Evaluation of calcium hydroxide dressing for short term prevention of coronal leakage

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ABSTRACT

Objective: The aim of this *in vivo* study was to evaluate the influence of coronal leakage on the apical dog's teeth healing, which were dressed with calcium hydroxide and kept or not in contact with oral environment. **Material and Methods:** After biomechanical preparation and filling with calcium hydroxide/saline paste, twenty six root canals were randomly divided into two experimental groups: Group 1 - coronally sealed with temporary restorative material; Group 2 - coronally unsealed. The animals were sacrificed after 7 days and the specimens were prepared for histological analysis. **Results:** In both groups the results were similar. Inflammatory cells were not present in the apical tissue or in the cementum. Besides, it was observed necrosis in the coronary third surface of the pulp stump and microorganisms were noted just in contact with debris, which were present in the specimens pulp chamber without sealing but not in the root canal. **Conclusion:** It was concluded that the calcium hydroxide used as dressing prevented the contamination of the root canal and keeps its mechanism in the apical tissues even under defective sealing in a period of at least 7 days.

Keywords: Coronal leakage. Calcium hydroxide. Dressing. Healing process.

Introduction

The use of intracanal medication has been advocated in the treatment of infected root canals. It may help to eliminate remaining viable bacteria unaffected by the chemomechanical preparation of the root canal[6,25] acting as a physicochemical barrier precluding the proliferation of residual microorganisms and also preventing the reinfection of the root canal by bacteria from the oral cavity.[1]

Instrumented root canals can be recontaminated between appointments in clinical situations by leakage through the temporary filling material, breakdown or loss of the temporary filling, or fracture of the temporary filling material and/or tooth structure. The root canal system then becomes exposed to oral microbiota, which jeopardizes the outcome of endodontic treatment. In these situations, intracanal medications that have antibacterial properties might be helpful in preventing bacterial invasion of the root canal system.[24]

Intracanal medications should have a broad antibacterial spectrum, no cytotoxicity, and should possess physiochemical properties that permit diffusion through the dentinal tubules and lateral ramifications of the root canal system.[3] However, whether interappointment temporary filling materials provide an adequate seal of the root canal system from contamination between sessions may still be questionable.[20]

Among the root canal dressings, calcium hydroxide ($Ca(OH)_2$) is considered to possess many properties of an ideal material[5] and has become popular because of its antimicrobial and biological properties.[9,10,15,17] The antimicrobial action of $Ca(OH)_2$ is related to its ionic dissociation in calcium and hydroxyl ions, and their toxic effects on bacteria which inhibits cytoplasmatic membrane enzymes with consequent changes in the organic components and nutrient transport.[10] Materials containing $Ca(OH)_2$ have been used to promote formation of hard tissue in apexification, perforations, fractures, resorptions.[5] $Ca(OH)_2$ is also related to the neutralization of lipopolysaccharides,[22] helping in the root canal cleansing.[14]

Some *in vitro* studies reported the time-dependent delay of coronal leakage with the use $Ca(OH)_2$ as dressing.[8,24] However, no *in vivo* study was found in the literature to demonstrate the ability of $Ca(OH)_2$ as dressing to prevent coronal bacterial leakage simulating a clinical situation where the inter-appointment restorative material had been displaced or fractured allowing a possible bacterial infiltration. So, the aim of the present study was to evaluate the effectiveness of $Ca(OH)_2$ dressing in the prevention of coronal leakage in unsealed dog's teeth.

Material and Methods

This study was conducted on 26 roots of premolar and incisor teeth from 1 adult mongrel dog aged 2-3 years old and weighing about 25 Kg. The use of animal for this research was in accordance to the guidelines approved by the Research Committee of São Paulo State University, Brazil, in compliance with the applicable ethical guidelines and regulations of the international guiding principles for biomedical research involving animals.

The animals were anaesthetized with 2 mL of a mixture of xylazine (Rompum; Bayer do Brasil S/A, São Paulo, Brazil) and ketamine hydrochloride (Ketalar; Park Davis-Aché Laboratórios Farmacêuticos S/A, São Paulo, Brazil), in a 1:1 ratio, administered intramuscularly and maintained with subsequent anesthetic injections. The animals were intubated with a cuffed endotracheal tube before beginning the experimental procedures.

