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CLINICAL RESEARCH

An Evidence-Based Analysis of the Antibacterial Effectiveness of Intracanal Medicaments

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The authors reviewed the literature evaluating the antibacterial effectiveness of intracanal medicaments used in the management of apical periodontitis. A PICO (problem, intervention, comparison, outcome) strategy was developed to identify studies dealing with calcium hydroxide, phenolic derivatives, iodine–potassium iodide, chlorhexidine, and formocresol. The final inclusion/exclusion criteria eliminated all papers except five that evaluated calcium hydroxide. The total sample size in the included studies was 164 teeth. Microbiologic sampling was performed before endodontic treatment (S1), after instrumentation and irrigation (S2), and after intracanal medication (S3). At S2, 62% of canals were positive. After medication, 27% still showed detectable growth. Of cultures that were positive at S2, 45% were still positive at S3. Most studies did not address issues of culture reversals or false positive and false negative cultures. The main component of antibacterial action appears to be associated with instrumentation and irrigation, although canals cannot be reliably rendered bacteria free. Calcium hydroxide remains the best medicament available to reduce residual microbial flora further.

The basis of endodontic treatment depends on identifying and eliminating the causative factors in the development of apical periodontitis so that optimal healing can be achieved. The role of bacteria and their byproducts in the pathogenesis of apical periodontitis has been clearly established (1–3). An improved prognosis has been shown for teeth after a negative culture has been obtained compared with teeth having a positive culture at the time of obturation (4, 5). Therefore, the prime objective of treatment is to eliminate bacteria and sources of nutrient supply from the root canal system.

Byström and Sundqvist (6–8) performed a series of landmark studies to evaluate the antibacterial effectiveness of mechanical instrumentation and irrigation. They found a 100- to 1000-fold reduction in bacterial counts after instrumentation and irrigation with saline; yet, all teeth still had a positive culture after the first appointment (8). The combined use of sodium hypochlorite and ethylenediaminetetraacetate (EDTA) as irrigants was shown to improve bacterial elimination significantly, but approximately 50% of teeth still harbored detectable bacteria after instrumentation (6, 7). The number of residual bacteria is usually low, but should the canal be left empty between appointments, the remaining bacteria can multiply to nearly the original levels (6, 8).

Antibacterial intracanal medication has been advocated to eliminate remaining bacteria after canal instrumentation and irrigation. Many medicaments have been used as intracanal dressings (9, 10) and according to their chemical basis, generally fall into the following categories: phenolic derivatives (eugenol, camphorated para-monochlorophenol, camphorated phenol, metacresyl acetate, beechwood creosote), aldehydes (formocresol), halides (iodine–potassium iodide), calcium hydroxide, antibiotics, and various combinations. The most popular intracanal medicament in use currently is calcium hydroxide.

Despite conflicting claims, no medicament appears to be ideal, and there is controversy over their use (11–13). Significant variability exists in clinical dental practice in treating teeth with infected, necrotic pulps and apical periodontitis.

Against this backdrop of clinical variability, there has been a move toward evidence-based dentistry, wherein clinical practice relies on the diligent and judicious use of the best available evidence from human clinical studies, combined with clinical expertise to formulate new or improved modes of intervention or treatment specific to the needs of the patient. Evidence-based dentistry focuses on answering clinical questions using a critical appraisal of published research. Hence, the purpose of this study was to analyze the endodontic literature evaluating the antibacterial effectiveness of intracanal medicaments in clinical studies. The ideal clinical question to be answered in this context can be framed in terms of a PICO question (problem [P], intervention [I], comparison [C], and outcome [O]), as follows: in patients undergoing endodontic treatment for apical periodontitis, does an intracanal medicament, compared with no intracanal medicament, result in elimination of bacteria from the root canal system, as measured by a negative culture? Because it has been shown that bacteria multiply rapidly when canals are left empty between appointments (6, 8), the design of a clinical study is unlikely to follow this format. Thus, the question was restated: In patients undergoing endodontic

TABLE 1. MEDLINE (OVID) search strategy developed to find articles related calcium hydroxide

