The effect of the renewal of calcium hydroxide paste on the apexification and periapical healing of teeth with incomplete root formation

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Abstract

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Aim To evaluate the influence of renewing calcium hydroxide paste on apexification and periapical healing of teeth in dogs with incomplete root formation and previously contaminated canals.

Methodology Forty premolars from four 6-month-old dogs were used. After access to the root canals and complete removal of the pulp, the canal systems remained exposed to the oral environment for 2 weeks. Canal preparation was then carried out using Hedströem files, under irrigation with 1% sodium hypochlorite, 1 mm short of the radiographic apex. After drying, the canals of one premolar in each dog were left empty (group 4-control), and those of the other nine teeth in each animal were filled with a calcium hydroxide-propylene glycol paste. All teeth were restored with reinforced zinc oxide cement (IRM) or IRM and amalgam (group 4). The paste was renewed and the teeth restored again 1 week later. Then, the nine teeth in each animal were divided into three experimental groups: group 1 – paste not changed; group 2 – paste renewed every 4 weeks for 5 months; and group 3 – paste renewed after 3 months had elapsed. The teeth were restored with IRM and amalgam (groups 1 and 3) or IRM (group 2). The animals were killed 5 months later, and blocks of the teeth and surrounding tissues were submitted to histological processing. The sections were studied to evaluate six parameters: apical calcified tissue barrier, inflammatory reaction, bone and root resorption, paste extrusion and microorganisms. Results of experimental groups were analysed by Kruskal–Wallis nonparametric tests and by the test of proportions. The critical value of statistical significance was 5%.

Results Significant differences ($P < 0.05$) were found in relation to the presence of bone resorption and paste in the periradicular area, the formation of a calcified tissue barrier at the apex, and the intensity of the apical inflammatory reaction. Bone resorption was more evident in group 1 (medicament not changed), and the presence of paste in the periodontal tissues was more common in groups 2 and 3. Renewal of the paste reduced the intensity of the inflammatory reaction (groups 2 and 3), but the formation of apical calcified tissue was more noticeable in the teeth where the paste had not been renewed.

Conclusions Replacement of calcium hydroxide paste was not necessary for apexification to occur, however, it did reduce significantly the intensity of the inflammatory process. Monthly renewal of calcium hydroxide paste reduced significantly the occurrence of apexification.

Keywords: apexification, calcium hydroxide, open apices, periapical healing.

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Introduction

Calcium hydroxide pastes have been used to induce apexification in pulpless teeth with incomplete root formation (Fujii & Machida 1991, Leonardo *et al.* 1993, Chosack *et al.* 1997, Finucane & Kinirons 1999, Kinirons *et al.* 2001). However, no agreement has been reached about the need to renew the paste or the frequency with which it should be done. Some claim that a single application is sufficient to induce apical closure, because the paste acts as a catalyst for the deposition of calcified tissue (Chawla 1986, Gupta & Sharma 1996). Others believe that the presence of a very wide foramen and inflammatory exudate in the apical region increases the rate of dissolution of the paste and makes its renewal necessary in the initial stages (Yates 1988, Leonardo *et al.* 1993), or when disappearance of the paste in the canal is detected radiographically (Webber 1984, Chawla 1986, Yates 1988).

Few controlled studies have evaluated the effect of paste renewal upon the apexification process. In follow-up studies, Finucane & Kinirons (1999) and Kinirons *et al.* (2001) observed that the formation rate of the apical calcified tissue barrier was directly proportional to the renewal frequency of the calcium hydroxide paste. On the other hand, in a study on monkey teeth, Chosack *et al.* (1997) concluded that for at least 6 months after mechanical preparation and initial placement of calcium hydroxide paste, there were no advantages in renewing the medication. In that study, the treatment was completed in non-contaminated canals. Therefore, the role of calcium hydroxide was evaluated on apical tissues exhibiting inflammatory reactions due only to pulp extirpation and canal preparation, without the presence of microorganisms or pre-existing apical periodontitis. In more normal clinical conditions, it is likely that the responses of the apical tissues would be different.

