Clinical Efficacy of Treatment Procedures in Endodontic Infection Control and One Year Follow-Up of Periapical Healing

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Abstract

The objective was to evaluate the clinical efficacy of chemomechanical preparation of the root canals with sodium hypochlorite and interappointment medication with calcium hydroxide in the control of root canal infection and healing of periapical lesions. Fifty teeth diagnosed with chronic apical periodontitis were randomly allocated to one of three treatments: Single visit (SV group, $n = 20$), calcium hydroxide for one week (CH group $n = 18$), or leaving the canal empty but sealed for one week (EC group, $n = 12$). Microbiological samples were taken to monitor the infection during treatment. Periapical healing was controlled radiographically following the change in the periapical index at 52 wk and analyzed using one-way ANOVA. All cases showed microbiological growth in the beginning of the treatment. After mechanical preparation and irrigation with sodium hypochlorite in the first appointment, 20 to 33% of the cases showed growth. At the second appointment 33% of the cases in the CH group revealed bacteria, whereas the EC group showed remarkably more culture positive cases (67%). Sodium hypochlorite was effective also at the second appointment and only two teeth remained culture positive. Only minor differences in periapical healing were observed between the treatment groups. However, bacterial growth at the second appointment had a significant negative impact on healing of the periapical lesion ($p < 0.01$). The present study indicates good clinical efficacy of sodium hypochlorite irrigation in the control of root canal infection. Calcium hydroxide dressing between the appointments did not show the expected effect in disinfection the root canal system and treatment outcome, indicating the need to develop more efficient interappointment dressings.

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Apical periodontitis is caused by root canal infection usually dominated by obligate anaerobes, and the number of cultivable species varies from two to eight (1). Recent molecular analyses of the microflora suggest a much higher number of microbial species (2). Control and elimination of the root canal infection is achieved by the combined action of several treatment procedures. During treatment, ecological conditions change remarkably in the root canal system contributing to the elimination of the microflora. Chemomechanical preparation aims at removing necrotic pulp tissue and infected dentine, and mechanical instrumentation augmented with antimicrobial irrigation kills the majority of the microorganims from the root canal. However, some microorganims may survive and therefore, an interappointment dressing, commonly calcium hydroxide, is often used to complete the disinfection of the root canal system before obturation.

Sodium hypochlorite is an effective disinfectant used for irrigation during mechanical preparation of the root canals. It has a wide antimicrobial spectrum and is potent also in low concentrations (3, 4). Calcium hydroxide is a commonly used root canal dressing. It has been found useful in treatment of immature roots for completion of root end formation, of root open teeth for the formation of an apical barrier, in traumatized teeth for the prevention or arrest of inflammatory root resorption, and of infected pulps with apical periodontitis (5). The antibacterial properties of calcium hydroxide are of primary importance, and several laboratory and clinical studies have testified to the efficacy of calcium hydroxide in the treatment of infected root canals (6–9). Its antimicrobial activity in the root canal is supposedly based on its high alkalinity and mechanical blocking of nutrients from the periapical area. Calcium hydroxide is effective on the majority of bacteria isolated from infected root canals (6, 10). However, the microflora of infected root canal is, occasionally, resistant against routine treatment procedures and medicaments. Microbiological investigations have shown that *Enterococcus faecalis* and *Candida albicans* can often be isolated from such persistent infections (6, 11–13). Several factors may contribute to the survival of these microorganisms during the treatment. They may for example tolerate high alkalinity caused by calcium hydroxide or they may be capable to penetrate into dentinal tubules and thus avoid effective concentrations of therapeutic agents (10, 14). It may be seen as a problem that a medicament, in this case calcium hydroxide, indirectly may favor the growth of relatively resistant organisms, which may maintain the infection and be ever more resistant to therapeutic efforts. The tooth may also be susceptible to reinfection through the temporary filling and dressing during the interim period.

Many in vitro studies have focused on the efficacy of different irrigants and interappointment dressing against microorganisms isolated from root canal infections. The aim of the present study was to evaluate the clinical efficacy of chemomechanical preparation with sodium hypochlorite and interappointment medication with calcium hydroxide in the control of the root canal infection. The hypothesis of the study was that using calcium hydroxide as an interappointment dressing would result in a higher percentage of root canals with no cultivable microorganisms in comparison to such root canals treated only with chemomechanical preparation using sodium hypochlorite but no interappointment dressing. Furthermore, better treatment outcome was expected for the teeth treated with calcium hydroxide interappointment dressing than for

the teeth without the dressing or for the teeth subjected to treatment completed in one appointment.

