The mechanism of action of Ca(OH)₂ is attributed directly to its ability of dissociating into calcium and hydroxyl ions resulting in increased pH locally.³ Many substances have been added to the calcium hydroxide powder to improve properties such as the antibacterial action, radiopacity, flow and consistency.⁶ Ca(OH)₂ kills bacteria through the effects of the hydroxyl ions; its efficacy depends largely on the availability of these ions in the solution, which, in turn, is dependent on the vehicle in which the calcium hydroxide is carried.⁷ The medicament vehicle plays a very important role in the overall disinfection process because it determines the velocity of ionic dissociation, causing the paste to be solubilized and resorbed at various rates by the periapical tissues and from within the root canal.⁸⁻¹⁰ Ca(OH)₂ has been shown to be inefficient in eradicating of both facultative anaerobes and yeasts, whilst other medications or irrigating solutions have been shown to be more effective on these microbiota in vitro.¹¹,¹² Renewed interest has been generated regarding the association of calcium hydroxide with other antimicrobial substances, such as camphorated paramonochlorophenol (CMCP), chlorhexidine (CHX), iodine potassium iodide (IPI), and antibiotics.¹³,¹⁴ Clindamycin is effective against many of the usual endodontic pathogens, particularly black-pigmented Prevotella and Porphyromonas species.¹⁵ Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Known for its strong antibacterial action against anaerobic cocci as well as Gram-negative and Gram-positive bacilli, it has been used both systemically and topically in the treatment of periodontal disease.¹⁶ To date, there is no study comparing the sustained release of Ca²⁺ from Ca(OH)₂ mixed with clindamycin or metranidazole in the endodontic literature. The aim of this study was to determine the sustained release of Ca²⁺ from Ca(OH)₂ in different vehicles like anesthetic solution, clindamycin and metranidazole. Also, this study measured the pH change in the environment over a period of 30 days.

MATERIALS AND METHODS

Preparation and Use of the Pastes

Forty-five polyethylene tubes having a length of 12 mm and a standardized foramen diameter of 0.4 mm were used in this study. Five empty polyethylene tubes were assigned as negative control and only sealed with 2 mm layer of Cavit (3M, ESPE, St. Paul, USA). The empty weight of the tube was measured as 0.05 gr with an analytical scale (Mettler Toledo, AB204-S, Switzerland). The Ca(OH)₂ combinations, except Group 1 water-soluble commercial premixed Ca(OH)₂ based-paste (Sure-Paste, Sure-Endo, Seoul, Korea. Excipients: Ca(OH)₂ barium sulfate, glycol derivatives, thickening agent, purified water), were prepared by adding Ca(OH)₂ powder (Merck, Darmstadt, Germany) into the following vehicles: Group 2: articain hydrochloric acid plus epinephrine (Maxicaïne fort 80 mg articain HCl+ 0.010 mg epinephrine/2 ml VEM Chemical, Ankara, Turkey), Group 3: clindamycin phosphate (Clindan 600 mg Bilim Pharmaceuticals, Istanbul, Turkey) and Group 4: metranidazole (excipients: disodium phosphate, sodium chloride, citric acid monohydrate and distilled water, Flagyl 0.5mg/100 ml Eczacibasi Baxter, Istanbul, Turkey). The weight of polyethylene tubes filled up to 10 mm with commercial premixed calcium hydroxide paste, which had the densest viscosity, were 0.11gr. In other groups, 3.2 mg Ca(OH)₂ powder, measured with an analytical scale, added to 2 mL of the vehicle until the solution formed a paste.¹⁷ Ten samples were prepared for each group. Each sample was weighed by using analytical scale in order to eliminate the differences as a result of the sample mass. Weights of samples were standardized as 0.11 gr ± 0.01 (Figure 1). The Ca(OH)₂ pastes were placed in the tubes using a Lentulo. The wider ends of all tubes were sealed with 2 mm layer of Cavit. The tubes were placed in individual vials containing 10 mL of distilled water (pH=7.2) and stored at 37 °C during experimental period.

Analyses of pH and Calcium Ion Release

The change in pH of all the different formulations was determined by dipping the electrode into the distilled water using pH meter, stirring the solution for 5 seconds to ensure uniform distribution of the hydroxyl ions, and then recording the pH at different time intervals up to 30 days (1 hour, 1 day, 1 week, and 1 month). The pH meter was calibrated with known standard pH solutions of 4, 7 and 10. Care was taken to see that the electrode was fully dipped in the medium before taking the readings. Between each reading, the
Electrode was cleaned with distilled water and dried with sterile tissue paper to ensure that no Ca(OH)$_2$ particles were left behind, because this could potentially interfere with the next reading. The Ca$^{2+}$ level of each group was determined by flame photometry (Jenway PFP7, Milano, Italy). At each period 0.1 ml sample was taken for analysis.

**Statistical Analysis**

The Ca$^{2+}$ level and pH values were expressed as the arithmetic mean ± standard deviation. Data were analyzed statistically by two way ANOVA with repeated measures on one factor. Interaction between Ca(OH)$_2$ pastes and periods was significant. The periods within the groups were compared separately by using Bonferroni test ($p=0.05$).

**RESULTS**

Table 1 shows the means and standard deviations for pH and Ca$^{2+}$ release (ppm) of the pastes in the different periods. According to statistical analysis, differences among the pH and Ca$^{2+}$ values of all groups were found to be statistically significant ($p<0.05$). The pH values and Ca$^{2+}$ release of all groups were gradually increased over time, except negative control group. After 30 days, all four experimental groups showed a significant increase in pH of the surrounding medium compared with the baseline values ($p<0.05$). After the first hour; Group 4 showed the highest pH level (5.59) and Group 2 reached the highest Ca$^{2+}$ level (20.15 ppm). Group 2 showed the highest pH value (10.54) and Ca$^{2+}$ release (200.39 ppm) after 30 days. Also this group showed the greatest pH variation ranging from 5.52 to 10.54.

