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Microbiological Evaluation of One- and Two-Visit Endodontic Treatment of Teeth with Apical Periodontitis: A Randomized, Clinical Trial

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The antimicrobial efficacy of endodontic procedures performed in one-visit (including a 10-min intraappointment dressing with 5% iodine-potassium-iodide) was compared with a two-visit procedure (including an interappointment dressing with calcium-hydroxide paste). Teeth with apical periodontitis (*n* = 96) were randomly assigned to either group. Root canal sampling and culturing were performed before and immediately after instrumentation, and after medication. Initial sampling demonstrated the presence of microorganisms in 98% of the teeth. Postinstrumentation sampling showed reduction of cultivable microbiota. Antibacterial dressing further reduced the number of teeth with surviving microbes. In the postmedication samples, residual microorganisms were recovered in 29% of the one-visit teeth and in 36% of the two-visit treated teeth. No statistically significant differences between the groups were discerned. It was concluded that from a microbiological point of view, treatment of teeth with apical periodontitis performed in two appointments was not more effective than the investigated one-visit procedure.

Periapical pathology evolves as a response to microorganisms present in the root canal. Consequently, endodontic treatment tries to eradicate the microbes from the root-canal system to promote periapical healing.

The intracanal microbiota is combated by mechanical instrumentation in combination with antimicrobial substances. Historically, several appointments were used, taking advantage of interappointment dressings (1). Gradually the number of sessions has been reduced, and a two-visit model using an interappointment dressing with calcium hydroxide (CH) has been proposed as a standard (2–4). Mechanical instrumentation is the most important step in the antimicrobial treatment of the root canal (5, 6). However, studies have shown that the adjunct of intracanal antimicrobial agents will add significantly to its effectiveness (2–4, 7).

In recent years, rotary nickel-titanium systems have facilitated the mechanical instrumentation of the canal and made the procedure less time consuming, and the potential for completion of the treatment in one visit has increased. Several authors have suggested such a rationale (8, 9), whereas others have questioned the antimicrobial efficacy of a one-visit procedure (10).

Although CH is routinely used as an interappointment dressing, it has been proven to be ineffective in short applications (10 min) (3) and should not be included in a one-visit procedure. It has been suggested that iodine-potassium-iodide (IPI) compounds be used as short duration antimicrobial agents (11). To reduce the time necessary to achieve and enhance the disinfecting effect, removal of the smear layer is important (12).

This study was designed to compare the microbiological outcome of a one-visit treatment regime, including a 10-min intraappointment dressing with 5% IPI, after removal of the smear layer with a standard two-visit procedure, including an interappointment dressing with CH.

MATERIALS AND METHODS

Approval for the project was obtained from the Göteborg University committee for research on human subjects. The experimental material was acquired from patients referred to the Department of Endodontology, Göteborg University. Asymptomatic teeth with necrotic pulps and apical periodontitis as verified radiographically were consecutively enrolled in the study and were assigned to one- or two-visit treatment at random. The randomization procedure was performed before clinical examination using the "minimization method" as described by Pocock (13). Two randomization factors were considered: tooth group and size of periapical lesion (Table 1). Teeth with unfavorable conditions for rubber-dam application were not accepted. Eighty-five patients (45 females; mean age, 55 yr) with 96 eligible teeth consented to participate in the study. Seven patients contributed more than one tooth.

Intracanal Procedures

Each tooth was isolated with a rubber dam and disinfected with 30% hydrogen peroxide and 10% iodine tincture according to the protocol proposed by Möller (14). After inactivation with 5% sodium thiosulphate fluid, the sterility of the operative field was checked. The tooth surface was scrubbed with a charcoal-impregnated cotton pellet, which was transferred to a bottle of transport medium (VMGA III) (14, 15). The pulp chamber was accessed and the working length established radiographically.

INITIAL SAMPLE

Sampling fluid (VMGA I) was introduced and the canal sequentially enlarged with nickel-titanium instruments for rotary (GT®/Profile® Dentsply-Maillefer, Ballaigues, Switzerland) and/or hand use (Nitiflex® Dentsply-Maillefer), reaching size ISO #25 at the working length. The entire canal content was absorbed by means of charcoal points and transferred to VMGA III. The canals were then further enlarged and prepared apically to between size ISO #40 and #60 while irrigating with 0.5% NaOCl.

POSTINSTRUMENTATION SAMPLE

To inactivate the NaOCl the canals were filled with 5% sodium thiosulphate for 30 s. Using a new set of sterile instruments, the canals were filled with VMGA I and dentinal shavings were produced with Hedstrom files ISO#25 to the working length. The entire canal content was absorbed by means of charcoal points and transferred to VMGA III. Up to this point, treatment and sampling procedures were identical for both groups.