Reducing the time needed for apexification enhances patient cooperation, reduces treatment costs, and facilitates the placement of the final restoration. The aim of this study was to evaluate the influence of the renewal of calcium hydroxide paste on the apexification and periapical healing of dogs' teeth with incomplete root formation and previously contaminated canals.

Material and methods

The research project was approved by the Animal Ethics Screening Committee of the Federal University of Santa Catarina (Florianópolis, Santa Catarina, Brazil).

A total of eighty root canals from 40 maxillary and mandibular premolars of four 6-month old Beagle dogs were used. After intramuscular sedation with an association of Rompum (Abbott, São Paulo, SP, Brasil) and Ketamin (Aster, São Paulo, SP, Brasil), the dogs were anaesthetised intravenously with 5% Thionembutal solution (Abbott) at the dose of 5-mg kg^{-1} body weight. During the operative procedures, the animals received an infusion of saline solution and intravenous anaesthetics, as required.

Pre-operative radiographs were exposed to confirm the presence of open apices. After endodontic access and determination of canal length, pulp tissue was removed with K files. Size 60 Hedströem files were introduced to the radiographic apex and were used in a filing action to totally remove the remnants of apical pulp tissue. The canals were irrigated with distilled water and after haemostasis, a cotton pellet was placed in the entrance of each canal and the teeth were left without a coronal restoration for 2 weeks.

After this period, radiographs were exposed that demonstrated the presence of apical lesions of variable size in all roots. The canals were cleaned under aseptic conditions to the radiographic apex using size 50–60 K-files. Hedströem files sizes 70 and 80 were subsequently utilized 1 mm short of the radiographic apex, using gentle filing movements. During preparation, the canals were irrigated with 1% sodium hypochlorite solution. After drying with sterile paper points, the canals of one premolar of each dog were left unfilled (group 4-control). The coronal restoration was achieved using reinforced zinc oxide cement (IRM) and amalgam. For the other nine premolars in each animal, the canals received a calcium hydroxide paste (pure calcium hydroxide, 0.612 mg; Reagen, Rio de Janeiro, RJ, Brazil) mixed with propylene glycol (0.4 mL; Quimidrol, Joinville, SC, Brazil). The paste was placed into the canals with a lentulo spiral, introduced 3 mm short of the radiographic apex. The teeth were radiographed for complete filling of the canals, and sealed with IRM. After 1 week, the canals were reopened and the paste was renewed for all teeth to assure the calcium hydroxide did not have its properties jeopardized by exudate from the initial apical inflammation. The teeth were then randomly divided into three experimental groups of 24 each: group 1 – without paste renewal for 5 months; group 2 – with paste renewal every 4 weeks for 5 months; and group 3 – with paste renewal after 3 months. The coronal seal was provided by IRM (group 2) or with IRM and amalgam (groups 1 and 3).

In the monthly reviews, periapical radiographs of all premolar teeth were exposed. To renew the paste (groups 2 and 3), the canals were irrigated with saline solution, and a size 80 K-file was introduced 1 mm short of the radiographic apex and gently manipulated. Irrigation was repeated until the solution was clear of any calcium hydroxide residue. After drying, the canals were filled again with calcium hydroxide paste, as described previously.

Five months after mechanical canal preparation, the animals were killed and the jaws dissected and fixed in 10% formol solution. After decalcification in formic acid-sodium citrate solution, the specimens were reduced, soaked in paraffin, and trimmed until the apical foramen was exposed. The blocks were cut into 6-μm thick sections and stained with haematoxylin–eosin (HE) and by Brown–Hopps technique (BH).

Typical sections from each root were analysed by two examiners under light microscopy for six parameters: apical calcified tissue barrier, which was classified as (1) absent – there was no new calcified tissue in the apical foramen; (2) incomplete – apical calcified tissue was interrupted by fibrous or inflammatory tissue; or (3) complete – apical calcified tissue extending from one to the other root canal wall; and inflammatory reaction, which was classified as (1) absent or mild – scattering of inflammatory cells with no structural damage; (2) moderate – focal accumulations of inflammatory cells, no tissue necrosis with some disruption of structure; or (3) severe – extensive inflammatory cell infiltrate with replacement of tissues, abscess. The presence of microorganism, paste extrusion, bone and root resorption was also recorded when detected.

