

Penetration Ability of Different Irrigants into Dentinal Tubules

Elio Berutti, MD, DDS, Riccardo Marini, MD, DDS, and Alessandra Angeretti, MD

Dentinal tubules of human root canal walls were infected with a known bacterial isolate. The teeth were divided into two groups and the root canals instrumentated. Different types of canal irrigant were used for each group. In group A, 5% NaOCl was followed by a 10% EDTA rinse and neutralized with a final physiological solution rinse. In Group B, 10% EDTA, a tensioactive agent (TRITON), and 5% NaOCl were used in sequence, with a final physiological solution rinse to neutralize the action of the agents used. Histological examination of group A specimens showed a residual area of infection extending from the canal lumen to a mean depth of 300 μm . Histological examination of group B specimens showed an infection-free area of tubules to a mean depth of 130 μm . Below this was an infected area of variable extent. In some group B sections, no infection was found.

The success of endodontic treatment depends on the dentist's ability to clean and disinfect the complex canal system three-dimensionally, and then to fill and seal this space completely (1). Shaping the canal, both manually and mechanically, opens this complex space to the action of the irrigant. Important requirements of an endodontic irrigant include properties such as antimicrobial activity, tissue-dissolving capability, and nontoxicity to the periapical tissues (2). NaOCl has to date been found to be the best canal irrigant. Hand et al. (3) showed that NaOCl exerts its maximum capabilities as an antibacterial agent and solvent of organic substances at a concentration of 5.25%.

Microbial infection of the pulp, leading subsequently to endodontic treatment, is frequently the consequence of dental caries, mechanical injury, or coronal microleakage; the success of endodontic therapy depends on eliminating or reducing these microorganisms. The microflora of the necrotic pulp has long been studied: primarily, it comprises anaerobic bacterial combinations in synergism (4). Once pulp necrosis has occurred, another important reservoir of micro-organisms from which canal reinfection may occur is the infected dentinal tubules (5).

The aim of this research was to verify in vitro the capability of NaOCl alone, or in combination with EDTA plus a tensioactive

agent, to penetrate the dentinal tubules of the root canal during endodontic instrumentation, and thus exercise its bactericidal and solvent actions inside the dentinal tubules.

MATERIALS AND METHODS

Twenty-four extracted human upper central incisors, completely free of caries and with only one root canal, were selected for study. They were stored in 0.9% physiological solution refrigerated at 4°C. Conventional access cavities were prepared and the dental pulp extirpated; a small retentive cavity was prepared at the apical foramen (6). Specimens were immersed in a 5% solution of NaOCl for 10 min; the root canals were dried with sterile paper points, and the small apical cavity was plugged with a cotton pledget (6). The prepared teeth were sterilized in ethylene oxide gas.

As controls, two teeth were transferred to a yeast extract/glucose broth (YG broth) (10 g/L of yeast extract (Difco Laboratories, Detroit, MI) and 10 g/L of glucose) and incubated for 24 h at 37°C as a test for sterility. The recently isolated bacterium *Streptococcus faecalis* was used as the test organism and maintained by regular transfer in YG broth. Pulp canals were inoculated with ~0.1-ml aliquots of fresh turbid culture of the test organism, and the access cavity was sealed with a sterile cotton pledget. Bacterial vitality was tested at the moment of inoculation by seeding into the blood agar plate and incubating for 24 h at 37°C. The two inoculated teeth were then immersed in the YG broth in a Petri dish and incubated for 20 days at 37°C. After 20 days' incubation, two tests were conducted: (i) the liquid inside the root canals was seeded onto a blood agar plate and incubated for 24 h at 37°C to ascertain the permanence of bacterial vitality; and (ii) four histological specimens were prepared to test for bacterial penetration inside the dentinal tubules. The remaining 16 teeth were divided into two groups of eight: group A and group B.

Instrumentation

The root canals of the two groups of teeth were instrumentated using a technique described by Ruddle (7) and Scianambolo (8). K-type files from #15 to #70 (Brasseler USA, Savannah, GA) were used; Gates Glidden burs were avoided. The working length was established at the end of the canal, when the endodontic instrument could be seen through the small cavity prepared at the apical foramen. All specimens were instrumented apically up to #40;

instrumentation was done with maximum control of sterility in a biological safety cabinet.

Irrigation

A carefully controlled amount of irrigant (33 ml) was used in each root canal; a syringe with a 22-gauge needle was used for each irrigant. After every two file sizes, recapitulation was done with a #10 file, and canals were flushed with 3 ml of irrigant. Each root canal was kept flooded with irrigant during the instrumentation phase. The total time that irrigant was present in the root canal system was precisely 11 min/specimen.

