EFFECT OF CALCIUM HYDROXIDE PASTES ON UNINSTRUMENTED CANAL WALL STUDIED WITH SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Calcium hydroxide ($Ca(OH)_2$) paste has been used as a root canal dressing for long time and promotes healing of vital pulp and periapical tissues. The aim of this study was to evaluate the dissolving effect of calcium hydroxide paste mixed with different vehicles on uninstrumented canal walls using scanning electron microscopy (SEM). Twenty one recently extracted single-root teeth were used. The crowns were removed and the root sections were divided longitudinally into two halves. The pulp tissue was removed and the specimens were randomly divided into 7 groups. The control group was immediately fixed without any treatment. The canals in the other groups were filled with the different calcium hydroxide pastes: group 1(n=6), with saline solution; group 2 (n=6) with propylene glycol; group 3 (n=6) with propylene glycol and camphorated p-monochlorophenol; group 4 (n=6) with sodium hypochlorite; group 5 (n=6) with chorhexidine 1%, group 6 (n=6) with iodine potassium iodide 0.1/0.2%. The specimens were kept in an incubator at 100% humidity and 37ºC, removed after 14 days, and washed ultrasonically for 10 min. Then they were fixed with glutaraldehyde and examined with scanning electron microscopy The percentage of organic remains and paste were evaluated and scored. The data were analyzed using the Kruskal- Wallis test. In groups 1, 5 and 2 there were more cavities without organic remains than in the other groups ($p<0.05$). However, there was no difference among them ($p>0.05$). The paste in group 3 cleaned only 5%. In groups 4, 6 and 7 (control) the canals walls were wholly covered with fiber, cells, and residual paste, the percentage cleaned was 0.

This study shows that $Ca(OH)_2$ pastes with saline solution, with chlorhexidine and with propylene glycol have a greater dissolving effect on the canal walls than the other pastes evaluated.

Key words: calcium hydroxide, chlorhexidine.

EFECTO DE PASTAS DE HIDRÓXIDO DE CALCIO SOBRE PAREDES DENTINARIAS SIN INSTRUMENTAR EVALUADAS CON MICROSCOPIO ELECTRÓNICO DE BARRIDO

RESUMEN

La pasta de hidróxido de calcio ($Ca\ (OH)_2$) es utilizada como medicación intermedia, por sus propiedades biológicas y antibacterianas disminuyendo clínicamente los síntomas entre sesiones. El objetivo de este trabajo fue evaluar la acción de disolución sobre el tejido pulpar de pastas de $Ca(OH)_2$ en paredes dentinarias radiculares sin instrumentar, mediante Microscopio Electrónico de Barrido (MEB). Se utilizaron 21 dientes humanos unirradiculares recientemente extraídos, a los cuales se les eliminó la corona y se cortaron longitudinalmente en mitades. La pulpa se extrajo con pinza de algodón. Luego fueron divididas al azar en 7 grupos, al grupo control, no se le realizó ningún tratamiento; a los restantes grupos se les colocó las distintas pastas de $Ca(OH)_2$; con solución fisiológica (n=6), con propilenglicol (n=6), con propilenglicol + paramonoclorofenol alcanforado (n=6), con hipoclorito de sodio (NaOCl) 1% (n=6), con gluconato de chorhexidina (CHX) 1%(n=6) y con solución iodada de ioduro de potasio (IKI) 0,1% / 0,2% (n=6). Las piezas se mantuvieron a 37ºC en condiciones de 100% de humedad durante 14 días. Luego se lavaron con ultrasonido durante 10 minutos y se fijaron con glutaraldehído para ser observadas al microscopio electrónico de barrido (MEB). El porcentaje de restos orgánicos y pasta fueron evaluados mediante un score. Los datos se analizaron con el test de Kruskal- Wallis. Los grupos 1, 5 y 2 presentaron mayores porcentajes de limpieza con respecto a las demás pastas utilizadas ($p<0.05$), sin encontrarse diferencias significativas entre ellas ($p>0.05$). La pasta 3 mostró un porcentaje de un 5% de limpieza. En las pastas 4, 6 y el control, el porcentaje de disolución de restos pulpares fue 0.

Este trabajo mostró que las pastas de $Ca(OH)_2$ con solución fisiológica, con CHX y propilenglicol fueron mas efectivas con respecto a la acción de disolución sobre las células odontoblásticas de las paredes dentinarias.