Kruskal–Wallis non-parametric tests were used to determine significant differences between the groups for the formation of apical barrier and the intensity of the inflammatory reaction. The other parameters were analysed by the test of proportions. The critical value of statistical significance was 5%.

Results

Before the conclusion of the experiment, four roots from group 4 were lost because of severe destruction of the periodontal support tissues. Another six roots were lost during the histological processing. The histological analysis was carried out on 70 specimens; 20 specimens from group 1 (without renewal), 23 specimens from group 2 (monthly renewal), 23 specimens from group 3 (renewal after 3 months) and four specimens from group 4 (control).

The histological data obtained are shown in Tables 1 and 2. In relation to the experimental groups, the statistical analysis revealed significant differences in the histological results in four of the six parameters studied.

Apical calcified tissue barrier

In all specimens from group 1, a complete apical barrier of calcified tissue, with characteristics similar to cementum (Fig. 1) or a partial barrier (Fig. 2) was observed. The Kruskal–Wallis test revealed significant differences ($\chi^2 = 23.1023$; $P < 0.0001$) between the results from group 1 and the results from groups 2 and 3, which were similar. In 11 specimens from group 2 and 7 specimens from group 3, the presence of fibrous cellular connective tissue was observed in contact with the calcium hydroxide paste, without any sign of calcification (Fig. 3).

	Calcified tissue barrier			Intensity of inflammatory reaction		
Groups (*n*)	Absent	Incomplete	Complete	Absent or mild	Moderate	Severe
1 (20)	0	11	9	4	6	10
2 (23)	11	12	0	18	5	0
3 (23)	7	15	1	8	8	7
4 (4)	4	0	0	0	0	4

Table 1 Absolute frequency, for calcified tissue barrier and intensity of inflammatory reaction, for each group studied

Groups (*n*)	Microorganism	Paste extrusion	Bone resorption	Root resorption
1 (20)	1	11	15	1
2 (23)	0	23	5	0
3 (23)	0	20	7	0
4 (4)	4	–	3	3

Table 2 Absolute frequency, for presence of microorganisms, extrusion of paste, bone and root resorption, for each group studied

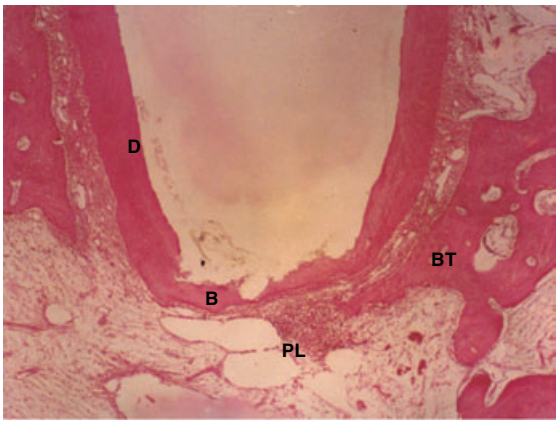

Figure 1 Apical area of a group 1 tooth. Complete barrier (B) and moderate inflammation in periodontal ligament (PL) are seen. BT, bone tissue; D, dentine (HE, X12.8).

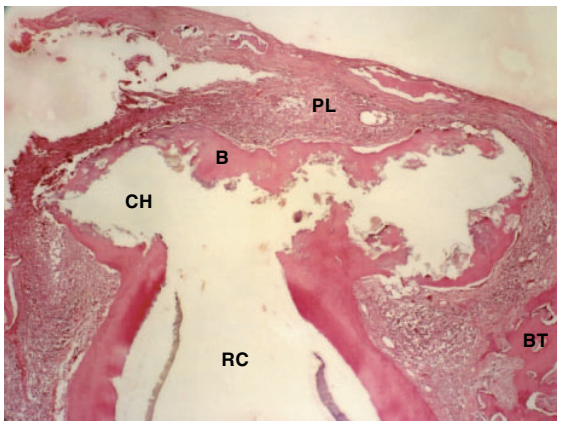

Figure 2 Apical area of a group 1 tooth. Incomplete barrier (B), and severe inflammation in periodontal ligament (PL) are seen. Note the presence of calcium hydroxide (CH) beyond the root canal. RC, root canal; BT, bone tissue (HE, X12.8).