Materials and Methods

Fifty teeth diagnosed with chronic apical periodontitis with periapical index (PAI) score 3, 4, or 5 (15), and with a positive microbiological sample in the beginning of the treatment were included in the present study. Trope and co-workers analyzed previously radiological outcome of these cases after three different treatment strategies (16). In the present study, the case selection criteria were different, and therefore, the number of cases is smaller. This study focuses on the microbiological status at the time of root filling and its impact on radiological healing of apical periodontitis. The teeth were randomly subjected to one of three different treatment strategies. The root canal treatment was carried out as single visit treatment (single visit, SV group, $n = 20$), or using calcium hydroxide as root canal dressing between the first and second appointment (CH group, $n = 18$), or leaving the canal empty between the appointments (Empty canal, EC group, $n = 12$). The project was approved by the Committee on Investigations Involving Human Subjects at the University of North Carolina, School of Dentistry. All patients read and signed a consent form before initiation of the treatment.

Examination and Diagnosis

The initial examination entailed registration of soft tissue status, percussion sensitivity, tooth mobility, coronal, and radicular restorations present, presence of approximal contacts, antagonists, and prosthetic involvement, and marginal bone level. The pulpal diagnosis was made based on anamnesis, X-ray examination using individual bite-mount radiography, examination of pulpal contents, and patient response to initial instrumentation as described previously (7)

Endodontic Treatment and Microbiological Sampling

A rubber dam, clamp and ligature were used to establish the working field. Preparative crown build-up was performed whenever necessary for clamp and rubber dam retention. The access cavity was prepared before or after the application of the rubber dam, but the dam was always placed before entry into the pulp chamber. On completion of the access cavity and before entry into the pulp chamber, preparations for asepsis were carried out. The tooth, cavity, and one inch of the dam surrounding the tooth were disinfected for 1 min by vigorous swabbing with 0.12% chlorhexidine gluconate and thereafter only sterile instruments were used. Microbiological control samples taken from the working field after surface disinfection were uniformly negative.

Microbiological samples were taken twice during each appointment. After the initial access the first access sample (A1) was taken from the pulp cavity and/or the pulp canal with one or several sterile paper points. In some cases access into the canal space was eased by prior insertion and withdrawal of a small size K-type file. Mechanical root canal instrumentation was performed with stainless steel instruments according to a standardized method as previously described (7). Sodium hypochlorite (2.5%) was used as an irrigant during the preparation rinsing the canals thoroughly after each file size. When instrumentation was complete, the canal was dried using sterile paper points. To neutralize the antimicrobial action of sodium hypochlorite, an excess of sodium thiosulphate was used to irrigate thoroughly the entire canal, and the canal was dried again. A sterile reamer or file one size larger than the last apical file was chosen, inserted to the full canal length, turned clockwise 360 degrees and withdrawn. The apical 5 to 8 mm of the tip of the instrument was cut off with a sterilized or flamed cable cutter, making sure that the cut end fell into a vial with RTF [first post irrigation sample (Pi1)] (17). Flamed cable cutter was controlled not to cause contaminations during the sampling. At the second appointment, the root canals in the CH group were rinsed and neutralized with 0.5% citric acid and then with saline. The canals in the EC group were rinsed with saline. The canal walls in both groups were vertically worked on with a reamer the same size as the last used at the previous appointment. The liquid in the canal was soaked up in paper points until dry, and the points transferred aseptically to a vial with 1 ml RTF with glass beads [second access sample (A2)]. A second post irrigation sample (Pi2) was then taken with a reamer one size up as at the first appointment.

Laboratory Procedures

The bacteriological specimens were processed within 2 h. They were vortexed for 30 s and then transferred to an anaerobic chamber, where all further processing took place. The samples were serially diluted in RTF and cultivated anaerobically on sheep blood cell agar and incubated at 37°C for 7 days. Representatives of observed colony types were selected and pure cultures were made. The identification was done by routine biochemical procedures designed for clinical purposes. Therefore, some bacteria are not identified in detail.