After the placement of a rubber dam, the teeth were submitted to crown opening and pulp extirpation up to the apical barrier. The root canal was explored up to the apical level by using a 15 K-file (Dentsply Maillefer, Catanduva, Brazil), and removal of the root pulp was performed with a #20 Hedstrom file (Dentsply Maillefer, Catanduva, Brazil). Root canals remained exposed to the oral cavity for 7 days to achieve bacterial contamination. Due to the absence of a main apical foramen in dog's teeth but only an apical delta, an experimental model was employed. The root canals were biomechanical prepared up to a 40 K-file (Dentsply Maillefer, Catanduva, Brazil) at the level of the apical barreir, with abundant irrigation with 1.0% sodium hypochlorite (Biodinamica Química e Farmacêutica, Ibiporã, Brazil). The teeth were overinstrumented up to a #25 K-file (Dentsply Maillefer, Catanduva, Brazil) to obtain a cementum canal and a main foramen. After final irrigation with saline, the root canals were dried with sterile paper points and dressed with a calcium hydroxide P.A. in distilled water.[8,11]

After biomechanical preparation and filling with calcium hydroxide/saline paste, the teeth were randomly

divided into two experimental groups: Group 1 - coronally sealed with temporary restorative material (Coltosol, Vogodent, Rio de Janerio, RJ, Brasil) (n=13); Group 2 - coronally unsealed (n=13).

Seven days after root canal treatment, the animals were sacrificed by an intramuscular anesthetic overdose. The specimens were fixed in 10% neutral-buffered formalin solution and decalcified in formic acid-sodium citrate. Segments of the jaws, each containing one root, were prepared for histological examination. The specimens were embedded in paraffin, serially sectioned to an average thickness of 6 μm and stained with hematoxylin and eosin (H&E) and Brown and Brenn staining techniques. Severity and extent of inflammation, as well as predominant inflammatory cell type in the periapical tissues, were recorded. Data were submitted to statistical analysis by Kruskal Wallis and Dunn tests. Significance level was set at 5%.

Results

The Brown and Brenn staining evidenced large amount of bacteria only in the pulp chamber of Group 2 formed basically from the scarps of the regular diet, which were not found in Group 1 (Fig 1A). Both experimental groups presented similar results in relation to pulp stump and periapical tissues. It was observed vitality of the middle and apical third of the pulp stump, but the coronal portion which was in close contact with $Ca(OH)_2$ dressing, was necrotic with an usual observation of basophilic line separating the material from a mineralized tissue (Fig 1B and C). The vital portions of the pulp stumps were in continuation with a periodontal ligament with no inflammatory reaction and normal thickness with no statistically significant difference (p>0.05) (Fig 2 and Table 1). It was also possible to note that periodontal fibers were inserted into the cementum and adjacent bone tissue (Fig 1D).

Figure 1. Group 2 **A**) Debris in the pulp chamber with Gram-positive microrganisms (Brown and Brenn, x200). **B**) Note basophilic line (arrow) delimiting the necrotic upper portion of the pulp stump (hematoxilin-eosin, x200). **C**) Cementum-Dentin limit (CDL). Note vital pulp stum (hematoxilin-eosin, x100). **D**. Panoramic view showing organized periodontal ligament without inflammatory cells and periodontal fibers inserting in the cementum and bone (hematoxilin-eosin, x100).

Table 1. Frequence of histopatologic findings in each group.

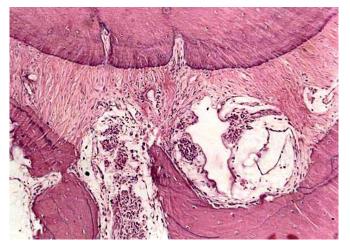| Event	Group 1	Group 2
Cementum resorption		
Active	0	0
Inactive	0	0
Mineralized tissue		
Present	13	13
Absent	0	0
Periodontal ligament		
Thin	13	13
Thick	0	0
Periodontal ligament organized		
Present	13	13
Absent	0	0
Ankylosis		
Present	0	0
Absent	13	13
Dentinal resorption		
Active	0	0
Inactive	0	0
Bone resorption		
Active	0	0
Inactive	0	0
Inflammatory infiltrate		
Absent	13	13
Slight	0	0
Moderate	0	0
Severe	0	0
Bacteria		
Present	0	0
Absent	13	13

*Statistically significant.

Figure 2. Group 1. Organized periodontal ligament without inflammatory cells (hematoxilin-eosin, x100).