ONE-VISIT GROUP

To remove the smear layer, the canals were filled with Tubulicid Plus® (Dental Therapeutics AB, Nacka, Sweden) for 20 s, dried with paper points, and refilled for an additional 20 s. Subsequently, the canals were filled with 5% IPI solution for 10 min. The IPI was inactivated with 5% sodium thiosulphate and the canals sampled for microorganisms according to the same protocol as earlier described. Finally, root canals were obturated.

TWO-VISIT GROUP

CH was placed meticulously by means of a Lentulo-spiral, and the access cavity sealed with Coltosol®. One week later, root canal instruments and simultaneous irrigation with VMGA I were used to remove the CH. Sampling was subsequently performed as described above. Finally, root canals were obturated. All samples reached the laboratory within 24 h.

Microbiological Examination

The samples were processed at the laboratory as outlined by Möller (14, 15). Anaerobic incubation was performed on a Brucella blood agar using hydrogen combustion in jars for 5 to 7 days while aerobic incubation was made on a blood agar plate for 2 to 3 days and a semiliquid medium (Hunton medium) (14) inoculated under flow of oxygen-free gas.

The initial samples were incubated undiluted and diluted 10^{-2} for estimation of total viable counts expressed as the number of colony-forming units per milliliter of transport medium.

The bacterial species or groups were identified on the basis of colony morphology, Gram-stain characteristics, and their ability to grow in the absence or presence of oxygen. Streptococci were speciated by the API-system (API-Strep and API-Zym, Les Balmes de Grottes, Montalieu, France). Special attention was paid to isolates producing polysaccharides based on their colony morphology on Mitis-Salivarius agars (MS Difco Laboratories, Detroit, MI) plates. Staphylococci were identified after growth on *Staphylococcus* agar (Difco). Enterococci were identified after growth on tellurite agar (Difco) plates. Lactobacilli were identified after growth on Rogosa agar (Difco) plates. Suspected enterics were identified by growth in Drigalski agar and speciated using the API system (API 20E). Anaerobic bacterial species were further identified after gas-chromatographic analyses of metabolic end products and grouped by genus. Black-pigmented, Gram-negative bacteria were further identified as *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, or *Prevotella* species on the basis of their auto fluorescence and their ability to agglutinate erythrocytes. Growth was semiquantified as very heavy, heavy, moderate, sparse, and very sparse on the agar plates corresponding approximately to $>10{,}000$, $<10{,}000$, $<1{,}000$, <100, and <10 colony-forming units per 0.1 ml VMGA III, respectively.

Statistical Methods

Chi-square test was used for comparisons between groups. Mantel-Haensel Chi-square test was used to test trend in contingency tables. All hypothesis tests were conducted at the 0.05 level of significance.

RESULTS

Teeth from different tooth groups and different lesion sizes were evenly distributed between the two groups (Table 1). Sterility checks of the operative field were negative in 91% of 140 samples taken. The results of sampling and culturing are displayed in Tables 2 to 6.

Initial sampling from the root canals demonstrated the presence of microorganisms in 98% of the investigated teeth. Strains classified as anaerobes were present in 74% of the samples and constituted 66% of 297 recovered strains. On comparison, the

TABLE 1. Distribution of teeth by randomization factors

	One Visit (n = 52)	Two Visits (n = 44)
Tooth group		
Incisors and canines	26 (50%)	25 (57%)
Bicuspids	16 (31%)	10 (23%)
Molars	10 (19%)	9 (20%)
Size of lesion		
2 mm diameter	5 (10%)	2 (5%)
>2 to 5 mm diameter	29 (56%)	23 (52%)
>5 mm diameter	18 (35%)	19 (43%)

TABLE 2. Classification of bacterial strains isolated from initial, postinstrumentation, and postmedication samples (n = 288)