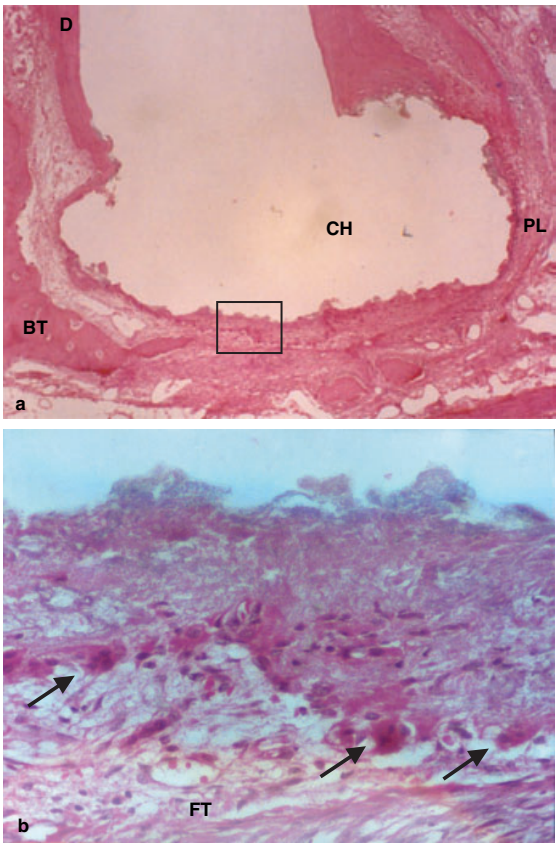

Figure 3 (a) Apical area of a group 2 tooth. Absence of barrier, and mild inflammation in periodontal ligament (PL) are seen. Note the presence of calcium hydroxide (CH) beyond the root canal. BT, bone tissue; D, dentine (HE, X12.8). (b) Higher magnification of (a) (box) showing the presence of multinucleate cells (arrows). FT, fibrous tissue (HE, X12.8).

Inflammatory reaction

All specimens from the experimental groups exhibited chronic inflammation in the proliferative stage. Besides the large quantities of endothelial cells and fibroblasts, characterizing granulation tissue, varying numbers of mononuclear cells (lymphocytes, plasmocytes, and macrophages) were also observed. Regarding the intensity of the inflammatory reaction, the Kruskal–Wallis test revealed significant differences ($\chi^2 = 19.4948$; $P < 0.0001$) between group 2 and groups 1 and 3, which were equivalent. Multinucleated cells, near the calcium hydroxide paste, which had leaked through the limits of the canal, or near to what appeared to be a calcification focus disseminated into the connective tissue, were detected in three specimens from group 1, 18 specimens from groups 2 and eight specimens from group 3 (Figs 3b and 4b).

Bone resorption

Bone resorption was observed in 15 (75%), 5 (22%) and 7 (30%) specimens from groups 1, 2 and 3, respectively. The test of proportions revealed that the results from group 1 were statistically different from group 2 ($P < 0.0026$) and group 3 ($P < 0.01$).

Paste extrusion

The presence of calcium hydroxide paste in the apical region was verified in 11 (55%), 23 (100%) and 20