No.	Search history	Results
1	Endodontics/or root canal therapy/or root canal treatment/or pulpectomy.mp.[mp=title, abstract, cas registry/ec number word, mesh subject heading]	9146
2	Limit 1 to human	7317
3	Calcium hydroxide.mp.[mp=title, abstract, cas registry/ec number word, mesh subject heading]	2540
4	Limit 3 to human	1586
5	2 and 4	419
6	Dressing/or medicament.mp.[mp=title, abstract, cas registry/ec number word, mesh subject heading]	423
7	5 and 6	8
8	Apical periodontitis.mp.[mp=title, abstract, cas registry/ec number word, mesh subject heading]	283
9	Limit 8 to human	241
10	5 and 9	15
11	Antibacterial effect/or antimicrobial effect.mp.[mp=title, abstract, cas registry/ec number word, mesh subject heading]	1341
12	Limit 11 to human	612
13	5 and 12	6

treatment for apical periodontitis, does medication eliminate residual bacteria from canals, compared with the same canals before medication, as measured by the number of positive cultures?

MATERIALS AND METHODS

To answer the clinically relevant question, a four-step method of evidence-based analysis was applied: Step 1, a search for the best evidence, conducting a comprehensive search of the endodontic literature in electronic databases; Step 2, appraisal and selection of papers according to study validity and clinical importance; Step 3, collection and analysis of the published evidence; and Step 4, determining the clinical applicability of the results.

A MEDLINE search strategy was developed to identify articles dealing with intracanal medicaments and endodontics. The search included articles in MEDLINE from 1966 to 2003. Using the PICO formatted question, methodologic MeSH (medical subject heading) terms were generated to make the search strategy more sensitive in identification of studies.

A large proportion of the dental literature focuses on laboratory and animal research. Although this literature is applicable by extrapolation, it lacks immediate clinical relevance to humans. To overcome this problem, a limit was placed on the identified articles so that the search addressed only human studies.

An outline of the MEDLINE search strategy for calcium hydroxide is presented in Table 1. Additional separate searches were conducted for other medicaments in the following groupings: (A) phenolic derivatives (camphorated para-monochlorophenol, camphorated phenol, cresatin, cresol, beechwood creosote, thymol); (B) iodine–potassium iodide; (C) chlorhexidine; and (D) formocresol. Because the number of references in each category was much smaller than for calcium hydroxide, the search was completed manually after Step 5.

All articles electronically identified by the MEDLINE search were scanned independently on the basis of the title, keywords, and abstract (when available) by two reviewers. When it was not possible to classify a paper, the full paper was obtained. The references of all eligible papers were hand-checked for more relevant studies. Reference lists from review articles were also surveyed.

The identified articles were examined for inclusion and exclusion based on several criteria (Table 2). It was decided that there was a stringent requirement for both a postinstrumentation (S2) sample (i.e. immediately before the medicament was placed) and a postmedication (S3) sample so that the effect of the medicament, separate from instrumentation and irrigation, could be assessed.

TABLE 2. Inclusion and exclusion criteria used in the evidence-based analysis

Inclusion criteria
1. Subjects had a noncontributory medical history
2. Subjects presented with mature teeth with infected necrotic root canals and radiographic evidence of periapical bone loss (as an indication of preoperative canal infection)
3. All selected root canals had not received any endodontic treatment previously
4. Subjects underwent nonsurgical root canal treatment during the study
5. Teeth were dressed with an intracanal medicament sealed in the canals
6. Microbiologic sampling was undertaken during the course of treatment, before canal preparation (S1), after canal preparation (S2), and after canal medication (S3)
7. Aerobic and anaerobic culturing techniques were performed on all samples
8. Treatment outcome was stated; the primary outcome measure was the number of teeth that had a positive and negative canal culture

Exclusion criteria
1. Inclusion of test teeth without infected necrotic root canal systems and/or radiographic evidence of periapical bone loss (hence no preoperative canal infection)
2. Study carried out on failed, endodontically treated teeth (retreatment cases)
3. No postinstrumentation sample (S2)
4. Use of two different antibacterial medicaments in succession, within the time frame of the study, in the same canal
5. Postmedication sample (S3) not taken immediately after removal of the test medicament
6. Repeated cleaning and irrigation procedures
7. Final cleaning and irrigation procedures performed during the second visit

Studies that met the above criteria underwent critical analysis. Extracted data included the number of subjects in the group; the number of dropouts or withdrawals, if reported; a description of the materials and methods, with a detailed assessment of the type of medicament used and the duration of its use; the times at which the microbiology of the canals was evaluated via sampling; and the

outcome variables used to measure the antibacterial effectiveness of the intracanal medicament.