Radiological Follow-Up

The periapical conditions were assessed radiographically using the PAI-system (15). The radiological examination was done with individually bite-mounted radiograph holders throughout the follow-up period. Control radiographs were taken at 4, 12, 26, and 52 weeks after the therapy and the PAI scores was recorded. Periapical healing, calculated as a reduction in PAI score from baseline, in different treatment groups was compared. In addition, because of the aim to find associations between microbiological status at the time of root filling and periapical healing, the teeth in groups CH and EC were pooled and then subdivided in a group with bacteria present (BP group) and one with bacteria absent (BA group) according to whether or not A2 sample was positive for growth. The change of PAI was analyzed at 52 wk using one-way ANOVA.

Results

Microbiology

First Appointment

All three groups showed comparable proportions of growth positive samples taken during the first appointment. In the access sample (A1) all cases included the study were growth positive. Post irrigation samples (Pi1) in—the SV, CH, and EC groups showed growth percentages of 20, 22, and 33%, respectively (Table 1).

Second Appointment

CH group

Six A2-samples (33%) showed growth. Chemomechanical preparation during the second appointment eliminated microorganisms efficiently and no growth was detected in the Pi2 samples (Table 1).

EC group

The sampling taken in the beginning of the second appointment (A2) showed growth in eight samples (67%). After irrigation with sodium hypochlorite only two Pi2 samples (17%) showed growth (Table 1).

Microorganisms

Identification of the isolated microorganisms revealed a number of species. The access sample in the first appointment (A1) showed generally mixed infections dominated by anaerobic microorganisms. Although the total number of growth positive cases decreased approx-

TABLE 1. Number of cases (%) with microbial growth in samples taken during first and second appointment

Group	First appointment		Second appointment	
	Access (A1) n (%)	Post irrigation (Pi1) n (%)	Access (A2) n (%)	Post irrigation (Pi2) n (%)
Single visit	20 (100)	4 (20)	—	—
Calcium hydroxide	18 (100)	4 (22)	6 (33)	0 (0)
Empty canal	12 (100)	4 (33)	8 (67)	2 (17)

TABLE 2. Microbiological findings in growth-positive samples taken in the beginning of the treatment and after chemomechanical preparation during the first appointment

Species	A1, n (%) n^{tot} = 50 (100)	Pi1, *n* (%) n^{tot} = 12 (100)
Gram-positive anaerobic rods	33 (66)	8 (67)
Peptostreptococcus sp.	20 (40)	2 (17)
Gram-positive facultative rods	17 (34)	5 (42)
Gram-negative anaerobic rods	17 (34)	6 (50)
Veillonella sp.	13 (26)	3 (25)
Alpha-haemolytic *Streptococcus* sp.	12 (24)	2 (17)
Fusobacterium sp.	6 (12)	0 (0)
Prevotella sp.	5 (10)	1 (8)
Black-pigmented Gram-negative rods	4 (8)	1 (8)
Porphyromonas gingivalis	2 (4)	0 (0)
Enterococcus faecalis	1 (2)	0 (0)
Capnocytophaga sp.	1 (2)	0 (0)
Leptotrichia buccalis	1 (2)	0 (0)
Pseudomonas aeruginosa	1 (2)	0 (0)
Enterobacter cloacae	1 (2)	0 (0)
Candida albicans	1 (2)	0 (0)

Sp. = species
A1 = Access sample 1
Pi1 = Post irrigation sample 1.

TABLE 3. Species isolated in the beginning of the second appointment (A2-sample) in Calcium hydroxide- and Empty canal– groups

Species	Calcium hydroxide, *n* (%), n^{tot} = 6 (100)	Empty canal, *n* (%), n^{tot} = 8 (100)
Gram-positive facultative rods	3 (50)	3 (38)
Gram-negative anaerobic rods	3 (50)	2 (25)
Alpha-haemolytic *Streptococcus* sp.	2 (33)	2 (25)
Peptostreptococcus sp.	1 (17)	2 (25)
Veillonella sp.	1 (17)	1 (13)
Prevotella sp.	1 (17)	1 (13)
Enterococcus faecalis	1 (17)	0 (0)
Gram-positive anaerobic rods	0 (0)	4 (50)
Bacteroides sp.	0 (0)	1 (13)

sp. = species.