	IS		PIS		PMS	
	One Visit	Two Visits	One Visit	Two Visits	One Visit	Two Visits
Anaerobs						
Fusiform rods	22	19	7	6	3	2
Prevotella spp.	34	29	8	7	5	1
Porphyromonas spp.	5	3	1		1	
Campylobacter spp.	1	1		1		
Capnocytophaga spp.	2		1			
Actinomyces spp.	1	1		1	1	1
Anaerobic lactobacilli	1		1		1	
Bifidobacterium sp.		1				
Other G+ anaerobic rods	20	20	9	10	4	4
Veillonella spp.		1	1			
Peptostreptococcus spp.	20	15	10	7	3	1
No. of anaerobs	106	90	38	32	18	9
Facultatives						
Coliform rods		3				
Klebsiella spp.	1		1			
Proteus spp.	1		1		1	
Pseudomonas spp.	1		1			
Other G− aerobic rods		1				
Lactobacillus spp.	6	15	2	5		4
Other G+ aerobic rods	1	2	1	2	1	
G+ aerobic spore-forming rods	3		1			1
Streptococcus spp. (NPSP)*	17	13	6	7	5	2
Streptococcus spp. (PSP)†	13	6	5	2		4
Enterococcus spp.	4	4	1	3	1	3
Staphylococcus spp.	5	3	5	5	3	7
Micrococcus spp.	1	1	1			
No. of facultatives	53	48	25	24	11	21
Total no. of strains	159	138	63	56	29	30
Semiquantification						
Very sparse growth	13	12	19	23	11	11
Sparse growth	25	31	28	25	11	6
Moderate growth	73	68	11	4	6	11
Heavy growth	47	26	4	4	0	2
Very heavy growth	1	1	1	0	1	0

* Nonpolysaccaride-producing species.
† Polysaccaride-producing species.
IS = initial sample; PIS = postinstrumentation sample; PMS = postmedication sample.
Results of the semiquantification also are displayed.

groups exhibited only minor differences regarding the quantity and quality of the microflora revealed.

The postinstrumentation sampling showed reductions of cultivable microbiota. However, bacteria were still found in 62% of teeth in the one-visit group and 64% in the two-visit group. The composition of the microbiota in the two groups remained similar and no statistically significant differences were found.

The postmedication sampling revealed residual microorganisms in 29% of teeth in the one-visit group and 36% of two-visit group. In the former, facultatives were present in 8 teeth (15%), and in the two-visit group, facultatives were found in 12 teeth (27%). However, no statistically significant differences between groups were discerned.

DISCUSSION

The outcome of endodontic treatment procedures is commonly determined by means of clinical and radiographic examination and

TABLE 3. Results of initial, postinstrumentation, and postmedication sampling

	IS		PIS		PMS	
	One Visit	Two Visits	One Visit	Two Visits	One Visit	Two Visits
No growth	1 (2%)	1 (2%)	20 (38%)	16 (36%)	37 (71%)	28 (64%)
Anaerobs only	15 (29%)	10 (23%)	12 (23%)	10 (23%)	7 (13%)	4 (9%)
Facultatives only	15 (29%)	8 (18%)	15 (29%)	10 (23%)	6 (12%)	11 (25%)
Mixed flora*	21 (40%)	25 (57%)	5 (10%)	8 (18%)	2 (4%)	1 (2%)
Statistics**	p = 0.42 (NS)		p = 0.64 (NS)		p = 0.36 (NS)	

IS = initial sample; PIS = postinstrumentation sample; PMS = postmedication sample; NS = not significant.
* Facultatives and anaerobes found together.
** Chi-square test (DF = 3).

TABLE 4. Distribution of initial, postinstrumentation, and postmedication samples according to number of recovered strains per sample

No. of Strains	IS		PIS		PMS	
	One Visit	Two Visits	One Visit	Two Visits	One Visit	Two Visits
None	1 (2%)	1 (2%)	20 (38%)	16 (36%)	37 (71%)	28 (64%)
One	15 (29%)	5 (11%)	16 (31%)	7 (16%)	7 (14%)	6 (14%)
Two	3 (6%)	10 (23%)	8 (15%)	15 (34%)	5 (10%)	7 (16%)
Three	11 (21%)	9 (20%)	4 (8%)	5 (11%)	1 (2%)	2 (4%)
Four or more	22 (42%)	19 (43%)	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Statistics*	p = 0.49 (NS)		p = 0.63 (NS)		p = 0.5 (NS)	

IS = initial sample; PIS = postinstrumentation sample; PMS = postmedication sample; NS = not significant.
* Mantel-Haenzel Chi-square test.

often is described in terms of "success" and "failure." However, periapical healing is sometimes slow and final results may not be possible to assess until 5 to 10 yr after treatment (1). Furthermore, authors have drawn attention to the difficulties in defining and maintaining criteria in radiographic evaluation of the periapical tissues (16). A more immediate outcome of endodontic treatment is the reduction or elimination of microorganisms from the root canal. Some studies have shown that periapical healing is more predictable in teeth that yield a negative culture at the time of root filling (17, 18), whereas other authors have challenged the significance of a negative culture (19). In any case, the elimination of cultivable microorganisms from the root canal system remains an important objective of endodontic treatment and may serve as a surrogate end point in clinical trials.

