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Residual Antimicrobial Activity After Canal Irrigation with Chlorhexidine

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We previously reported that the in vitro antimicrobial activity of a 2.0% chlorhexidine endodontic irrigant was equivalent to that of 5.25% sodium hypochlorite. The purpose of this study was to determine if chlorhexidine irrigants could instill substantive antimicrobial activity in instrumented root canals in vitro. Human teeth were instrumented using 2.0% or 0.12% chlorhexidine as irrigants. After instrumentation, the root canals were filled with sterile water, and samples of the root canal fluid were absorbed with paper points at 6, 12, 24, 48, and 72 h after treatment. The paper points were assayed for antimicrobial activity by placing them on agar plate surfaces inoculated with *Streptococcus mutans* and measuring zones of inhibition. Antimicrobial activity was present in all 2.0% chlorhexidine-treated teeth throughout the 72-h testing period and in most teeth, in relatively lower concentrations, for 6 to 24 h after irrigation with 0.12% chlorhexidine. These results indicate that chlorhexidine instills substantive antimicrobial activity when used as an endodontic irrigant.

A major objective in endodontic therapy is to disinfect the root canal system before obturation of the canal. Sodium hypochlorite is the current irrigant of choice, but we have reported that a 2.0% chlorhexidine gluconate irrigant, a less malodorous and toxic agent, possessed in vitro antimicrobial activity equivalent to that of 5.25% sodium hypochlorite (1). In addition to its immediate killing action, chlorhexidine is recognized for its antimicrobial substantivity, i.e. residual action (2, 3). Others (4, 5) have reported that chlorhexidine binds to, and is subsequently released from, dentin and enamel, but the presence of substantiveness within an instrumented root canal has not been demonstrated. The purpose of this study was to determine if substantive antimicrobial activity could be induced with chlorhexidine irrigants.

MATERIALS AND METHODS

Two chlorhexidine gluconate irrigants were tested; a 2.0% solution prepared by diluting a 20% stock solution of chlorhexidine (Sigma, St. Louis, MO) in sterile deionized water on the day of use and a 0.12% commercial oral rinse ("Peridex," Proctor & Gamble, Cincinnati, OH). Negative control teeth were irrigated with sterile deionized water.

Freshly extracted, single-rooted teeth were obtained from the University of Texas Dental Branch clinics. The teeth were stored in tap water at 4°C until used. Before instrumentation the apex of each tooth was sealed with TRIAD VLC resin (Dentsply Int., York, PA), and the tooth was secured in a wooden rack. The root canal system was accessed using a high speed handpiece (Star Syntex, Lancaster, PA), and the root canal was instrumented biomechanically using a step-back technique with Flex R files (Union Broach Health-Chem Co., Emittsville, PA). With each change in file size the canal was irrigated with 1 ml of irrigant. After completion of file instrumentation, the canal was further enlarged with a 0.050" para post system drill (Whaledent Int., Mohawk, NJ) to increase the reservoir of intracanal fluid for testing purposes. Following enlargement, each canal was again irrigated with 1 ml of irrigant. The canal was then irrigated with 3 ml of sterile deionized water to flush out the original irrigant and dried with endodontic paper points (Kerr, Romulus, MI). Each canal was then filled with sterile deionized water. The instrumented teeth were held in a humidifier at room temperature.

Six hours after instrumentation, specimens of the instrumented root canals were taken as follows. The broad end of a #80 endodontic paper point trimmed to 1.8 centimeters was inserted into the instrumented canal and left for approximately 2 min. The paper point was removed and stored in a cryogenic vial (Nalge, Rochester, NY) at −20°C. The canal was then irrigated with three 1 ml sterile deionized water rinses, filled with sterile deionized water, and returned to the humidifier. This procedure was repeated 12, 24, 48, and 72 h after instrumentation.

Within 24 h after the last specimen was taken, the paper points were tested for antimicrobial activity as follows. A 24-h Todd-Hewitt (Difco, Detroit, MI) broth culture of *Streptococcus mutans*, strain 6715, cultivated on Mitis-Salivarius agar (Difco) containing bacitracin (200 units/ml, Sigma) and streptomycin (200 mg/ml, Sigma) (MS-BS plates) was used as the target organism for antimicrobial activity. It was chosen because it could be selectively cultured in the presence of contaminant bacteria as a result of its resistance to the inhibitors present in MS-BS medium. This negated the need for sterilization or other processing that might adversely affect specimens. Tests in our laboratory indicated that the inhibitors did not affect the antimicrobial activity of chlorhexi-

FIG 1. Chlorhexidine standards. Beginning at "12:00 o'clock" and going clockwise, the paper points contained zero, 0.0002%, 0.002%, 0.02%, 0.2%, and 2.0% chlorhexidine gluconate.

dine. With the use of a sterile swab, a lawn of *S. mutans* was spread over an MS-BS plate and allowed to dry for 30 min at room temperature. Paper points containing root canal contents were then placed on the plate. The plates were incubated at 37°C in an increased CO_2 atmosphere. After 48 h of incubation the zones of inhibition around the paper points were measured perpendicular to the paper point.