Data from the studies concerning the number of teeth infected at the start of treatment, after chemomechanical preparation, and after an interappointment period of antibacterial medicament were tabulated for comparative analysis.

To focus attention on valid and applicable results, it was crucial to understand the strength of evidence of the studies identified in the search. There is a hierarchy of research design, with the highest levels of evidence preferred over lower levels of evidence. High levels of evidence (e.g. randomized controlled trials or systematic reviews with meta-analysis, prospective longitudinal studies) use control groups and randomization procedures, include adequate sample size, eliminate the effects of multiple variables or control for them, and are reproducible (14, 15). Consequently, as part of the analysis, the level of evidence of the studies was determined.

RESULTS

Included and Excluded Studies

The primary search from all sources identified 152 related references. After scanning of the titles and abstracts, the full text of 17 studies was obtained and underwent the data extraction process. These articles covered calcium hydroxide (13 papers), camphorated paramonochlorophenol (3 papers), camphorated phenol (1 paper), iodine–potassium iodide (3 papers), chlorhexidine (1 paper), and formocresol (2 papers). Based on the inclusion and exclusion criteria developed at the start of the literature search, all 17 studies were subsequently scrutinized for ultimate inclusion in the evidence-based analysis.

Several well-known studies met some but not all inclusion criteria and were excluded because of their research design and presentation of results. The main reasons for exclusion can be related to each excluded study. A list of these papers is given in Table 3. Consequently, of the 17 studies, only 5 met our final inclusion criteria. All 5 studies investigated the antibacterial effect of calcium hydroxide (Table 3).

Levels of Evidence

The results of the search found that the best available evidence was low, with all studies characterized as prospective pretest/posttest experimental trials. In this model, the outcomes were measured in the same human subjects before and after exposure to the intracanal dressing for comparison. No control participants were used—that is, patients with infected necrotic root canals, with radiographic evidence of periapical bone loss, treated by the same method of instrumentation and irrigation, but without dressing sealed in the canal between appointments.

Calcium Hydroxide: Analysis of Included Studies

The most significant of the criteria to be met was microbiologic sampling taken at two time points in the treatment sequence: postinstrumentation (S2), to take into account the antibacterial effect of instrumentation and irrigation of infected root canals; and postmedication (S3), to determine separately the antibacterial effect of the intracanal medicament. All 5 studies also included a preinstrumentation sample (S1).

The number of teeth observed per study ranged from 18 to 60, with a combined overall sample size of 164 teeth. Most of the teeth evaluated were single-rooted, single-canal incisors, canines, and premolars, although distal roots of mandibular molars (16) and mesiobuccal roots of mandibular first and second molars (17) were also included. The results were stated in terms of canals that had positive or negative cultures, rather than teeth.

TABLE 3. Excluded and included studies in the evidence-based analysis

Excluded studies	Reasons for exclusion	Included studies
Calcium hydroxide		Calcium hydroxide
Peciuliene et al. 2001	2	Peters et al. 2002
Molander et al. 1999	4,5	Shuping et al. 2000
Sundqvist et al. 1998	2,3	Yared and Bou Dagher 1994
Barbosa et al. 1997	4	Ørstavik et al. 1991
Reit and Dahlén 1988	3	Sjögren et al. 1991
Safavi et al. 1985	1,6	
Byström et al. 1985	3	
Cvek et al. 1976	1	
Iodine-potassium iodide		
Peciuliene et al. 2001	2	
Reit et al. 1999	3	
Safavi et al. 1985	1,6	
Camphorated paramonochlorophenol		
Barbosa et al. 1997	4	
Bystrom et al. 1985	3	
Koontongkaew et al. 1988	3	
Camphorated phenol		
Byström et al. 1985	3	
Chlorhexidine 0.2%		
Barbosa et al. 1997	4	
Formocresol		
Simon et al. 1979a	1,2,7	
Simon et al. 1979b	1,2,4	

