Vol. 96 No. 5 November 2003

ORAL SURGERY
ORAL MEDICINE
ORAL PATHOLOGY

ENDODONTICS

Editor: Larz S. W. Spångberg

The effect of chlorhexidine as an endodontic disinfectant

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Objective. The purpose of this study was to establish whether addition of a 2% chlorhexidine rinse to a conventional treatment protocol enhances the rate of the successful disinfection of the root canal system in vivo.
Study design. Twenty-four teeth with infected necrotic pulps and resorbing apical periodontitis were treated with a conventional technique in which 1% NaOCl as irrigant was used. Half of the cases received an additional rinse with 2% chlorhexidine. Prereduced thioglycollate medium was used to take cultures that were incubated for 4 weeks.
Results. Cultivable bacteria were retrieved at the conclusion of the first visit in 1 out of 12 chlorhexidine cases whereas in the control group 7 out of 12 cases showed growth. This difference was significant ($P < .05$).
Conclusion. The findings are clinically important.
(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:578-81)

Bacteria have long been recognized as the primary etiology in the development of periapical bone lesions.[1] When endodontic treatment is performed under aseptic conditions and according to accepted clinical principles, the success rate is generally high.[2,3] However, despite optimal endodontic therapy, some cases fail because there are root canal regions that cannot adequately be debrided and disinfected with existing instrumentation techniques and chemical agents. It is generally acknowledged that most treatment failures are caused by microorganisms persisting in the apical parts of root canals of filled teeth.[4,5]

Previous studies have shown that instrumentation and antibacterial irrigation with sodium hypochlorite will render about 50% to 75% of infected root canals free from bacteria at the end of the first treatment session, while the remaining root canals contain recoverable bacteria.[6,7] Consequently, the biomechanical preparation is usually combined with an intracanal antimicobial dressing such as $Ca(OH)_2$ in a multivisit procedure to reliably eliminate bacteria from root canal systems.[8,9]

The issue of single-visit and multiple-visit endodontic treatment of teeth with necrotic infected pulp has been the subject of debates for many years.[10,11] Studies comparing long-term outcomes after single-visit and multiple-visit endodontic treatment have shown that there are significantly fewer failures with 2-visit treatment.[3,12] However, a recent study suggested that there was no difference between single and multiple visits.[13]

One-step treatment of teeth with infected necrotic pulps, if successful, has the advantage of being less time-consuming because fewer visits for the patient are required. The goal of 1-visit treatment of teeth with necrotic infected pulps might be accomplished with a more effective antiseptic irrigant, used in conjunction with biomechanical procedures. This could render the dentin walls, pulp fragments, and any remaining organic debris free of viable bacteria to achieve a higher percentage of clinically predictable disinfection of pulp space.

Chlorhexidine is a potent antimicrobial agent that is particularly effective against *Enterococcus faecalis*, a microorganism that has been implicated in treatment failures.[14,15] Chlorhexidine has also been shown to have long-term antimicrobial properties because of its unique ability to bind to hydroxyapatite.[16] A gradual

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Received for publication Nov 1, 2002; returned for revision Jan 15, 2003; accepted for publication Feb 14, 2003.

1079-2104/2003/$30.00 + 0
doi:10.1016/S1079-2104(03)00168-9

release of this bound chlorhexidine could maintain an even level of the molecule sufficient to create a bacteriostatic milieu in the root canal over a prolonged period of time. This is in contrast to the effect of other disinfectants, which rapidly dissipate from the pulp space and have no residual antimicrobial effects.[17]

The purpose of this study was to establish whether the addition of 2% chlorhexidine to the conventional treatment protocol enhances the rate of the successful disinfection of the root canal system in vivo.

MATERIALS AND METHODS

Patient selection

The project was approved by the Committee on Investigations Involving Human Subjects at the University of Connecticut Health Center. The experimental subjects for the study were selected from referrals to the Department of Endodontology, University of Connecticut School of Dental Medicine. Primary criterion for inclusion of subjects in the study was the presence of a radiographically demonstrable apical periodontitis on a single-rooted tooth with a necrotic pulp. Patients were excluded from the study if (1) they were on antibiotics for the 2 weeks prior to treatment, (2) the tooth was not suitable for good rubber dam isolation, (3) the root canal in question had been entered or medicated before the inclusion stage, (4) there was a sinus tract, or (5) the initial culture resulted in no growth. The age of the patients ranged from 12 to 70 years. Informed consent was obtained from all subjects who participated in the study. A total of 24 teeth were treated.