Paper points immersed in ten-fold dilutions of 2% chlorhexidine were tested as above to evaluate the relative sensitivity of the antimicrobial assay. Negative controls consisted of paper points immersed in sterile deionized water.

The two chlorhexidine irrigants were compared using a two-way analysis of variance with repeated measures (SuperANOVA, Abacus Concepts, Berkeley, CA).

RESULTS

The antimicrobial assay could detect as little as 20 μg of chlorhexidine/ml (0.002%) (Fig. 1). However, on different MS-BS plates there was some variation in the size of zones around paper points immersed in identical concentrations of chlorhexidine, and therefore the assay was used only as a semiquantitative assay. Paper point specimens obtained from the teeth irrigated with sterile deionized water did not produce zones of inhibition.

Twenty-three teeth were treated with 2.0% chlorhexidine. Antimicrobial activity was detected in all specimens (6 through 72 h) taken from the 2.0% chlorhexidine-treated teeth (Table 1).

Twenty-one teeth were treated with 0.12% chlorhexidine. Antimicrobial activity was detected in the 6- and 12-h specimens of all teeth irrigated with 0.12% chlorhexidine and in 15, 6, and 2 of the specimens collected at 24, 48, and 72 h, respectively (Table 1).

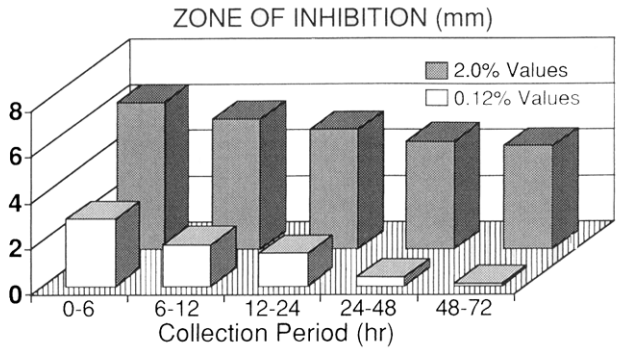

FIG 2. Comparison of the antimicrobial activity within canals following irrigation with 2.0% and 0.12% chlorhexidine gluconate.

Comparison of the two groups of chlorhexidine-treated teeth (Fig. 2) revealed that the antimicrobial activity remaining in the 2.0% chlorhexidine-treated teeth was significantly greater ($p < 0.05$) than in the 0.12% chlorhexidine-treated teeth at all collection times.

DISCUSSION

Chlorhexidine, especially because of its substantive antimicrobial properties, has become recognized as an effective oral antimicrobial agent for use in periodontal therapy and caries prevention and as a therapeutic agent for oral infections in general (2, 3). The results of this study indicate that chlorhexidine can also instill substantive antimicrobial activity when used as an endodontic irrigant in vitro. Although others (4, 5) have demonstrated that chlorhexidine adsorbs to, and is released from, dentin and enamel, this is the first demonstration of chlorhexidine's substantivity within instrumented root canals. Furthermore, the study reveals that chlorhexidine continues to be released as long as 48 to 72 h after instrumentation.

Of the two formulations of chlorhexidine used in this study, the 2.0% solution instilled greater and longer lasting antimicrobial activity. Although statistically significant, these differences are of unknown clinical importance. However, the fact that Ringel et al. (13) and Delaney et al. (14) reported viable organisms remained within root canals irrigated with 0.2% chlorhexidine suggests that the higher concentration would be preferred. Other differences between the two formulations, relevant to their potential use as endodontic irrigants, are that the 0.12% irrigant is readily available as oral rinses ("Peridex," Proctor and Gamble, Cincinnati, OH and "PeriGard," Colgate, Canton, MA), whereas the 2.0% irrigant is not available commercially and must be prepared by the operator

TABLE 1. Mean zones of inhibition around paper points

Irrigant	Collection period (hrs) 0–6	6–12	12–24	24–48	48–72
2.0% chlorhexidine	6.4 + 0.9* (100%)**	5.7 + 1.0 (100)	5.2 + 0.9 (100)	4.7 + 1.0 (100)	4.5 + 1.2 (100)
0.12% chlorhexidine	2.9 + 0.9 (100)	1.8 + 0.8 (100)	1.4 + 1.0 (70)	0.4 + 0.7 (30)	0.1 + 0.3 (10)