Palabras clave: hidróxido de calcio, clorhexidina.

INTRODUCTION

Instrumentation and irrigation solutions significantly reduce the number of germs in the root canal[1,2]. However, it has been shown that it is difficult to achieve total disinfection due to the complex morphology of the root canal system[3,4], particularly in molars, in which the presence of anastomosis, isthmuses and deltas that residual bacteria can colonize, reaching the depth of the dentine, periapical tissues and outer surface of the root, act as a selective habitat (bacterial biofilm), enabling the development or persistence of apical periodontitis[5-7]. The use of $Ca(OH)_2$ as a topical medication has been known for a long time, and provides very good results in infected canals[8] because it diffuses through dentine walls towards the periodontal tissue, changing the pH of the dentin[9].

Nevertheless, $Ca(OH)_2$ alone proved to be inefficient for destroying these bacteria[1]. The selection and use of alternative disinfectants is difficult because their mode of action, activity and inhibition within the root canal are unknown. Other irrigation solutions or medications proved to be more effective for acting on this microbiota *in vivo* [10], suggesting that calcium hydroxide powder could be mixed with different substances such as propylene glycol, camphorated paramonochlorophenol[11] or irrigation solutions such as chlorhexidine gluconate (CHX), sodium hypochlorite (NaOCl) or iodine potassium iodide (IKI), to achieve a wider antibacterial spectrum and a more long-lasting effect[12-14]. The dissolving action of $Ca(OH)_2$ paste was described by Hasselgren et al. in 1988[15]. They found that treatment with $Ca(OH)_2$ dissolved necrotic porcine muscle tissue, thus, when used as a topical medication it would reinforce the solvent effect of NaOCl.

The aim of this study was to evaluate the dissolving action of $Ca(OH)_2$ pastes with different vehicles on the line of odontoblasts and predentin of non-instrumented root canal walls, using Scanning Electro Microscopy (SEM).

MATERIALS AND METHODS

We used 21 anterior human teeth, which were kept in saline solution until they were used. The crowns were removed and the root sections were cut longitudinally into two halves: mesial and distal. The pulp tissue was removed using cotton tweezers. The 42 specimens were washed with ultrasound in distilled water for 10 minutes and then randomly divided into 7 groups – 6 treated with calcium hydroxide pastes and one control group.

The following calcium hydroxide pastes were used:

Group 1 - $Ca(OH)_2$ + saline solution
Group 2 - $Ca(OH)_2$ + propylene glycol
Group 3 - $Ca(OH)_2$ + propylene glycol + camphorated p-monochlorophenol
Group 4 - $Ca(OH)_2$ + NaOCl 1%
Group 5 - $Ca(OH)_2$ + CHX 1%
Group 6 - $Ca(OH)_2$ + IKI 0.1/0.2%
Group 7 - Control, no treatment

The pastes were prepared in a proportion of (1: 1.5 W/V). The 1% chlorhexidine solution (pH 6.5) was prepared just before using from a concentrated 20% solution and distilled water. The IKI solution (pH 6.7) was prepared with 0.1% iodine, 0.2% potassium iodide and 99.7% distilled water. The 1% NaOCl solution (pH 11.9) was prepared from a concentrated 5% solution diluted in distilled water, and the saline solution (pH 7.6) was of the usual type for medical use, purchased on the market.

The specimens were kept at 100% humidity and 37ºC for 14 days, then washed ultrasonically with distilled water for 10 minutes and fixed in glutaraldehyde to be mounted on aluminum cylinders 3 cm in diameter and gold- coated. They were observed using SEM (Fine Coat ION SPUTTER. JFC-1100, Jeol) at 20X magnification to select an observation area; a line was drawn on the major root axis in the middle of the canal, and a perpendicular line was drawn at 2 mm from the apex. Photomicrographs were taken at 780X of the dentin wall of the canal and observed by two evaluators who determined the amount of pulp remnants, presence or absence of predentin and amount of residual $Ca(OH)_2$. They were scored on a scale of 0 to 3[16], with zero (0) assigned to walls free of remnants of open dentinal tubules (100% clean), one (1) to 50% clean, two (2) to 25% clean and three (3) to 0% open tubules.

The data were statistically analyzed using the Kruskal-Wallis test.