Group A (8 Specimens)

The irrigant used during instrumentation was 5% NaOCl (OGNA, Milan, Italy). At the end of instrumentation, canals were flushed with 3 ml of 10% EDTA (Sigma Chemical Co., St. Louis, MO). After 3 min, a final flush with 3 ml of physiological solution was done to halt any chemical activity of the irrigants.

Group B (8 Specimens)

During instrumentation, each irrigation consisted of 1 ml of 10% EDTA (Sigma), followed 15 s later by 1 ml of a solution of 1% Triton X-100 as tensioactive agent (Sigma), then followed immediately by 1 ml of 5% NaOCl (OGNA). In this case also, chemical activity of the irrigants was halted with a final flush with 3 ml of physiological solution.

In both groups, the canals were then dried with sterile paper points, and the teeth were immediately placed in 10% formalin for 1 wk.

Specimens were decalcified in 5% formic acid, dehydrated, and then embedded in paraffin. The clinical crown and the apical 2 mm of each tooth were removed, and the specimens were serially sectioned longitudinally through the root canals, at a thickness of 7 μm . The resulting slides were stained with the Brown and Brenn technique for histological demonstration of bacteria.

RESULTS

The experimental model proposed by Akpata and Blechman (6) was found to be a simple and reliable system to obtain a tubular infection in vitro. The ethylene oxide sterilization of the specimens before infection produced complete sterility, a condition indispensable to the success of the experiment.

After 20 days incubation, histological examination of the two test specimens showed that *S. faecalis* had produced a dense and deep tubular infection, more conspicuous at the coronal and median thirds of the dentin abutting the canal lumen. The degree and depth of penetration of the bacteria were less in the apical third of the specimens, wherein the lumen of the dentinal tubules is more narrow.

Cementum was confirmed as a valid barrier against the penetration of bacteria. In no cases where it was intact was any penetration of bacteria into the underlying dentin detected.

Histological examination of the specimens in group A (in which NaOCl was used alone as irrigant during canal instrumentation)

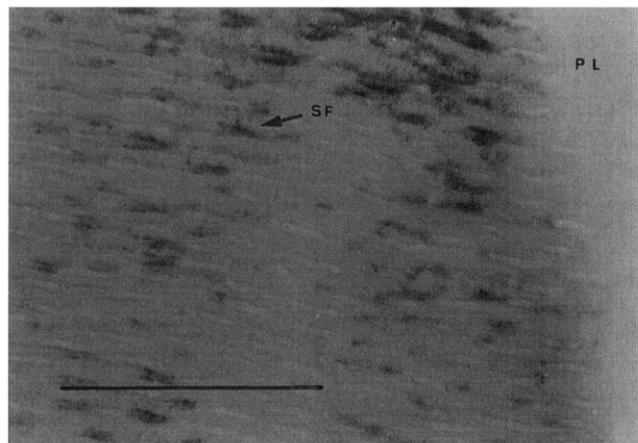


FIG 1. Group A: Histological section at the median third of the canal. Residual area of infection of dentinal tubules by *S. faecalis*. SF, cells of *S. faecalis*; PL, pulp lumen. Bar = 100 μm . (Brown and Brenn stain; original magnification $\times 400$.)

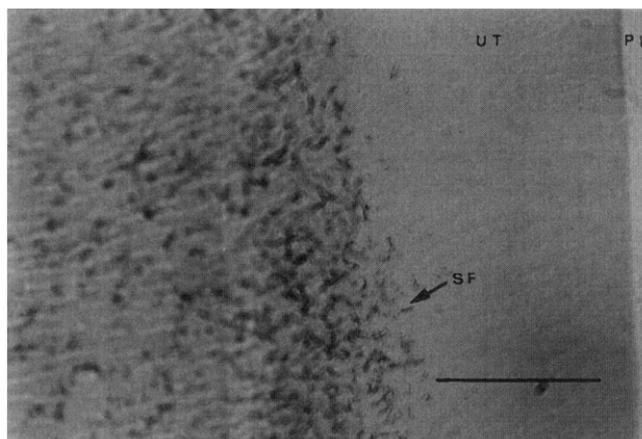


FIG 2. Group B: Histological section at the median third of the canal. Area of infection-free dentin abutting the canal lumen, below which is a residual area of tubular infection by *S. faecalis*. SF, cells of *S. faecalis*; UT, uninfected tubule; PL, pulp lumen. Bar = 100 μm . (Brown and Brenn stain; original magnification $\times 250$.)

revealed a residual area of tubular infection that, from the wall of the canal lumen, extended to an average depth of 300 μm (Fig. 1).

Histological examination of the specimens in group B (in which EDTA, a tensioactive agent, and NaOCl were used in sequence as irrigants during instrumentation) showed an area of dentin free from tubular infection that extended to an average depth of 130 μm from the canal lumen.

Beyond this first zone in group B, there was a band of moderate tubular infection of variable extension (Fig. 2). In some histological sections of dentin abutting the canal lumen in its coronal and median thirds, no tubular infection was found (Fig. 3).

