Braz Dent J (2002) 13(1): 17-22 ISSN 0103-6440

Calcium Hydroxide Root Canal Dressing. Histopathological Evaluation of Periapical Repair at Different Time Periods

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The objective of this study was to evaluate periapical and apical repair using calcium hydroxide root canal dressings for different lengths of times in teeth with induced chronic periapical lesions. A total of 61 root canals of maxillary and mandibular premolars from 4 dogs were used. After mechanical preparation of the root canals using the crown-down technique, and 5.25% NaOCl as irrigating solution, the apical foramen was enlarged in all cases. A calcium hydroxide root canal dressing was applied. The control group did not receive a root canal dressing. The animals were killed at 7, 15 or 30 days. After histological preparation, serial sections were stained with hematoxylin-eosin and Mallory's trichrome. The best histopathological results occurred at 15 and 30 days, and the worst results occurred at 7 days and in the control group.

Key Words: calcium hydroxide, root canal dressing.

INTRODUCTION

It has been reported that bacteria lodged in the root canal system plays an important role in the development and maintenance of periapical lesions. Thus, the elimination of these bacteria is of great importance for apical and periapical healing after endodontic treatment (1,2).

Considering the complex anatomy of root canals, and that certain areas are not accessible during mechanical preparation, the use of a root canal dressing has been recommended in teeth with chronic periapical lesions to reach areas not accessible by instrumentation (3-5). Calcium hydroxide has been recommended because of its antibacterial (3,6,7) and biological (8,9) properties, which have been exhaustively studied. However, literature is deficient on the length of time the dressings should remain in the root canal.

In bacterial cultures, Sjögren et al. (10) demonstrated the inefficiency of calcium hydroxide used for only 10 min. This may have occurred because the medication did not reach the intended area in this short time period. Nerwich et al. (11) demonstrated, *in vitro*, that even though the OH^- ions diffuse into the periapical area in a matter of hours, 2 to 3 weeks are necessary for them to reach a greater depth in the dentinal tubules. Takahashi et al. (12), analyzing the pH and the concentration of calcium ions in the periapical area, concluded that at least 2 weeks are necessary for calcium hydroxide bactericide activity.

Considering that elimination of bacteria is necessary for healing, and that this elimination also depends on the period of time the medicament remains in the root canal, we proposed to study the effect of time on apical and periapical healing in induced chronic periapical lesions in dogs.

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MATERIAL AND METHODS

This study was conducted on 61 root canals of the premolars from four mongrel dogs aged approximately 1 year. The animals were injected intravenously with 3% nembutal sodium (30 mg/kg body weight; sodium pentobarbital, Abott do Brasil Ltda., São Paulo, SP, Brazil) and access cavities were made on the occlusal surface. After pulp removal, the root canals were left exposed to the oral environment for 7 days to allow microbial contamination. Anesthesia was again induced, and the access openings were sealed with zinc oxide-eugenol cement (IRM-Caulk-Dentsply, Petropolis, RJ, Brazil). Standard radiographs were taken at 15-day intervals for the observation of radiolucent periapical areas, which usually occurred at about 45 days.

After isolation of the dental area with a rubber dam, the teeth were disinfected with 70% alcohol and 0.3% iodine and the zinc oxide-eugenol cement was removed. The septic-toxic content of the root canals was removed using K files in the crown-apex direction, using 5.25% sodium hypochlorite for irrigation (Chemical Institute, UNESP, SP, Brazil). Working length was established at 2 mm short of the radiographic apex and the apical foramen was enlarged using K files up to size 30. Further instrumentation was performed sequentially at the working length with K files to size 70, with irrigation using 3.6 ml of 5.25% sodium hypochlorite solution at every change of file.