Collection of root canal samples

Once teeth were deemed acceptable for study, they were treated as follows: After rubber dam isolation, the tooth was cleaned with 30% hydrogen peroxide followed by an application on the tooth surface and surrounding rubber dam of 5% tincture of iodine.[18] The disinfectant residue was inactivated with 10% sodium thiosulfate. The tooth surface in the area of the access opening was sampled and cultured in prereduced thioglycollate medium. An access opening was made with sterile burs. The working length was established by placing a sterile #15 K-file in the root canal and adjusting the file length to within 1 mm of the apex with the aid of an apex locator (Root ZX, J. Morita Inc, Irvine, Calif). To confirm the working length, a radiograph was taken with CDR digital radiography (Schick Technologies, Long Island City, NY). A sterile syringe and needle were used to introduce sampling fluid into the canal. Care was taken not to overfill the canal. A #20 K-file was manipulated to the working length by using the pumping maximum recovery (PMR) method for 1 minute with an effort to contact all canal walls and to suspend as many bacteria as possible.[18] The entire content of the root canal was absorbed by sterile paper cones and transferred to test tubes containing prereduced thioglycollate broth (BBL, Cockeysville, Md). This culture sample was designated *C1*. The canal was then debrided and instrumented by using a crown-down technique with ProFile GT and ProFile 0.04 taper files (Dentsply Tulsa Dental Products, Tulsa, Okla), after which the canal was irrigated with copious amounts of buffered 1% sodium hypochlorite solution. The apical portion of the canal was enlarged to 3 sizes beyond the initial measurement file. The root canal was dried with sterile paper cones, and any remnant of sodium hypochlorite solution was inactivated with 5 mL of 10% solution of sodium thiosulfate. The canal was dried and a bacteriologic sample of its contents was taken in the previously described manner. This culture sample was designated *C2*.

At this point, a dental assistant randomly assigned the tooth either to the experimental group or to the control group by drawing from a pool of lots and then prepared the appropriate syringe containing either sterile saline or chlorhexidine. These solutions henceforward are called *irrigating solution*. The operator was blinded to the solution being used.

Four mL of the irrigating solution was used in the canal for 30 seconds. The irrigating solution was agitated with a sterile master apical file size file to working length to make sure that all parts of the canal were treated. The canal was dried with sterile paper cones, and any remnant of irrigating solution was inactivated with 3 mL of 0.3% L-α-lecithin in 3% Tween 80 (Sigma, St Louis, Mo). The canals were dried with sterile paper cones and a sample of the root canal content was taken. This culture sample was designated *C3*.

Finally the canal was dried and filled with Ca $(OH)_2$ paste (DT Temporary Dressing $Ca(OH)_2$; Dental Therapeutics, Nacka, Sweden). A subsequent appointment was scheduled within 7 to 10 days.

The thioglycollate broth cultures were incubated at 37°C and visually inspected daily for the first 7 days, after which the cultures were observed weekly for another 3 weeks.

Statistical analysis

Data were evaluated statistically with a χ^2 test to assess the difference between the experimental group and the control group at the end of the first appointment. A value of $P < 0.05$ was considered statistically significant.

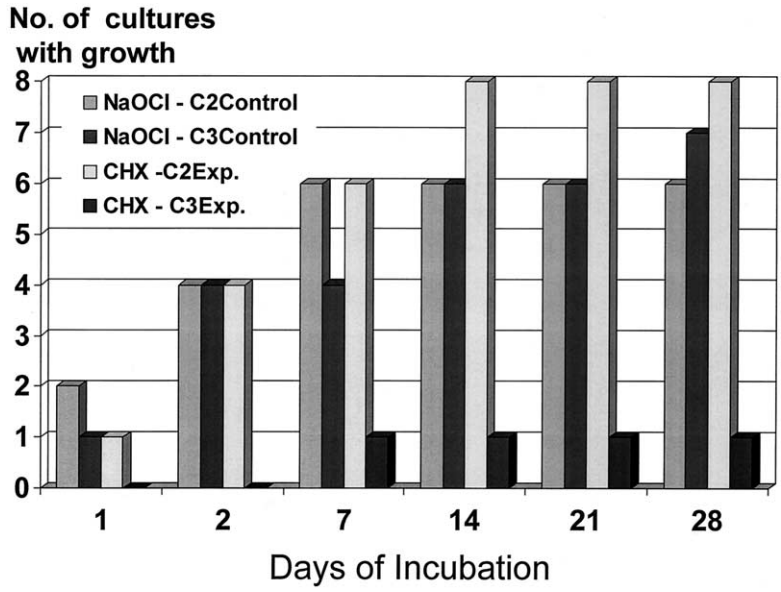

Figure. Number of samples with growth after completed mechanical instrumentation with 1% sodium hypochlorite (C2 control and C2 experimental) and after an additional irrigation with saline or 2% chlorhexidine (C3 control and C3 experimental). The samples were incubated for 28 days.