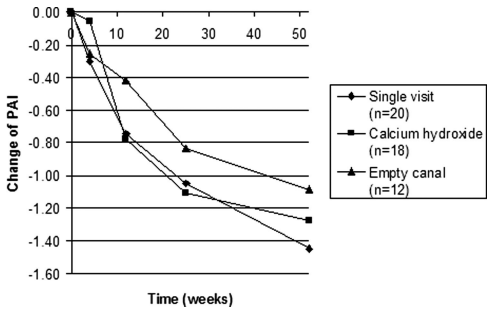

Figure 1. Change of PAI in different treatment groups.

imately to one fourth during the first appointment, the relative proportions of the different microbial groups remained comparable (Table 2).

The samples taken during the second appointment showed somewhat different relative proportions of microbes in comparison to the ones isolated during the first appointment. While many strict anaerobes were present, Gram-positive facultative microorganisms slightly dominated (Table 3).

Radiological Follow-Up

The SV, CH, and EC groups showed only minor differences in the radiological healing of the periapical lesion. The mean change of PAI in each group at 1 yr control was −1.45, −1.28, and −1.09, respectively (Fig. 1).

The bacteriological status at the appointment of root filling had a remarkable impact on the healing of the lesion. In the BA group the mean change of PAI at the 1-yr control was −1.53, whereas the BP group showed only −0.79 mean change of PAI. This difference was statistically significant ($p < 0.01$) (Fig. 2).

Discussion

The effect of different treatment procedures on the control of root canal infection and its impact on the radiologically observed healing of the periapical lesion was studied. The access sample (A1) taken during the first appointment showed generally mixed cultures dominated by anaerobes. The microbiological status found is in accordance to the other studies on the cultivable microflora of root canal infections before treatment (1). Chemomechanical preparation decreased the percentage of growth positive samples (Pi1) to 20 to 33%. This indicates a good efficacy of mechanical instrumentation and sodium hypochlorite against the infective microflora within a short period of time, and is in accordance with previous studies (3, 4, 8, 18). Microbes that survived during the chemomechanical preparation may have avoided efficient concentrations by penetration into dentinal tubules or by biofilm formation on dentin, or because of inactivation of medicaments (19, 21).

The EC group showed growth in 67% of the samples taken in the beginning of the second appointment (A2). This is clearly more than the percentage of growth positive samples after chemomechanical preparation during the first appointment (Pi1, 33%) and probably reflects regrowth of bacteria that were reduced below the detection limit, but not totally eliminated. Therefore, already during the first appointment all root canals should be carefully prepared to an adequate length and size, and irrigated with antimicrobial and tissue dissolving solution in before interappointment dressing or permanent obturation. Unfortu-

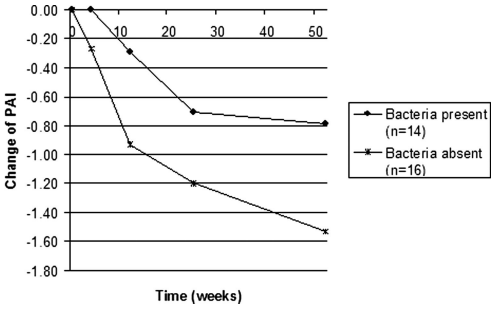

Figure 2. Change of PAI in cases with bacteria absent or present at the second appointment. Single visit cases are not included.

nately, this is not always the case in general practice and the microorganisms remaining in partly prepared root canals may cause an unfavourable treatment response.

A surprisingly high proportion (33%) of samples taken after calcium hydroxide dressing also showed growth. The antimicrobial activity of calcium hydroxide is based on its high alkalinity and it is effective on the majority of bacteria isolated from root canal infections, in vitro (6). However, some microorganisms often found in persistent root canal infections, such as *E. faecalis* and *C. albicans*, have been demonstrated to be resistant against calcium hydroxide (3, 6, 14). These organisms were, however, found only few times in the present study, whereas other microorganisms, such as *Veillonella* sp., *Peptostreptococcus* sp. and alpha-hemolytic streptococci, were isolated during the second appointment after calcium hydroxide dressing. Interestingly, in vitro studies have shown sensitivity of these organisms to calcium hydroxide (8). Possibly calcium hydroxide loses its antimicrobial activity in the root canal dependent on local conditions. This has been demonstrated in vitro (20, 21), and emphasizes the need for development of more effective medicaments for disinfection of the root canal system.