A rubber dam and an aseptic technique were used throughout treatment for all teeth, and the crown and surrounding rubber dam were disinfected before the pulp chamber was entered. Canals were prepared using rotary nickel titanium files (17), nickel titanium hand files (16), or conventional hand files (18–20). Irrigation of the canals involved the use of physiologic saline (19) or varying concentrations of sodium hypochlorite, 0.5% to 2.0%. The final file sizes for canal preparation between studies and within each study were variable, ranging from ISO size 25 to 80, or No. 6 to 8 *Profile* 0.04 Tapers Series 29 (Tulsa Dental Products, Tulsa, OK). After canal preparation, the teeth were dressed with calcium hydroxide paste for 1 (17–20) or 4 weeks (16). The calcium hydroxide was either placed into the canals with a spiral filler (17–20) or plugged with a sterile paper point (16). Temporary restoration of teeth between appointments involved the use of two layers of Cavit and glass ionomer cement (16), or zinc oxide eugenol cement (18–20). In the study by Shuping et al. (17), the nature and material(s) of the temporary restorations were not stated.

Microbiologic sampling was carried out on all the canals before instrumentation (S1), after instrumentation and irrigation (S2), and then immediately after removal of the calcium hydroxide medicament (S3). The incidence of cultures with detectable growth (positive cultures) was assessed, as was the number of bacterial cells in these samples ($\log_{10}$). Canals were prepared for postinstrumentation sampling (S2) by inactivation of the remaining sodium hypochlorite with sterile sodium thiosulfate. Samples were taken by absorbing reduced transport fluid (16, 17) or saline (18, 20) with sterile paper points. In the case of Ørstavik et al. (19), dry dentine shavings from the tips of the last two files were transferred to reduced transport fluid vials.

Initial cultures (S1) were positive in almost all canals (162 of 164) (Table 4). After instrumentation (S2), 101 canals (62%) registered a positive culture. The percentage of positive cultures at S2 ranged from 14% (16) to 100% (18) of test canals. After medication (S3), 45 (27%) canals of the overall test sample showed detectable bacterial growth (Table 4). It must be noted that all canals were sampled at S3, not just canals with a positive culture at S2.

When compared with the number of positive cultures at S2 (101), 45% (45) of canals still had positive cultures after medication with calcium hydroxide. None of the studies reported how many teeth with positive cultures at S3 also had positive cultures at S2 or negative cultures at S2 (i.e. culture reversals). All the studies except that of Peters et al. (16) showed a decrease in the percentage of teeth with a positive culture at S3 in comparison with S2. Peters et al. (16) reported a dramatic increase in the number of teeth recording detectable growth. At S2, 3 of 21 (14%) teeth had a positive culture; however, after 4 weeks of calcium hydroxide dressing, 15 of 21 (71%) teeth had a positive culture.

Other Medicaments

No study met the inclusion criteria for evaluation in this evidence-based analysis, although several reported the frequency of positive cultures after medication (S3). Three microbiologic studies on the antibacterial effects of iodine–potassium iodide were found, although one investigated the retreatment of root-filled teeth with persistent chronic apical periodontitis (21). S3 samples reported 34% to 44% positive cultures after a minimum of 3 to 7 days dressing (22, 23). The combined antibacterial effect of chemomechanical preparation and camphorated para-monochlorophenol or camphorated phenol medication showed similar efficacy to iodine–potassium iodide, with 31% to 33% positive cultures at S3 (24, 25). Barbosa et al. (24) reported 8 of 36 (22%) positive S3 cultures using chlorhexidine.

DISCUSSION

The studies evaluated in this analysis reinforce the strong correlation between apical periodontitis and the presence of bacteria in canals (162 of 164 teeth, or 99%). Cleaning, shaping, and irrigation greatly reduce the cultivable numbers of bacteria, typically by 99% to 99.9%. However, these studies have shown that it is impossible to achieve a sterile root canal system in all cases by cleaning and shaping alone (62% remaining positive), in agreement with the findings of Byström and Sundqvist (6, 8). Residual bacteria are most often located in inaccessible areas such as isthmuses, ramifications, deltas, accessory and lateral canals, and dentinal tubules (26). If bacteria persist in the root canal system at the time of obturation, there is a higher risk of failure (4, 5). Hence, attempts to eliminate remaining bacteria involve the use of an antibacterial intracanal dressing.