After instrumentation, the root canals were filled with buffered 14.3% EDTA which was agitated with a K file for 3 min. After irrigation with saline, the root canals were dried with paper points and filled with a calcium hydroxide-based paste (Calen-PMCC: 2.5 g calcium hydroxide, 1 g zinc oxide p.a., 0.05 g colophony, 2 ml polyethylene glycol 400, 0.04 g paramonochlorophenol; S.S. White Artigos Dentários, Rio de Janeiro, RJ, Brazil) using a threaded syringe (ML endodontic syringe, S.S. White Artigos Dentários) with a 27-gauge needle (Terumo, Tokyo, Japan) for 7 days (group I), 15 days (group II) or 30 days (group III). The pulp chamber was filled with a sterile cotton pledget and the crown opening sealed with zinc oxide-eugenol cement. In the control group (group IV), the root canals were left empty, a cotton pledget was placed in the canal entrance and the access cavity was sealed with the same cement.

The animals were killed at 7, 15, or 30 days and the teeth were individually separated, fixed in 10% formalin for 48 h and decalcified in formic acid-sodium citrate. After paraffin embedding, the blocks were cut into serial 6-µm sections and stained with hematoxylin-eosin and Mallory's trichrome for histopathologic analysis.

The histological sections were examined according to the following parameters: inflammatory infiltrate, subjectively classified as absent/mild, moderate, or severe; thickness of the periodontal ligament, subjectively classified as normal/mild, moderate, or severe; resorption of mineralized tissues (cementum, dentin and bone) classified as absent or present.

The exact test of Fisher was used for statistical analysis.

RESULTS

Group I (7 days)

In 18 root canals, severe pathological changes were observed in the apical and periapical regions (Figure 1). In all specimens the apical surface was irregular along its complete length with the presence of areas of active cementum resorption, which in 3 cases

Figure 1. Group I - 7 days. Presence of severe inflammatory infiltrate in the periapical region. Cementum reabsorption. H&E. Original magnification: 40X.

reached the radicular dentin. The ramifications of the apical delta were dilated and contained necrotic residue or were empty. The apical periodontal ligament was severely thickened in 13 roots and moderately thickened in 5 (Table 1). In this region, 14 root canals had severe and dense predominantly neutrophilic inflammatory infiltrate (Figure 1) and moderate infiltrate in 4 specimens. Twelve specimens had extensive areas of bone resorption, with a predominance of medullary spaces and the presence of osteoclasts (Table 1).

Group II (15 days)

Disorganized or necrotic connective tissue was found at the apex area where the ramifications of the apical delta persisted, in 8 of 16 specimens. The interstitial connective tissue of the apical opening was necrotic in 8 specimens, with moderate inflammatory infiltrate in 2 cases, and absence of inflammatory infiltrate in 6 specimens. The surface of the apical cementum was irregular, and in 8 specimens there was deep active cementum resorption, which in 2 cases reached the radicular dentin. The inflammatory infiltrate present in the periapical region was severe in 8 specimens, moderate in 5 and mild in 3 (Table 1). In 6 roots there were inflammatory foci, predominantly neutrophilic. In 10 specimens there was an extensive capillary network

Table 1 - Quantitative results of the histopathological analysis.

	7 days (N=18)	15 days (N=16)	30 days (N=17)	Control (N=10)
Inflammatory infiltrate				
Mild	0	3	8	0
Moderate	4	5	5	0
Severe	14	8	4	10
Ligament thickness				
Mild	0	0	2	0
Moderate	5	6	12	0
Severe	13	10	3	10
Cement resorption				
Passive	2	8	13	0
Active	16	8	4	10
Bone resorption				
Absent	6	8	15	0
Present	12	8	2	10
Dentin resorption				
Absent	15	14	17	4
Present	3	2	0	6

