Histological Periapical Repair after Obturation of Infected Root Canals in Dogs

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Histological periapical healing of infected roots obturated in one-step or with prior calcium hydroxide (Ca(OH)₂) disinfection was compared. Seventy-two roots of vital dog teeth were instrumented to ISO size 45. Sixty roots were infected with dental plaque and closed. Six weeks later, apical periodontitis was radiographically confirmed in the infected roots. The teeth were divided into the following groups: group 1, one-step (n = 24)-roots were irrigated with 10 ml of saline, obturated, and permanently restored; group 2, Ca(OH)₂ (n = 24)roots were treated as in group 1, except that after saline irrigation, Ca(OH), dressing was placed in the canal for 1 wk before obturation; group 3, positive control (n = 12)—the roots were irrigated with saline, but the canals were not obturated; and an additional group, group 4, served as a negative control (n = 12) – these teeth that were not infected with plaque were aseptically obturated. The dogs were sacrificed after 6 months. The roots and surrounding apical tissues were prepared and histologically examined by two independent evaluators blinded to the treatment groups. A two-way ANOVA test demonstrated that the four treatment groups were significantly different from one another. The positive control showed the most inflammation, the negative control the least, and the Ca(OH)₂ group had significantly less inflammation than the one-step group (p < 0.05). It is concluded that Ca(OH)₂ disinfection before obturation of infected root canals results in significantly less periapical inflammation than obturation alone.

Apical periodontitis is caused, primarily, by bacteria in the root canal space (1-4). If bacteria are removed to levels that are undetectable by bacteriological methods in use today, an extremely high success rate in the resolution of apical periodontitis can be expected (5, 6).

The scientifically documented procedure for the best results in canal disinfection of teeth with apical periodontitis is based on complete debridement and irrigation of the root canal during the first appointment, followed by the application of a calcium hydroxide $(Ca(OH)_2)$ dressing for 1 wk or more. Obturation of the root canal is then performed at the second or a later appointment (7). Mechanical instrumentation alone causes a 100- to a 1000-fold reduction in the number of bacteria, but complete elimination in only 20 to 43% of cases (8). Added antibacterial irrigation with 0.5% sodium hypochlorite solution provides disinfection in some 40 to 60% of the treated cases (9). The subsequent application of a Ca(OH)₂ dressing brings the percentage of bacteria negative teeth to 90 to 100% (10); this treatment regimen is thus the current standard for root canal disinfection.

One issue frequently debated in recent years is whether conscientious cleaning by instrumentation and irrigation reduces the number of bacteria to a point where the obturation of the canal at the same visit will ensure successful treatment. Many practitioners feel high success rates are possible with this technique based on patient acceptance, lack of significant flare-ups, and practice management considerations. Because current research findings indicate that one-step treatment of teeth with apical periodontitis is obturation of an infected canal in a relatively high percentage of cases, proponents of this technique rely on the lack of nutrition and space for bacterial multiplication to overcome the bacterial infection (11, 12). Matsumiya and Kitamura (13) suggested that entombment of bacteria will result in their death, even though in their study the canals were obturated with $Ca(OH)_2$, an effective antibacterial agent, and it was not clear if the bacteria were viable or not (13).

The purpose of present study was to determine histologically: (a) the role of obturation alone in the healing of teeth with apical periodontitis and (b) compare periapical healing of teeth with infected canals obturated with or without prior $Ca(OH)_2$ disinfection.

MATERIALS AND METHODS

Seventy-two vital roots of 24 premolars and 24 incisors in three adult dogs were used. The dogs were anesthetized throughout all experimental procedures. The induction was achieved by intravenous administration of 20 mg/kg of body weight of thiopental, followed by 1 to 2% halothane for maintenance of anesthesia. To take standardized radiographs throughout the experiment, pretreatment bite blocks were fabricated using Stat-BRTM bite registration material (Kerr Corporation, Romulus, MI). All procedures were performed under strict asepsis.



FIG 1. Radiographs taken 6 wk after root canal infection. Radiolucencies (*arrows*) can be clearly seen at the apices of lower premolars.

Each dog was treated with all four treatment methods. After coronal pulp exposure, all roots were instrumented to ISO size 45. Sixty roots were infected with dental plaque and temporized with IRM (Caulk Co., Densply, Milford, DE). Six weeks later, apical periodontitis was radiographically confirmed in the infected teeth (Fig. 1). Anaerobic cultures were obtained and confirmed bacterial infection in teeth with apical periodontitis. The teeth were then randomly assigned to three groups as follows: group 1, one-step (n = 24)—each root was irrigated with 10 ml of saline, obturated with gutta-percha, and Roth 811 sealer (Roth International Ltd., Chicago, IL) with lateral condensation technique, and temporized with IRM; group 2, $Ca(OH)_2$ (n = 24)—teeth were treated as in group 1, except that after saline irrigation, a Ca(OH)₂ dressing was placed in the canals using a lentulo spiral (14) and teeth were temporized with IRM for 1 wk (after 1 wk, the teeth were obturated as in group 1); and group 3, positive control (n = 12)—each root was irrigated with 10 ml of saline, canals were not obturated but left empty, and accesses were temporized with IRM. An additional group, group 4, served as a negative control (n = 12). These teeth were not infected. They were irrigated with 10 ml of sterile saline, obturated as in other groups, and temporized with IRM. At the time of obturation of group 2, temporary restorations from all teeth in all groups were replaced by permanent amalgam restorations. The dogs were fed a soft diet for 3 to 5 days immediately after dental procedures. At the end of the observation period (6 months), standardized radiographs were taken that were used for a radiographic analysis (part 2 of this study).