Only five studies, all involving calcium hydroxide, met our criteria for evaluating separately the antibacterial effectiveness of a medicament in an infected canal. Its antibacterial effect on primary infection of the root canal system in vivo is only partially effective, with 27% of canals still positive after medication (Table 3). Other studies not included in this analysis, but reporting the frequency of positive and negative cultures at S3, have indicated a comparable range of outcomes (25, 27–29). Those studies evaluated the combined effect of cleaning and shaping plus canal medication, with the canals medicated for as long as 3 months. Interestingly, the percentage of positive cultures at S3 with the use of other medicaments (iodine–potassium iodide, CMCP, and chlorhexidine) was also in a similar range (22% to 44%) (22–25).

The variability in clinical techniques of the included studies makes definitive conclusions difficult regarding the antibacterial effectiveness of calcium hydroxide. The divergence between the studies may be partly explained by such factors as number of test

TABLE 4. Treatment outcome of orthograde endodontic treatment using calcium hydroxide as an antibacterial intracanal medicament

Study	Medicament duration (wks)	Teeth observed (n)	Positive cultures n (%)		
			Preinstrumentation S1	Postinstrumentation S2	Postmedication S3
Peters et al. 2002	4	21	21 (100)	3 (14)	15 (71)
Shuping et al. 2000	1	42	41 (98)	16 (38)	3 (7)
Yared and Bou Dagher 1994	1	60	60 (100)	60 (100)	19 (32)
Ørstavik et al. 1991	1	23	22 (96)	13 (57)	8 (35)
Sjögren et al. 1991	1	18	18 (100)	9 (50)	0 (0)
Total		164	162 (99)	101 (62)	45 (27)

teeth and tooth/canal type, instrumentation technique (hand vs. rotary nickel titanium files) and final file size, irrigants (NaOCl vs. saline), presence or absence of the smear layer, duration of calcium hydroxide dressing or any other intracanal disinfectant, method of removal of calcium hydroxide, and type of temporary restoration. Microbial coronal leakage may occur during treatment or between appointments, especially if inadequate measures are taken to temporize the tooth. There are also intrinsic problems in the diagnostic accuracy of microbiologic root canal sampling (22, 28, 30). For example, the anatomic complexity of the root canal system may preclude thorough cleaning but also present difficulties in retrieval of bacteria in a sample, and, after the use of an intracanal medicament, remnants may enter the sample and interfere with the microbiologic examination, calling attention to the risk of false negative and false positive culture results (22).

Culture reversals and the incidence of false negative and false positive cultures are important issues. Four of the studies (16–19) did not address these issues. Most of the studies did not separately report outcomes for positive S2 canals versus negative S2 canals at S3. Hence, the question of culture reversals arises. Microbiologic examination should also determine the total number of bacteria and characterize the bacteria to genus level for individual cases. In the study conducted by Peters et al. (16), specific bacteria were identified, and the percentage prevalence for overall positive samples was given. However, these results were not case-specific for the 21 test teeth. The findings by Sjögren et al. (20) were supported by bacterial identification to genus level in each individual case.

The results presented by Peters and Wesselink (13) do not fit well with the other studies analyzed in this paper. First, the postinstrumentation samples demonstrated a very low number of positive cultures (14%). The difference in results has been postulated to be caused by a difference in concentration of NaOCl. However, it has been shown that there is no significant difference in in vitro bacterial reduction by instrumentation and irrigation with 1%, 2.5%, and 5.25% NaOCl (31). Second, many more S3 cultures were positive, and S3 samples showed significantly higher bacterial counts, indicating regrowth of bacteria despite the placement of calcium hydroxide in the canals. The possibility of inadequate placement of calcium hydroxide or of interappointment contamination cannot be dismissed. Recent debate has also arisen concerning the role that calcium hydroxide may play in selecting for resilient microorganisms that may cause recalcitrant infection (32, 33).

The studies evaluated are the best currently available clinical evidence addressing the antibacterial effectiveness of calcium hydroxide as an intracanal medicament, separate from instrumentation and irrigation. The data suggest that the main component of antibacterial action during endodontic treatment of apical periodontitis is associated with mechanical instrumentation and irrigation with sodium hypochlorite and ethylenediaminetetraacetate. This paper reinforces the evidence that canals cannot be reliably rendered free of bacteria in 100% of cases. However, it is essential to reduce the microbial flora to as low a level as possible to ensure a successful outcome. Calcium hydroxide currently remains the best medicament available. Therefore, to maximize reduction of bacteria in the root canal before root canal filling, calcium hydroxide should be used as an interappointment dressing for a minimum of 7 days.

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