RESULTS

None of the disinfection controls showed growth. The pulp space of all 24 teeth contained bacteria at the beginning of treatment.

At the end of the first appointment, after 4 weeks of incubation, irrigation with chlorhexidine yielded 1 positive culture as compared with 7 positive cultures in the control group. This difference was significant ($P < .05$).

All cultures taken after the instrumentation with 1% sodium hypochlorite showed similar frequency and rate of growth (Figure). The difference occurred after the experimental teeth were treated with chlorhexidine instead of saline, which was used on the control teeth.

DISCUSSION

The results from the present study showed that, at the end of the first appointment, 2% chlorhexidine was significantly more effective than the saline control in providing a bacteria-free root canal. However, total disinfection of the root canal system of all teeth was not achieved. One of the 12 root canals treated with chlorhexidine showed growth at the end of the first visit. This tooth was also the only tooth diagnosed with exacerbating apical periodontitis and swelling at the initial treatment session. In this case, the highly effective PMR sampling technique might have retrieved microbes that were not accessible to the chlorhexidine rinse.

It is of importance to notice that sampling after conventional instrumentation and irrigation with sodium hypochlorite (culture C2) showed similar frequency and rate of growth for both groups of teeth. Only after the treatment with 2% chlorhexidine did the shift occur (culture C3). This shows that there were no major difference in seriousness of infection between the control and experimental group.

Hard tissue binding of chlorhexidine, although beneficial from a microbiologic aspect, was recognized to be problematic when bacteriologic sampling of the root canal content was performed because a significant amount of chlorhexidine in the sample may cause false negative results. A mixture of 0.3% L-α-lecithin in 3% Tween 80 was used to inactivate any residual antimicrobial effect of chlorhexidine.[19]

The characteristics of the prereduced fluid thioglycollate medium in the screw-capped vials made this medium suitable for transport and culturing of specimens from root canals in the present study. This medium has a high capacity to reduce oxygen, and no toxic intermediates of oxygen are accumulated in the medium when it is exposed to atmospheric oxygen. In addition, the agar of the medium prevents the oxygen to which the medium is exposed during the sampling procedure to diffuse deep into the medium. When the vial is closed with the screw cap after the sampling, the enclosed oxygen is consumed by the medium and an anaerobic environment is maintained for the specimen.[20,21]

Furthermore, this medium has been shown to be as

good or even better than the more elaborate PRAS tryptone soy broth medium (Oxoid, London, England) for transport and cultivation of bacteria from root canal specimens.[22]

For optimum recovery of microorganisms residing in the root canal system, the PMR method as advocated by Strindberg[23] and Möller[18] was used. With the largest coarse file that can be used to instrument the canal wall, the content of the canal was agitated with a pumping motion that allowed the sampling of all retrievable microorganisms from the canal system, including those from the dentinal tubules and the very apical part of the root canal.[24,25]

It is important to recognize that in this study all samples were obtained through intact caries-free clinical crowns. Although this minimized the risk of leakage along the rubber dam, the risk of contamination could not be totally disregarded owing to extensive manipulation of the root canals during the experiment. Great care was taken to preserve the vitality of anaerobes by inoculating the samples straightaway, at the chair side, onto prereduced media, which were incubated under suitable conditions. In the present study, it was more important to reveal the presence of bacteria in the specimen than to demonstrate the actual bacterial species or their number.

Prolonged incubation was used to avoid overlooking slow-growing organisms or very small inoculates. Some samples showed growth first after 2 to 4 weeks. Thus, the positive sample remaining in the CHX group was first detected on the 7th day of incubation. Nevertheless, the medium employed could not be expected to be suitable for the growth of all microorganisms. To further characterize the changes in microflora occurring during this type of experiment, a detailed account of species and cell numbers would be valuable in future studies.

The sample size in this study was small. Although having a larger number of teeth would have been preferable, it was extremely difficult to achieve that goal in a prospective, controlled study such as this one with very strict inclusion criteria. Nevertheless, the findings of this study demonstrate that additional rinse with 2% chlorhexidine resulted in enhanced disinfection of the root canal system. This result is clinically important.

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