In negative control group pH change and Ca$^{2+}$ release was not observed. This result displayed that the only source of Ca$^{2+}$ ions was pastes.

**DISCUSSION**

It has been shown that the action of inflammatory and clastic cells is enhanced by an acidic pH, leading to disintegration and subsequent resorption of the hard tissues.$^{18}$ Hence, placing a formulation of Ca(OH)$_2$ with an appropriate vehicle, which can sustain high alkaline pH in the periapical area to inhibit the action of these inflammatory and clastic cells, can stop the resorption process. However, persistent infection might occur as a result of the presence of microorganisms that are refractory to the endodontic procedures.$^{19}$ For this reason, the association of Ca(OH)$_2$ with other antiseptic agents has been proposed to improve the action against the microorganisms.$^{15,20}$ When Ca(OH)$_2$ is used, calcium acts clearing the carbon dioxide used by bacteria for anaerobic respiration.$^{21}$ Another important property of Ca(OH)$_2$ is its ability to promote inactivation of bacterial lipopolysaccharides found in the outer membrane of gram-negative bacteria.$^{22-24}$ Previous studies testing antibiotics based root canal medicaments, such as clindamycin/ethylene vinyl acetate (EVA) fibres and a sustained release delivery gutta-percha point containing metronidazole (SRDGM), showed that these intracanal dressings inhibit the growth of bacteria.$^{20}$ Nevertheless, none of the previous studies combined the Ca(OH)$_2$ with this form (im/iv) of antibiotics. Therefore in the present study im/iv forms of antibiotics were used as vehicles to evaluate the effects of antibiotics on pH level as well as Ca$^{2+}$ release of pastes.

Nerwich et al.$^{25}$ measured pH changes in root dentin over a 4-week period and considered this as a reasonable time interval to expect effective therapeutic benefits from calcium hydroxide based materials. Gomes et al.$^{26}$ showed that the concentration of calcium ions peaked and stabilized at 2–3 weeks after packing root canals with Ca(OH)$_2$. Hence, in this study, we evaluated the Ca$^{2+}$ release and pH levels up to 30 days. The results of this study showed that, all the vehicles tested showed sustained release of the Ca$^{2+}$ up to 30 days. Regarding Ca$^{2+}$ release, Group 2 presented the highest values in the studied period, with significant differences compared with other groups; while at the first measurement Group 4 and at the last measurement Group 1 presented lowest value. Yucel et al.$^{27}$ verified extremely high pH readings for the association of Ca(OH)$_2$ powder with different substances and anesthetics, with the highest readings occurring after 24 hours. It should be mentioned that most studies found pH values between 11 and 12.
higher than the readings observed in the present study.\textsuperscript{27,28} This might be due to the fact that in these studies, the pH was measured either directly in the powder-vehicle mixture or in distilled water after direct placement of the paste in it. In the present study, the pastes were used to fill artificial root canals with apical foramens measuring 400 μm in diameter (equivalent to a size 40 K-file), and the coronal access was sealed before immersion in water. This allowed slower dissolution of the paste in water, and the pH was measured in the water in which the tube had been stored in an attempt to mimic the clinical situation more closely. There were no correlations between the pH of the pure vehicles (maxicaine 2.96, clindamycin 3.42 and metronidazole 4.56) and those mixed with Ca(OH)\textsubscript{2} for an extended period of time. This may be related to the Ca(OH)\textsubscript{2} powder being added to vehicles until the solutions formed a paste. Interestingly, the pH values of Group 2 were the highest when compared with the other groups. This might be due to aqueous property of maxicaine that promotes a high degree of solubility. In the present study, all the formulations showed a high alkaline pH (>10) at the end of 1 month. At these pH levels, most of the bacteria cannot grow.\textsuperscript{25} However, Byström et al.\textsuperscript{2} reported that Enterococcus faecalis (E faecalis) was a resistant bacterium in root canals, surviving at pH 11.5, but being killed at pH 12.5. So, a high pH value is desired for a long time period to achieve antibacterial effect. Evans et al.\textsuperscript{29} reported that E faecalis was resistant to Ca(OH)\textsubscript{2} at a pH of 11.1, but not pH 11.5. After 30 minutes exposure in Ca(OH)\textsubscript{2} pH 11.1, some cells (0.4%) survived; however, at pH 11.5, greater than 99.99% were killed. In light of these findings, we can assume that the pH values of the experimental groups were not strongly alkaline enough to eliminate E faecalis from the root canal in a week, even in a month. Anesthetic solutions with or without vasoconstrictors, have been used as a vehicle of Ca(OH)\textsubscript{2} paste because these solutions are readily available, sterile and easy to handle. Most of these solutions have an acidic pH. However, the final paste has a high pH that is maintained over time and promotes rapid ionic release.\textsuperscript{6} In the present study, Group 2 in which anesthetic solution was used showed the highest Ca\textsuperscript{2+} release at all times, and also the highest pH at the end of the study (p<0.05). Therefore anesthetic solutions can be recommended as vehicles to be mixed with Ca(OH)\textsubscript{2}. Namely, all pastes behaved differently in terms of pH and calcium ion release in the studied periods.

**CONCLUSION**

Within the limits of this study the pH values of the experimental groups were rather alkaline, nevertheless additional microbiologic culture studies can be done for more conclusive results. Antibiotic solutions can be used as an alternative vehicles to be mixed with Ca(OH)\textsubscript{2} powder also.

**REFERENCES**


<table>
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<th>Period</th>
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<th>Sure-paste</th>
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