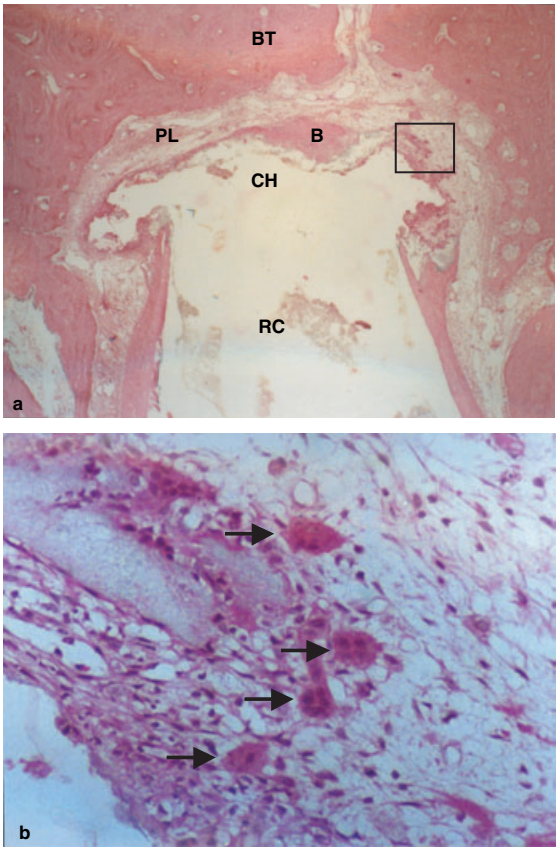

Figure 4 (a) Apical area of a group 2 tooth. Incomplete barrier (B) and mild inflammation in periodontal ligament (PL) are seen. Note the presence of paste (CH) beyond the limits of the root canal (RC). BT, bone tissue (HE, X12.8). (b) Higher magnification of (a) (box) showing the presence of multinucleate cells (arrows) (HE, X12.8).

(87%) specimens from groups 1, 2 and 3, respectively (Figs 2–4). The test of proportions revealed significant differences between groups 1 and 2 ($P < 0.0026$) and 1 and 3 ($P < 0.0454$).

There were no significant differences between the groups in terms of the presence of microorganisms and root resorption. Microorganisms were found in dentinal tubules of one specimen from group 1. The sections from this same specimen, stained with H.E., revealed that the root surface corresponding to the area where the microorganisms were detected was covered by reparative cementum. In another specimen from group 1, an area of active resorption was observed in one of the root surfaces. In the opposite root surface and in the remainder of the experimental teeth, previously reabsorbed areas were covered by reparative cementum.

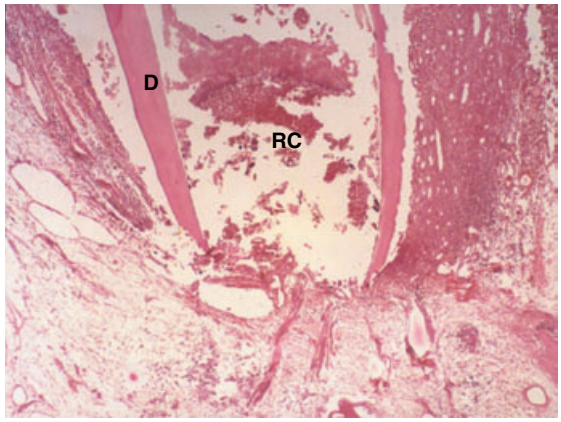

Figure 5 Apical area of a tooth from group 4. Absence of apical closure, severe root resorption, and severe chronic inflammatory reaction are seen. D, dentine; RC, root canal (HE, X12.8).

In the specimens from group 4, histological evaluation revealed the absence of apical closure, severe bone and root resorption, severe chronic inflammatory reaction, and connective tissue infiltrated with inflammatory cells (Fig. 5). The Brown-Hopps staining technique revealed the presence of microorganisms in all specimens. Microorganisms were located in the root canal, inside of dentinal tubules, and in the periodontal tissues, near the apical foramen.

Discussion

The results from group 4 (control) confirmed the difficulty of performing suitable root canal preparation in teeth with incomplete root formation (Cvek *et al.* 1976) and reinforce the need of an antibacterial dressing to eliminate the bacteria resistant to instrumentation and irrigation (Leonardo *et al.* 1993).

In the majority of the specimens from the experimental groups, the presence of reparative cementum in previously reabsorbed areas of the root surfaces, and the absence of microorganisms revealed that the calcium hydroxide paste was effective in the disinfection of the canals and in facilitating tissue repair, confirming the findings of other investigators (Torneck *et al.* 1973, Smith *et al.* 1984, Leonardo *et al.* 1993).

Although all efforts were made to limit the paste to the root canal by introducing the Lentulo spiral 3 mm short of the radiographic apex, the histological sections revealed that extrusion was a common occurrence, mainly in cases where replacement of the dressing had been performed. This finding corroborates those of

Chosack *et al.* (1997). Considering that the technique of inserting the paste into the canals was identical, it was expected that the incidence of paste extrusion in teeth from group 1 (without renewal) would be superior. However, the time from the initial placement of the dressing to the histological analysis was sufficient to allow the extruded material to be reabsorbed gradually, in many of the specimens. Since calcium hydroxide has low solubility, the greater presence of multinucleated cells in groups 2 and 3 can be explained by the frequent and inadvertent extrusion that occurred during medicament replacement.