* Results expressed as mean width of zones ± SD. All values for 2.0% chlorhexidine were significantly greater ($p < 0.05$) than the corresponding values for 0.12% chlorhexidine. ** Percent of teeth with detectable antimicrobial activity.

or a pharmacist. Secondly, 0.12% oral rinses have a long history of use without having caused significant adverse reactions that might affect their use as endodontic irrigants (2, 3). Although 2.0% preparations of chlorhexidine have not been used as extensively as the commercially available oral rinses, they have been used as oral rinses (15) and subgingival irrigants (16) without apparent adverse effects. Therefore, one would not expect adverse effects from the 2.0% formulation if it is used as an endodontic irrigant.

These results and those of our previous study (1) indicate that chlorhexidine rather than sodium hypochlorite may be preferred as an endodontic irrigant. On initial exposure chlorhexidine is at least as effective as sodium hypochlorite (1), and, as revealed in this study, it instills substantive antimicrobial activity potentially protective of the canal tissues for many hours after instrumentation. Although sodium hypochlorite may be equally effective on initial exposure, it is not a substantive antimicrobial agent. In addition, it is malodorous and very caustic, whereas the chlorhexidine formulations used in this study are relatively innocuous. The one advantage sodium hypochlorite may have is its reported (6, 7, 8) tissue-dissolving property, but this has been questioned (9, 10), especially with the lower sodium hypochlorite concentrations often used in endodontic irrigants (11). Furthermore, there is little clinical evidence that the reported debriding advantage of sodium hypochlorite is a factor in successful endodontic therapy (12).

Thus, based on actual evidence, the mechanical effects of instrumentation, coupled with the substantive antimicrobial activity of chlorhexidine, are probably at least as effective a modality as instrumentation with sodium hypochlorite, and the former does not suffer from the previously noted disadvantages of sodium hypochlorite. Further studies are needed to evaluate the two irrigants. But at this point, chlorhexidine appears to be an excellent, and possibly a preferred, alternative to sodium hypochlorite.

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References

1. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endodon 1994:20:276–78.

2. Fardak O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. J Am Dent Assoc 1985;112:863–69.

3. Greenstein G, Berman C, Jaffin R. Chlorhexidine: an adjunct to periodontal therapy. J Periodontol 1986;57:370–76.

4. Rolla G, Loe H, Rindom Schiott C. The affinity of chlorhexidine for hydroxyapatite and salivary mucins. J Periodont Res 1970;5:90–5.

5. Parsons GJ, Patterson SS, Miller CH, Katz S, Kafrawy AH, Newton CW. Uptake and release of chlorhexidine by bovine pulp and dentin specimens and their subsequent acquisition of antibacterial properties. Oral Surg 1980;49: 455–59.

6. Trepagnier CM, Madden RM, Lazzari EP. Quantitative study of sodium hypochlorite as an in vitro endodontic irrigant. J Endodont 1977;3:194–96.

7. Abou-Rass M, Piccinino M. The effectiveness of four clinical irrigation methods on the removal of root canal debris. Oral Surg 1982;53:524–28.

8. Gordon TM, Damato D, Christner P. Solvent effect of various dilutions of sodium hypochlorite on vital and necrotic tissue. J Endodon 1981;7:466–69.

9. Cohen S, Burns RC. Pathways of the pulp. 3rd ed. St. Louis: CV Mosby, 1984;411–12.

10. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigation solutions. J Endodon 1975;1:127–35.

11. Ingle JI, Bakland LK. Endodontics. 4th ed. Baltimore: Williams and Wilkins, 1994;181–82.

12. Walton RE, Rivera EM. Clearing and shaping. In: Walton RE, Torabinejad M, eds. Principles and practice of endodontics. 2nd ed. Philadelphia: W.B. Saunders, 1995:212.

13. Ringel AM, Patterson SP, Newton CW, Miller CH, Mulhern JM. In vitro evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. J Endodon 1982;8:200–04.

14. Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg 1982:53:518–23.

15. Loe H, Rindom Schiott C. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. J Periodont Res 1970;5:79–83.

16. Southard SR, Drisko CL, Killoy WJ, Cobb CM, Tira DE. The effect of 2.0% chlorhexidine digluconate irrigation on clinical parameters and the level of *Bacteroides gingivalis* in periodontal pockets. J Periodontol 1989:60:302–09.