The diagnostic accuracy of intracanal sampling and culturing has been questioned (20–22). To avoid false-negative results, residual microorganisms should be given the opportunity to recover after instrumentation and medication by leaving the canal empty for a period before sampling. In the present study, root canal samples were obtained directly after instrumentation and medication procedures. Dressing remnants also influence the diagnostic accuracy and "culture reversals" sometimes occur (20, 21, 23). "Culture reversals" were observed in the present study (Table 6). Hence, conclusions regarding the differences in the quality and quantity of the recovered microbiota before and after intracanal procedures must be made with caution.

In accordance with other findings, our initial samples were found to be dominated by anaerobes (5, 7, 24, 25) and instrumentation and irrigation procedures considerably reduced the cultivable microflora (4, 5). The antimicrobial medication further decreased the number of teeth with persistent, cultivable microorganisms. However, in contrast to the findings of Byström et al. (2), Sjögren et al. (3), and Shuping et al. (4), but in agreement with Reit & Dahlén (20), Ørstavik et al. (26) and Molander et al. (25), a considerable number of teeth, 29% in the one-visit group and 36% in the two-visits group, were found to still harbor survived microorganisms after completed antimicrobial treatment. The difference between the groups was not statistically significant.

Previous investigations have shown inferior antimicrobial efficacy of IPI compared with CH when used as interappointment dressings (25, 27). IPI is quite rapidly inactivated by remaining organic material in the root canal and its antimicrobial activity is reported to persist for between 1 and 3 days (14, 28). Consequently, IPI used as an interappointment dressing may increase the risk of multiplication of residual microorganisms or reinfection of the canal. In our study, IPI was used as a short-duration intraappointment dressing followed by immediate obturation. The antimicrobial effect of IPI in this study may have been enhanced by the use of Tubulicid Plus®, partly because of its smear layer removing capacity (29) and partly because of its having antimicrobial activity of its own (30). On the other hand, no smear layer removing procedure was used in the two-visit group; a protocol that might have negatively influenced the treatment outcome.

In agreement with other findings, the relative frequency of facultative anaerobic strains increased as a result of instrumentation and medication (20, 25, 31). In the one-visit group, 38% of the remaining strains were classified as facultatives, whereas the corresponding figure for the two-visit group was 70%. The difference between groups did not reach statistical significance (Table 3). However, IPI has a broader antimicrobial spectrum than CH and might serve as an important adjunct in the reduction of facultatives (25).

After medication, 29% and 36% of teeth in the one- and two-visit were found to harbor residual microbiota. Hence, the goal of obtaining a "sterile" canal before obturation was inconsistently reached. It has been suggested that the fraction of teeth with positive cultures may be further reduced by adding ultrasonics (32) and rinsing with more potent smear layer removing solutions (33). However, neither higher concentrations of NaOCl (7) or an ex-

TABLE 5. Distribution of initial, postinstrumentation, and postmedication samples according semiquantification of the dominating strain(s)

Growth	IS		PIS		PMS	
	One Visit	Two Visits	One Visit	Two Visits	One Visit	Two Visits
None	1 (2%)	1 (2%)	20 (38%)	16 (36%)	37 (71%)	28 (64%)
Very sparse	5 (10%)	2 (4%)	11 (21%)	10 (23%)	7 (13%)	8 (18%)
Sparse	8 (15%)	6 (14%)	11 (21%)	11 (25%)	5 (10%)	1 (2%)
Moderate	19 (36%)	19 (43%)	5 (10%)	4 (9%)	2 (4%)	6 (14%)
Heavy	18 (35%)	15 (34%)	4 (8%)	3 (7%)	0 (0%)	1 (2%)
Very heavy	1 (2%)	1 (2%)	1 (2%)	0 (0%)	1 (2%)	0 (0%)
Statistics*	p = 0.6 (NS)		p = 0.84 (NS)		p = 0.4 (NS)	

IS = initial sample; PIS = postinstrumentation sample; PMS = postmedication sample; NS = not significant.
* Mantel-Haenzel Chi-square test.