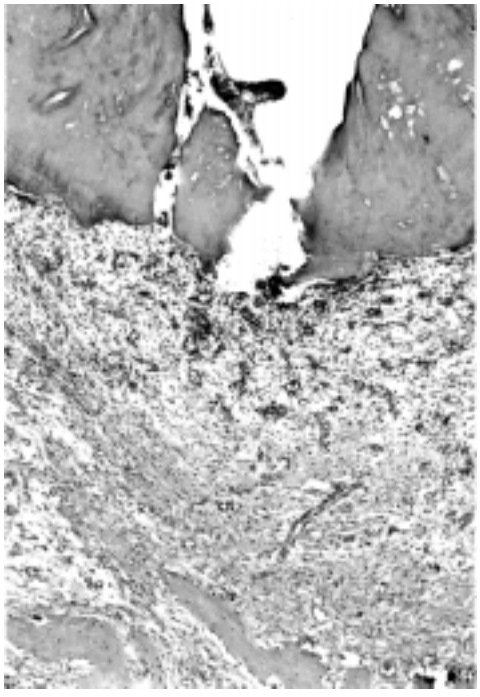

Figure 2. Group II - 15 days. Presence of moderate inflammatory infiltrate in the periapical region. Collagen fibers surround the inflammatory cells. H&E. Original magnification: 40X.

Figure 3. Group III - 30 days. Intense formation of collagen fibers and mild inflammatory infiltrate in the periapical region. H&E. Original magnification: 40X.

and collagen fibers surrounding the inflammatory cells (Figure 2). The apical periodontal ligament was thickened moderately in 6 cases and severely in 10 cases. Extensive areas of bone resorption persisted in 8 specimens, 8 of these presented empty medullary spaces and osteoclasts in its periphery. Five roots presented areas of newly-formed bone.

Group III (30 days)

In 17 root canals, the ramification of the apical delta was empty or partially obliterated by mineralized tissue in 3 specimens and presented connective tissue with few inflammatory cells in 14 specimens. The interstitial connective tissue was absent in 6 roots and presented mild/moderate mononuclear inflammatory infiltrate containing numerous fibroblasts in 11 specimens (Figure 3). The apical periodontal ligament had mild thickness in 2 roots, moderate thickness in 12, and severe thickness in 3 specimens (Table 1). The connective tissue in this area presented mild inflammatory infiltrate in 8 roots, moderate in 5, and severe in 4 (Table 1). Intense newly formed collagen fibers were found parallel to the apical surface. Newly formed bone was deposited near the alveolar process to reestablish normal periapical thickness. In this new mineralized tissue, the medullary spaces were filled by connective tissue with osteocytes in its interior and osteoblasts at its surface. In 2 specimens active bone resorption was found. In the 4 specimens where the inflammatory infiltrate was severe, the inflammatory foci were single, with reduced volume and density, and with a small amount of irregular thin collagen fibers. In 13 roots in which the cementum was previously reabsorbed, there was new cementum deposition. In only four specimens was there active cementum resorption (Table 1).

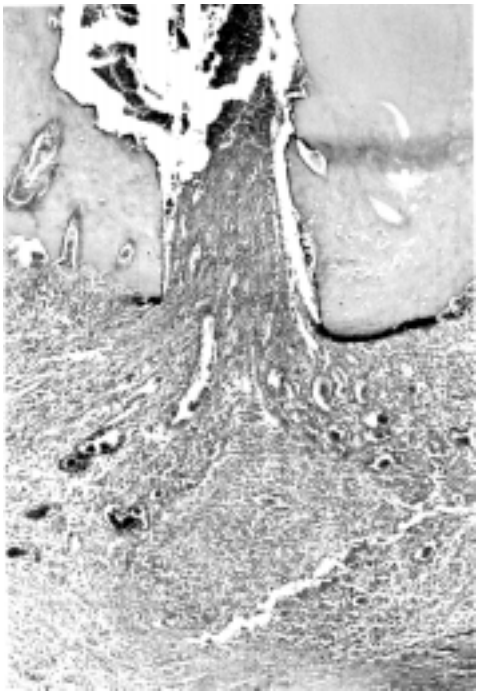

Figure 4. Group IV - Control. Areas of cementum reabsorption and severe inflammatory infiltrate in the periapical region. H&E. Original magnification: 40X.