Some authors claim that the extrusion of the calcium hydroxide paste is of no concern, because the paste is reabsorbed (Cvek *et al.* 1976, Webber 1984) and may even stimulate the deposition of hard tissue (Webber 1984). However, the results of the present study demonstrated that the continuous presence of calcium hydroxide in the apical tissues influenced positively the intensity of the inflammation and negatively the formation of calcified tissue.

Inflammation was significantly milder in teeth submitted to monthly paste replacement. However, it is likely that monthly replacement and probably extrusion interrupted or hampered the formation of calcified tissue. On the other hand, in teeth that had not been submitted to paste renewal, a complete or partial barrier of calcified tissue was observed in all specimens, in spite of the presence of a more intense inflammatory reaction. Other investigators also noticed apical closure in the presence of persistent inflammation of the tissues (Torneck *et al.* 1973, Yates 1988, Fujii & Machida 1991, Leonardo *et al.* 1993, Chosack *et al.* 1997).

These results suggest that a single application of calcium hydroxide paste acted as an apexification catalyst, as reported by Chawla (1986) and Gupta & Sharma (1996). However, they differ from the results of Finucane & Kinirons (1999) and Kinirons *et al.* (2001) who, after clinical follow-up studies, reported that the rate of formation of the barrier was directly proportional to the frequency of the paste renewals. In these studies, the treatments were carried out because the patients were suffering from dental trauma, but the authors did not mention whether the teeth had apical periodontitis. It has been suggested that the presence of inflammatory changes in the apical tissues can influence the formation time of the apical barrier (Webber 1984, Ghose *et al.* 1987).

The results of the present study also differ from Chosack *et al.* (1997) who compared the effect of a single application of calcium hydroxide paste versus monthly or 3 months renewals and reported that the amount of calcified tissue formed was similar in the three groups. However, in the present study calcium hydroxide was placed in canals which had been previously exposed to the oral cavity whilst, in the study of Chosack *et al.* (1997), the paste was inserted soon after the removal of the pulp. Holland *et al.* (1977) showed that, 30 days after pulp extirpation and filling of canals with calcium hydroxide paste, formation of cementum-like tissue had occurred, isolating the paste and the necrotic portion of the apical connective tissue. This explains the results obtained by Chosack *et al.* (1997): replacement of paste should not have influenced, at least not noticeably, the formation of the barrier, as 30 days had passed since the placement of the previous dressing and, as such, part of the apical periodontium could still have been separated from the canal by the layer of necrotic tissue and by previously calcified areas.

In the present study, the environment was different, because there was no healthy apical tissue. In the cases of a single application (group 1), the calcium hydroxide created favourable conditions for apexification. In the cases of replacements (groups 2 and 3), the apexification was partially or totally inhibited by the constant presence of the paste in the apical tissues.

Generally speaking, it was observed that the frequent renewal of calcium hydroxide paste resulted in detrimental effects on the repair and apexification processes. Although the intensity of the inflammatory reaction reduced with the increase in the number of renewals, the formation of a barrier of calcified tissue was much more evident in teeth in which a single dressing had been applied. The results suggest that, if it is necessary to renew the dressing where the sealing has failed, or when there is a flare-up, then it would be judicious to ensure the paste remains in the root canal space.

Taking into consideration that the endodontic treatment of teeth with open apices depends on the formation of calcified tissue, it seems reasonable to suggest that, at least for a period of 5 months, renewal of calcium hydroxide paste should not be performed, so that the deposition of hard tissue is not disturbed.

Conclusions

Calcium hydroxide paste aided apexification and the repair of apical tissues; replacement of calcium hydroxide paste was not required for occurrence of apexification; replacement of calcium hydroxide paste

reduced significantly the intensity of the inflammatory process; and monthly renewal of calcium hydroxide paste reduced significantly the occurrence of apexification.

Acknowledgements

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