RESULTS

Table 1 shows the percentage of dissolution of pulp remnants produced by the different pastes at 14 days. The $Ca(OH)_2$ pastes whose vehicles were distilled water, chlorhexidine and propylene glycol (groups 1, 5 and 2) produced significantly higher percentages of cleanliness than the other pastes

Table 1: Dissolution percentage of pulp remnants 14 days after applying the different pastes.

Pastes	Mean	Median	St. Dev,
1	82%	80%	3%
2	67%	70%	6%
3	5%	5%	5%
4	0%	0%	0%
5	77%	80%	6%
6	0%	0%	0%
7	0%	0%	0%

(1) $Ca(OH)_2$ with saline solution,
(2) $Ca(OH)_2$+ propylene glycol,
(3) $Ca(OH)_2$+ propylene glycol+ camphorated p-monochloroohenol,
(4) $Ca(OH)_2$ + NaOCl 1%,
(5) $Ca(OH)_2$ + CHX 1%,
(6) $Ca(OH)_2$ + IKI 0.1%/0.2% , (7) control.

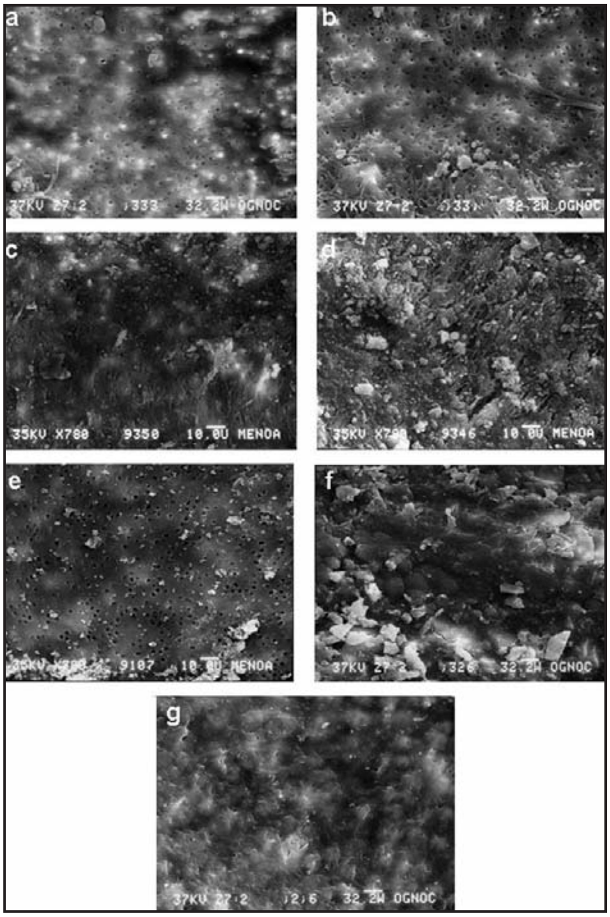

Fig. 1: SEM of dentinal walls (apical third), 14 days after applying the different pastes. Bar: 10 microns.
a) $Ca(OH)_2$ with saline solution, b) $Ca(OH)_2$ + propylene glycol, c) $Ca(OH)_2$ + propylene glycol + camphorated p-monochlorophenol, d) $Ca(OH)_2$ + NaOCl 1%, e) $Ca(OH)_2$ + CHX 1%, f) $Ca(OH)_2$+ IKI 0.1%/0.2%, g) untreated dentinal wall (control).

($p<0.05$), with no significant difference among them ($p>0.05$), (Fig. 1, a , e y b).

The $Ca(OH)_2$ paste with propylene glycol and paramonochlorophenol (group 3) achieved 5% cleanliness (Fig. 1, c). For the pastes whose vehicles were sodium hypochlorite and iodine potassium iodide, and the control (groups 4, 6 and 7) the percentage of dissolution of pulp remnants was 0 (Fig. 1, d, f y g).

DISCUSSION

$Ca(OH)_2$ has many properties in addition to its antibacterial effect. One of them is its ability to dissolve organic tissue[15]. Different authors suggest combining $Ca(OH)_2$ with other irrigation solutions in order to widen the antimicrobial spectrum and achieve a more long lasting effect. To do so, they propose the use of sodium hypochlorite and chlorhexidine solutions in combination with the calcium hydroxide powder[12,13,17,18]. However, these combinations have not been fully studied. The vehicle plays an important role because it determines the speed at which ions dissociate, causing paste reabsorption in periapical tissues and within the root canal[19].