At 6 months, the dogs were deeply anesthetized with 100 mg/kg of intravenous administration of pentobarbital. The left and right common carotid arteries were exposed/perfused with 4% neutralbuffered formaldehyde (pH 7.4), and jaws were resected, fixed in formaldehyde solution, and decalcified in 5% formic acid. Individual blocks containing the experimental roots and surrounding apical tissues were cut and paraffin-embedded. Approximately, 50 serial longitudinal sections of 5 to 7 μ m thickness were cut in a mesio-distal orientation to include the entire root canal system. Every other section was subsequently stained with hematoxylin and eosin using standard methods. An outside examiner selected five sections per root to be examined by two independent evaluators. Sections were selected that showed the full length of the canal to at least 1 mm from the end of the root. Sections were



Fig 2. Histological section of a root that received a score of "0" for no inflammation and normal width of the PDL space (H&E, \times 40).

evaluated with a light microscope at $\times 10$ magnification. The evaluators, one endodontist and one oral pathologist, were blinded to the treatment groups and evaluated the histological sections according to the following predetermined scale:

- 0 = no inflammation and normal width of the periodontal ligament (PDL) space (Fig. 2)
- 1 = mild inflammation and widened PDL space (Fig. 3)
- 2 = moderated inflammation and detectable loss of apical bone (Fig. 4)
- 3 = severe inflammation and severe destruction of apical and cortical bone (Fig. 5).

Statistical Analysis

A two-way ANOVA was used to test if the four treatment groups were different from one another and to test the influence of tooth and dog on the outcome. The level of significance for the overall differences and pair-wise comparisons of treatments was set at p < 0.05. The Bonferroni-Holm method was used to control for multiple comparisons. The κ -statistics were used to determine agreements between the two observers.

In addition, the Cochran-Mantel-Haenszel statistical test was used to compare the frequency in which a particular inflammation score was given to a treatment group. The level of significance was set at p < 0.05.



Fig 3. Histological section of a root that received a score of "1" for mild inflammation and widened PDL space (H&E, \times 40).

RESULTS

One root in each of the $Ca(OH)_2$ group, (+)-control group, and (-)-control group was lost in histological sectioning. One tooth (two roots) in the $Ca(OH)_2$ group was lost due to furcal perforation.

The dog associated with each tooth was included in the model to control for any effect of the dog on the outcome. No dog effect was found (p = 0.27), and the interactions of dog × treatment were not significant (p = 0.17). It was also confirmed that tooth was not a predictor of the outcome. The κ -statistics showed very good agreements between the two evaluators (weighted $\kappa = 0.852$).

The overall results for the four treatment groups are summarized in Table 1. The positive control group showed the most inflammation, with an average inflammation score of 2.75 \pm 0.55, whereas the negative control group showed the least inflammation with the average score of 0.065 \pm 0.70. The Ca(OH)₂ group had significantly less inflammation than the one-step group, with average scores of 1.36 \pm 0.80 and 1.73 \pm 0.80, respectively. All treatment groups were significantly different from each other when multiple comparisons were controlled for, using the Bonferroni-Holm method (p < 0.05). The *p*-values for these pair-wise comparisons are shown in Table 2.

The bar graph in Fig. 6 illustrates the distribution of group 1 (one-step) and group 2 (Ca(OH)₂) within the inflammation score categories. Overall, 79% of all sections in groups 1 and 2 were given a score of 1 (mild inflammation) or 2 (moderate inflammation). It was found that there was an overall difference between the two treatment groups (p < 0.05). Frequency of each inflammation

Journal of Endodontics



Fig 4. Histological section of a root that received a score of "2" for moderate inflammation and detectable loss of apical bone (H&E, \times 40).

score given to sections in groups 1 and 2 are compared in Table 3. Twenty-two percent of the sections in group 1 were given a score of 3 (severe inflammation), compared with 3% for group 2 (p = 0.001). In group 2, 48% of sections received a score of 2 and 32% received a score of 1, compared with 30% and 48% for group 1, respectively (p = 0.007 and p = 0.015). None of the sections in group 1 received a score of 0 (no inflammation), whereas 17% of sections in group 2 received a score of 0 (p = 0.001).