TABLE 6. Distribution of teeth according to the sequence of the results of the initial, postinstrumentation, and postmedication samples

	One Visit	Two Visits
IS, PIS, and PMS negative	1 (2%)	1 (2%)
IS, PIS, and PMS positive	13 (25%)	11 (25%)
IS and PIS positive; PMS negative	19 (36%)	17 (39%)
IS positive; PIS and PMS negative	17 (33%)	10 (23%)
IS positive, PIS negative, PMS positive "Culture reversals"	2 (4%)	5 (11%)
Statistics*	p = 0.16 (NS)	

IS = initial sample; PIS = postinstrumentation sample; PMS = postmedication sample; NS = not significant.
* Chi-square test.

tended dressing period with CH (3, 25) have been shown to increase the antimicrobial efficiency. The role of entombed microorganisms in the dentine body of obturated roots is still unclear (34, 35).

In conclusion, the findings of our study indicated that given a meticulously instrumented root canal, a one-visit antimicrobial treatment including 10-min dressing with 5% IPI, might be as effective as a two-visit procedure using CH.

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References

1. Strindberg LZ. The dependence of the results of pulp therapy on certain factors. Acta Odontol Scand 1956;14(Suppl):21.
2. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1:170–5.
3. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antibacterial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119–25.
4. Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endodon 2000;26:751–5.
5. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321–8.
6. Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endodon 1998;24:763–7.
7. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg 1983;55:307–12.
8. Weiger R, Rosendahl R, Löst C. Influence of calcium hydroxide intracanal dressings on the prognosis of teeth with endodontically induced periapical lesions. Int Endod J 2000;33:219–26.
9. Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. Int Endod J 2002;35:660–7.
10. Trope M, Bergenholtz G. Microbiological basis for endodontic treatment: can a maximal outcome be achieved in one visit. Endod Topics 2002;1:40–53.
11. Safavi E, Spångberg L, Langeland K. Root canal dentinal tubule disinfection. J Endodon 1990;16:207–10.
12. Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142–9.
13. Pocock SJ. Clinical trials. A practical approach. Chichester, UK: John Wiley & Sons, 1983.
14. Möller ÅJR. Microbial examination of root canals and periapical tissues of human teeth. Odontol Tidskr 1966;74:1–380.
15. Dahlén G, Möller ÅJR. Microbiology in endodontic infections. In: Slots J, Taubman MA, eds. Contemporary oral microbiology and immunology. St. Louis: Mosby Year Book, 1992:444–75.
16. Reit C, Hollender L. Radiographic evaluation of endodontic therapy and the influence of observer variation. Scand J Dent Res 1983;91:205–12.
17. Engström B, Hård af Segerstad L, Ramström G, Frostell G. Correlation of positive cultures with the prognosis for root canal treatment. Odontol Revy 1964;15:257–70.
18. 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.
19. Heling B, Shapira J. Roentgenologic and clinical evaluation of endodontically treated teeth, with or without negative culture. Quintessence Int 1978;11:79–85.
20. Reit C, Dahlén G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. Int Endod J 1988;21:291–9.
21. Reit C, Molander A, Dahlén G. The diagnostic accuracy of microbiologic root canal sampling and the influence of antimicrobial dressings. Endod Dent Traumatol 1999;15:278–83.
22. 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.
23. Olgart LG. Bacteriological sampling from root canals directly after chemomechanical treatment. A clinical and bacteriological study. Acta Odontol Scand 1969;27:91–103.
24. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy 1974;25:347–58.
25. Molander A, Reit C, Dahlén G. The antimicrobial effect of calcium hydroxide in root canals pretreated by 5% iodine-potassium-iodide. Endod Dent Traumatol 1999;15:205–9.
26. Ørstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J 1991;24:1–7.
27. Safavi E, Dowden ED, Introcaso JH, Langeland K. A comparison of antibacterial effect of calcium hydroxide and iodine-potassium-iodide. J Endodon 1985;11:454–6.
28. Engström B. On duration of the antibacterial efficiency of four antiseptics used in root canal treatment *in situ.* Svensk Tandläkare-Tidskrift 1958;51:1–6.
29. Liolios E, Economides N, Parissis-Messimeris S, Boutsioukis A. The effectiveness of three irrigating solutions on root canal cleaning after hand and mechanical preparation. Int Endod J 1997;30:51–7.
30. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35–40.
31. Chavez de Paz L, Dahlén G, Molander A, Möller Å, Bergenholtz B. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. Int Endod J 2003;36:500–8.
32. Sjögren U, Sundqvist G. Bacteriologic evaluation of ultrasonic root canal instrumentation. Oral Surg 1987;63:366–70.
33. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. J Endodon 2003;29:170–5.
34. Peters LB, Wesselink PR, Moorer WR. The fate and role of bacteria left in root dentinal tubules. Int Endod J 1995;28:95–9.
35. Siqueira Jr JF. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001;34:1–10.