According to different authors, calcium hydroxide paste can be used at different consistencies. Maisto[20] reports that the right consistency can be achieved by eliminating the necessary amount of water or adding $Ca(OH)_2$ powder[19]. Haenni uses a creamy consistency (1: 1,5. W/V)[13], while Zerella reports a "smooth slurry" consistency (0.5 g powder in 1 ml solution)[21].

In a previous study, we used $Ca(OH)_2$ powder with different vehicles *in vitro*, and evaluated its action on dentinal components. The pastes preserved their alkalinity for up to 30 days without altering dentinal structure[22].

Different studies have proved the cytotoxicity of camphorated p-monochlorophenol (CPMC), Soekanto et al.[23], and one of the requirements in the selection of a medication for intra-canal use should be biocompatibility. Results of experimental and clinical research have shown that the vehicle plays a part as an aid with the aim of increasing the biological properties of $Ca(OH)_2$ conferring upon it chemical characteristics such as dissociation, diffusion and filling capacity. Hydrosoluble vehicles favor these properties. This is not true of CPMC, because camphor makes it greasy, hindering ion diffusion within the root canal, Estrela et al.[24].

According to Andersen, the dissolving action of calcium hydroxide described by Hasselgreen[15] is neither as strong nor as immediate as that of sodium hypochlorite[25]. Considering these reports, it might be assumed that the combination of $Ca(OH)_2$ with sodium hypochlorite might increase the dissolving effect compared to using each one separately[26]. However, this study showed that when they are combined, the paste takes on a consistency that makes it difficult to eliminate, and it remains adhered to the dentinal walls with no effect visible on tissues. When paste with either hypochlorite or iodine potassium iodide was used, $Ca(OH)_2$ remained on the walls even after ultrasound washing.

Wadachi et al. claim that an intermediate medication with $Ca(OH)_2$ applied for 7 days plus irrigation with sodium hypochlorite for 30 seconds might produce optimum results[25].

In a similar study, Wakabayashi et al. conclude that conventional $Ca(OH)_2$ paste applied for 7 days can dissolve odontoblastic cells but not odontoblastic processes, and that applying the paste for 28 days increases its dissolving capacity[27].

After the chemical-mechanical preparation of the root canal, the amount of detritus is much lower than what we found in this study for the initial state of the teeth, as they had not been instrumented. Moreover, the dissolving capacity of $Ca(OH)_2$ is completed by the action of sodium hypochlorite used as an irrigation solution[15].

Calcium hydroxide and chlorhexidine paste proved to have satisfactory physicochemical properties when used as a topical medication[14]. When $Ca(OH)_2$ and chlorhexidine were mixed, it was found that the antimicrobial activity of chlorhexidine was inhibited by the high pH and buffer capacity of the suspension. The reduction of efficacy may be explained by the deprotonation of biguanide at pH values higher than 10. Ionization decreases and CHX precipitates, Haenni et al. 2003[13,14]. Chlorhexidine has antioxidant and pro-oxidant properties alone or combined with $Ca(OH)_2$ and at high concentrations it may be potentially genotoxic and damaging to periapical tissues, Yeung et al.[28]. Studies of chlorherxidine concentration are therefore ongoing, with the aim of reducing its cytotoxic action to a minimum, da Silva et al.[29].

The combination of calcium hydroxide and sodium hypochlorite has been shown to be stable at high pH values[13]. Such a mixture may have better properties for dissolving tissue than calcium hydroxide with conventional saline solution, shown by Hasselgren et al.[15]. Little is known regarding the mixture of these two alkalis. Because as they are both cations, when ions are exchanged, another strong alkali is produced: sodium hydroxide.

In a previous *in vitro* study on bovine pulp tissue, we showed that the dissolving capacity of calcium hydroxide pastes mixed with sodium hypochlorite or chlorhexidine was similar to that of calcium hydroxide paste with saline solution at 72 hours[30].

Conventional paste prepared with calcium hydroxide and distilled water and applied for 14 days, had greater dissolving capacity on odontoblastic cells of the dentinal walls, and was followed in efficacy by $Ca(OH)_2$ pastes with chlorhexidine and with propylene glycol. This was not true of the proposed pastes with propylene glycol + camphorated p-monochlorophenol, sodium hypochlorite and iodine potassium iodide solution, after which cell remnants and calcium hydroxide plaque were observed adhering to the walls.

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