DISCUSSION

To our knowledge, no study has evaluated the role of obturation (alone) in healing of apical periodontitis. To do this, we needed to obturate canals known to be infected to simulate 40 to 60% of necrotic cases where a one-step procedure is done and the canal is still infected (9). To perform a histological evaluation in a prospective fashion, an animal model had to be used.

The benefit of using dogs in our study was primarily that 24 roots (two incisors and two premolars per quadrant) are available for endodontic treatment. Thus, we were able to have an adequate number of observations for statistical comparisons using only three dogs. The disadvantage of using dogs is that the web-like anatomy of their apices is different from apical structures in humans. Whereas this fact makes direct correlation of the results to humans difficult, it still allows us to legitimately compare treatment methods. The assumption is then made that the superior treatment



Fig 5. Histological section of a root that received a score of "3" for severe inflammation and destruction of apical and cortical bone (H&E, \times 40).

TABLE 1. Overall results for the groups

Treatment	No. of Observations	Mean	SD	Minimum	Maximum
(+)-Control	55	2.75	0.55	1.0	3.0
(-)-Control	55	0.64	0.70	0	2.0
One step	120	1.73	0.80	1.0	3.0
Ca(OH) ₂	105	1.36	0.80	0	3.0

TABLE 2. p-Values for the pair-wise comparisons of the four groups

	(+)-Control	()-Control	One step
(-)-Control	0.0001		
One step	0.0002	0.0001	
Ca(OH) ₂	0.0001	0.0097	0.0345

All groups are significantly different from one another.

method tested in the dog model will also be superior in the human model.

Histological healing was evaluated using a subjective scale. Previous investigations have demonstrated the outcomes of subjective and objective methods to be similar (15). This is probably due to the fact that a truly objective method of counting inflammatory cells does not exist. Even in the objective methods described, the examiner must identify the histological structures and the computer counts the cells. So, the method is no more than an



Fig 6. Distribution of group 1 (one-step) and group 2 (Ca(OH)₂) within the inflammation score categories (*p < 0.05).

TABLE 3. Frequency (%) of each inflammation score received by sections in groups 1 and 2

	Score					
	"0"	"1"	"2"	"3"		
Group 1	0	48	30	22		
Group 2	17	32	48	3		
p-Value	0.001	0.015	0.007	0.001		
Total	8	41	38	13		

p-Values for corresponding categories are shown.

automatic counting method rather than an objective evaluation. Two independent and blinded evaluators were used in our study, including an experienced pathologist. The κ -statistics showed very good agreement between the evaluators, which makes us confident that the evaluation was as accurate as possible.

The aim of this study was to evaluate the role of obturation of an infected canal in healing of apical periodontitis. All other variables, including instrumentation and antibacterial irrigation, were eliminated. The mechanical instrumentation necessary for adequate obturation was performed before infection of the canals, and only sterile saline that has no antibacterial properties was used for irrigation. Thus, the canals were expected to be infected at the time of obturation. Therefore, healing that was observed was attributed to the obturation in the one-step group or to the Ca(OH)₂ disinfection and obturation in the Ca(OH)₂ group.

The highest inflammation scores were recorded in the positive control group, wherein the infected canals were not disinfected or obturated and the lowest in the negative control group where vital teeth were instrumented and obturated aseptically. These results confirm the role of canal infection in the induction and persistency of apical periodontitis.

Interestingly though, the negative control group did have mild inflammation present in a surprisingly high number of cases. Thus, it seems that instrumentation and obturation of a vital case is somewhat irritating to the periapical structures. The high clinical success rate that we expect from vital cases is probably due to the fact that our clinical evaluations are symptomatic and radiographic, and the mild inflammation seen histologically in this study is below any threshold for those evaluations.

Obturation alone significantly reduced inflammation, compared with the positive control group. This result could be explained by the suggestion that obturation reduces the space and nutrition for multiplication of bacteria and that less bacteria will result in reduced inflammation (11, 12). Also, whereas inflammatory stim-

368 Katebzadeh et al.

ulants might still be present in the root canal space, they might not be able to communicate with the periapical tissues due to the mechanical barrier created by the obturation material. Still, the best results were obtained when the infected canals were disinfected with Ca(OH)₂ before obturation, and this treatment form should be performed as a routine in cases with apical periodontitis.

The fact that obturation has an effect on reduction of inflammation can explain the fairly high success rate of one-step endodontics in teeth with apical periodontitis. The increased success afforded by the use of Ca(OH)₂ disinfection is therefore difficult to appreciate clinically, which contributes to the "illusion" that onestep treatment is as successful as Ca(OH)₂ disinfection before obturation. However, recent controlled studies confirm the clinical benefit of reducing the number of bacteria in the root canal before obturation (6, 10). A prospective radiographic study on humans by Trope et al. (16) showed superior clinical results for Ca(OH)₂ disinfection, compared with one-step treatment of teeth with apical periodontitis.

In conclusion, in our study, obturation alone significantly reduced inflammation, compared with an empty canal and $Ca(OH)_2$ disinfection before obturation of infected root canals resulted in significantly less periapical inflammation than obturation alone.

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Journal of Endodontics

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