Discussion

Intracanal medications may prevent saliva bacteria penetration in the root canal in two ways: Chemically and/or physically.[24] The contamination of the root canal system occurs when the number of bacteria cells exceeds the antibacterial medication activity. Moreover, medications that fulfill the root canal act as a physical barrier against bacteria penetration. The canal contamination will only occur with the solubilization by saliva, the medication permeability to saliva, or percolation of saliva in the interface between the medication and the root canal walls. However, in any case, if the medication has antibacterial effects, neutralization may occur

during the bacteria invasion.[26] Due to the alkaline pH, calcium hydroxide inhibits the bacterial enzyme activity and cellular membrane permeability[8] resulting in a direct and indirect antimicrobial effect over different microorganisms,[10,11] as well as hydrolysis of lipopolysaccharides.[22,23]

Calcium hydroxide paste acts as physicochemical barriers against infection with a marked pH-dependent antibacterial activity. The antimicrobial action of $Ca(OH)_2$ is related to its ionic dissociation in calcium and hydroxyl ions and their toxic effects on bacteria inhibiting cytoplasmatic membrane enzymes with consequent changes in the organic components and nutrient transport.[10] Saliva has buffer activity provided by proteins, phosphate and bicarbonate buffer system.[24] Thus, when exposed to saliva, it is likely that the calcium hydroxide chemical effect should be neutralized by its buffer ability. However, in the present study, bacteria was not seen in the root canal walls and the periodontal tissue surrounding the root was histologically normal, from which it can be inferred that calcium hydroxide/saline paste was able to prevent the contamination of the root canal system during a period of 7 days. These findings can be attributed to both, the chemical and physical characteristics of calcium hydroxide paste. The pH changes in the radicular dentin occur when calcium hydroxide is used and needs from 1 to 7 days to reach the external dentin.[21]

It was not possible to find in the literature *in vivo* results to be compared with the present ones. However, *in vitro* results have demonstrated that the root canals were completely contaminated after 19-day exposure to Staphylococcus epidermidis or after 42-day exposure to Proteus vulgaris.[26] Canals filled with calcium hydroxide/saline showed entire recontamination with an average of 14.7 and 16.5 days.[24] Recontamination was detected after an average time of 1.8 days in the unsealed canals medicated with calcium hydroxide paste.[8] These results evidenced differences in the time required for contamination or recontamination of the canals medicated with calcium hydroxide, mainly in detriment of the methodologies employed. The present study results showed that even in a critical situation, when the effectiveness of the restorative material is not present, the contamination did not occur for at least 7 days with the use of

calcium hydroxide/saline paste as dressing.

Calcium hydroxide itself is a white odorless powder with a molecular weight of 74.08. It has a low solubility in water and a high pH (12.5–12.8).[9] When the powder is mixed with a suitable vehicle, a paste is formed. Three types of vehicle have been used: Aqueous, viscous or oily,[4] being the selection of the appropriate vehicle dependent on the clinical situation. If rapid ionic liberation at the beginning of treatment is required, an aqueous vehicle is indicated; whilst a viscous vehicle is appropriate when a more gradual and uniform release is necessary. Oily vehicle pastes have limited application. Another form to use calcium hydroxide is in points which are relatively recent and designed to release calcium hydroxide from a gutta-percha matrix. However, the rise in pH of root dentine at apical and cervical sites was significantly greater in teeth dressed with a aqueous calcium hydroxide paste material compared with teeth dressed with calcium hydroxide points.[4] In the present study, calcium hydroxide was used in a paste form from the mixing of calcium hydroxide powder with distilled water to allow a rapid ionic releasing, which can partly explain the results.

Another interesting point to be discussed is the biological property which is related to the periapical healing found in the present study. Calcium oxide may react with water or tissue fluids forming calcium hydroxide, which in contact with water dissociate in calcium ions and hydroxyl ions. The calcium ions react with the carbon dioxide in the tissues and form calcium carbonate granulations presented as calcite crystals birefringent to polarized light, which stimulates hard tissue deposition,[16] which aids its clinical use.[2,12,13,18,30] The diffusion of hydroxyl ions from the root canal raises the pH at the surface of root adjacent to the periodontal tissues, thereby possibly interfering with osteoclastic activity, and promotes an alkalinization in the adjacent tissues favoring the healing process.[29] Calcium ions are important due to their participation in the activation of calcium-dependant adenosine triphosphatase.[25] Calcium reacts with carbonic gas to form calcium carbonate crystals (birefringent to polarized light), which serve as a nucleus for calcification, and favors mineralization.[25] A rich extra-cellular network of fibronectin in close contact with these crystals strongly support the role

of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue.[25] Calcium is also needed for cell migration and differentiation.[24] This biological action can help to explain the vitality of the pulp stumps in continuation with a periodontal ligament with no inflammatory reaction and normal thickness, which is in accordance to other studies.[17,18,19]

Conclusion

Due to the present study results, it was possible to observe that calcium hydroxide/saline paste as dressing can promote an effective barrier against microbial invasion into the root canal system of dog's teeth in a period of at least 7 days, even if the coronal restoration fails to help the healing process of the periapical tissues.

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