The present study is an extension of a study outcome after single-visit vs. two-appointment therapy of chronic apical periodontitis (15). The teeth included in the present study were those for which both bacteriological and radiological data were complete. Similar to Trope et al. (15), we found no remarkable differences between the treatment groups. However, the bacteriological status at the second appointment had a remarkable impact on the healing of the periapical lesion. Irrespective of the treatment group, the change of PAI at 52 wk control was significantly better in the cases with no cultivable microorganims at the appointment of root filling compared with cases in which bacteria were isolated. These results concur with the findings of Sjögren et al. (22).

The present results question the efficacy of calcium hydroxide as an interappointment dressing. However, calcium hydroxide is an efficient antimicrobial agent and tissue solvent and remains a recommended interappointment dressing, not least in cases with complicated root canal anatomy that confounds efficient chemomechanical preparation (4, 23). Because of the various good properties of calcium hydroxide, a combination with, e.g., chlorhexidine, to improve the antimicrobial activity, should be investigated clinically (3).

It must be emphasized that an absence of bacteria before obturation resulted in the best treatment results. Therefore, rather than to conclude on the relevance of single vs. multiple visit protocols, one should continue to search for better antibacterial protocols to ensure that canals are predictably rendered bacteria-free before root filling.

References

1. Dahlén G, Haapasalo M. Microbiology of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds. Essential Endodontology, 1st ed. Oxford, UK: Blackwell Science, 1998.
2. Munson MA, Pitt Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 2002;81: 761–6.
3. Waltimo TMT, Ørstavik D, Sirén EK, Haapasalo MPP. In vitro susceptibility of *Candida albicans* to four disinfectants and their combinations. Int Endod J 1999;32:421–9.
4. Zehnder M, Grawehr M, Hasselgren G, Waltimo T. Tissue dissolution capacity and dentine disinfecting potential of calcium hydroxide mixed with irrigating solutions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:608–13.
5. Fava LRG, Saunders WP. Calcium hydroxide pastes: classification and clinical indications. Int Endod J 1999;32:257–82.
6. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in treatment of infected root canals. Endod Dent Traumatol 1985;1:170–5.
7. Kerekes K, Tronstad L. Long-term results of endodontic treatment performed with a standardized technique. J Endod 1979;5:83–90.
8. Byström A. Evaluation of endodontic treatment of teeth with apical periodontitis. University of Umeå, Umeå, Sweden, 1986.
9. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119–25.
10. Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375–9.
11. Waltimo TMT, Sirén EK, Torkko HLK, Olsen I, Haapasalo MPP. Fungi in therapy-resistant apical periodontitis. Int Endod J 1997;30:96–101.
12. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1–7.
13. Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001;34: 429–34.
14. Waltimo TMT, Sirén EK, Ørstavik D, Haapasalo MPP. Susceptibility of oral *Candida* species to calcium hydroxide *in vitro.* Int Endod J 1999;32:94–8.
15. Ørstavik D, Kerekes K, Eriksen HM. The periapical index: scoring system for radiographic assessment of apical periodontitis. Endod Dent Traumatol 1986;2:20–34.
16. Trope M, Delano EO, Orstavik D. Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. J Endod 1999;25:345–50.
17. Syed SA, Loesche WJ. Survival of human dental plaque flora in various transport media. Appl Microbiol 1972;24:638–44.
18. Spångberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro.* Oral Surg Oral Med Oral Pathol 1973;36:856–81.
19. Sen BH, Safavi KE, Spångberg LS. Colonization of *Candida albicans* on cleaned human dental hard tissues. Arch Oral Biol 1997;42:513–20.
20. Portenier I, Haapasalo H, Rye A, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxyapatite and bovine serum albumin. Int Endod J 2001;34:184–8.
21. Haapasalo H, Sirén E, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of local root canal medicaments by dentin. Int Endod J 2000;33:126–31.
22. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.
23. Law A, Messer H. An evidence-based analysis of the antibacterial effectiveness of intracanal medicaments. J Endod 2004;30:689–94.