Control Group (IV)

In this group (N = 10), the apical delta ramifications were empty or filled by necrotic residue. The surface of the apical cementum was irregular in all cases with the presence of areas of active cementum resorption, plus the formation of apical craters of different depths which, in 6 specimens, exposed the root dentin. The interstitial connective tissue of the apical opening was necrosed in 6 and absent in 4 cases. The apical periodontal ligament in all roots was severely thick, disorganized and with severe inflammatory cell infiltrate that at times was diffuse and at times formed several focal points containing predominantly neutrophils (Figure 4). The bone was extensively reabsorbed in all cases with frequent osteoclasts and medullary spaces were absent.

Statistical Analysis

Statistical evaluation of the results showed that groups I and IV were statistically similar ($p>0.05$) and these two groups were statistically different from groups II and III ($p<0.05$). Group III had significantly less inflammatory infiltrate than the other 3 groups ($p<0.05$)

DISCUSSION

The use of root canal dressings between sessions in root canal treatment of teeth with chronic periapical lesions is important for reducing bacteria beyond levels obtained with mechanical preparation, particularly by penetration of areas that are unreachable by instruments or irrigation solutions, such as dentinal tubules

and ramifications (6,13-16). These dressings are chosen based on diffusion, toxicity, and inflammatory potential (17). However, most medications are nonspecific, and may cause inflammation and discomfort for the patient (18).

Calcium hydroxide has shown clinical efficiency in reducing exudate due to its hygroscopic properties (19) and in stimulating apical and periapical repair, with no discomfort (20). However, these clinical conditions have not been substantially supported by histological studies based on the length of time of use necessary for root canal dressings.

We observed an accentuated decrease in inflammation at 30 days compared to control and 7 days, periods in which there was edema, severe mixed inflammatory infiltrate containing mononuclear cells and polymorphonuclear neutrophils. At 30 days, the inflammation was mild, without neutrophils and there was an intense neoformation of collagen fibers indicating evolution of the repair process ($p<0.05$). We believe that high alkalinity and antibacterial activity were factors which aided in the reduction of the inflammatory reaction because tissue destruction occurs mainly in acid pH, and calcium hydroxide acts as a buffer. Nerwich et al. (11) reported that calcium hydroxide reached a pH of 9.0 in the region of apical cement after only 2-3 weeks. Thus, we can affirm that as a function of the time the calcium hydroxide dressing remained in the root canal, the periapical region showed different histological aspects.

Also at 7 days, the periodontal ligament presented severe thickness, intense dissociation of collagen fibers, generalized edema and few fibroblasts in contrast to 30 days where there was a dense network of collagen fibers and fibroblasts. At 7 days, the thickness of the periodontal ligament was increased due to intense bone reabsorption while at 15 and 30 days, bone neoformation caused a decrease in this thickness ($p<0.05$). Thus we conclude that at 30 days more advanced repair was seen compared to the other two periods.

RESUMO

Leonardo MR, Silveira FF, Silva LAB, Tanomaru Filho M, Utrilla LS. Curativo de demora à base de hidróxido de cálcio. Avaliação histopatológica do repara apical e periapical em diferentes períodos de tempo. Braz Dent J 2002;13(1):17-22.

O objetivo deste estudo foi avaliar o reparo apical e periapical após uso de curativo de demora com hidróxido de cálcio por diferentes períodos de tempo em dentes de cães com lesão periapical induzida. Um total de 61 canais radiculares de prémolares superiores e inferiores de cães foram usados. Após preparo biomecânico dos canais radiculares usando técnica coroa-ápice e solução de hipoclorito de sódio a 5,25% como solução irrigadora, o forame apical foi dilatado (patência apical) em todos os casos. Curativo de demora à base de hidróxido de cálcio foi usado. O grupo controle não recebeu curativo de demora. Os animais foram sacrificados aos 7, 15 e 30 dias. Depois da preparação histológica, cortes seriados foram corados com hematoxilina e eosina e Tricrômico de Mallory. O melhor reparo apical ocorreu nos grupos de 15 e 30 dias e e os piores resultados ocorreram nos grupos de 7 dias e controle.

Unitermos: hidróxido de cálcio, curativo de demora.

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Accepted September 26, 2001