DISCUSSION

The experimental model proposed by Akpata and Blechman (6) was found to be an extremely effective and easy way of simulating and investigating the effects of intracanal irrigants on microorganisms lodged in the dentin wall. *S. faecalis* was chosen as a test organism primarily because it is among the few facultative

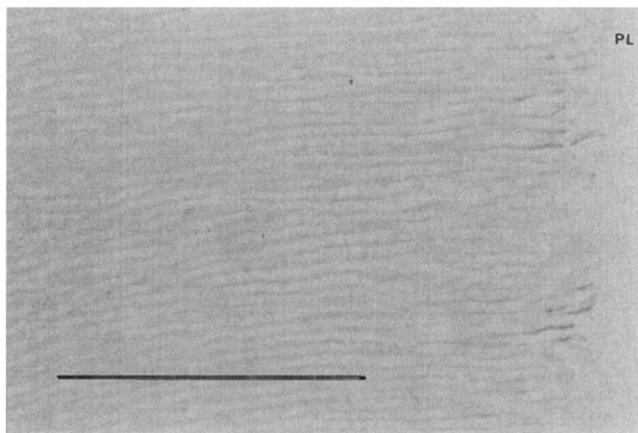


FIG 3. Group B: Histological section at the median third of the canal. Infection-free dentinal tubules. PL, pulpal lumen. Bar = 100 μm . (Brown and Brenn stain; original magnification $\times 400$.)

anaerobic organisms associated with persistent apical periodontitis (9) and also because previous experimental studies have used this organism (6, 10).

NaOCl is still the best known irrigant for its solvent action on pulp tissues, its marked bactericidal action, and its action as a lubricant. However, many studies have shown that, despite correct instrumentation and correct use of the irrigant (NaOCl), it is almost impossible to obtain a canal system free from bacteria and pulp residues (11, 12).

Other studies have shown that bacteria may invade the dentinal tubules and accessory canals, wherein they are protected from the action of both instrumentation and irrigants that are only active on the surface (13, 14). A possible cause of failure in treatment is thus the persistence of an endodontic infection sustained by bacteria that have colonized the dentinal tubules (15). We know that NaOCl is unable to remove the smear layer produced during canal instrumentation (16). The bactericidal effectiveness of irrigants obviously depends on their ability to penetrate the infected dentinal tubules, a process that is clearly influenced by the presence or absence of a smear layer. It has been shown that the formation of this smear layer reduces root dentin permeability by between 25% and 49% (17). Thus, the smear layer protects the underlying microbes and may contain microbes itself.

Alternating NaOCl and a chelating agent, such as EDTA, which can remove the smear layer, has been found to be extremely effective and superior to the use of NaOCl alone in cleaning the canal system (18).

The association between NaOCl and EDTA has been shown to produce a stronger bactericidal action than NaOCl alone (19). Use of a chelating agent is important to prepare the canal surface for the NaOCl, so that the latter can exert its action at a depth, within the accessory canals and within the dentinal tubules. From this study, it seems clear that the use of EDTA allows NaOCl to act at depth within the dentinal tubules. Histological slides from group A show bacteria on the canal surface and lodged within the dentinal tubules abutting the canal lumen. Thus, the smear layer produced during instrumentation occludes the dentinal tubules and protects them from the action of NaOCl.

Analysis of the histological preparations from group B shows that the irrigants were able to exert their cleaning action inside the dentinal tubules. Results were of two types: (i) the dentinal tubules were perfectly free of bacteria from the canal lumen to a depth of

$\sim 130 \mu\text{m}$, beyond which a band of moderate infection appeared, revealed by the Brown and Brenn staining of surviving bacteria and corresponding to the extreme penetration of the tubular infection; and (ii) the dentinal tubules were perfectly clean and bacteria-free for their entire length. This was more frequently the case in the histological sections of the coronal and median third of the canal. It may be hypothesized that the regularity and larger size of the dentinal tubules in this area, as well as the well-demonstrated greater efficiency of the irrigants in these portions of the root canal, cleared out the dentinal tubules more thoroughly. The operational sequence used for group B was aimed at encouraging as much as possible the action of NaOCl in depth. EDTA, used first, aimed to remove the smear layer; a concentration as high as 10% was necessary, because it was to be expected that it would be diluted by the NaOCl already present in the canal. This was followed by a tensioactive agent to lower the surface tension and prepare the canal walls for the NaOCl; at this point, some of the NaOCl introduced into the canal might presumably be sucked into the dentinal tubules by capillary action and fluid dynamics, thus enabling it to exert its effect, as revealed by the histological preparations.

Drs. Berutti and Marini are assistant professors of Endodontics, Department of Endodontics, School of Dentistry, Turin University, Turin, Italy. Dr. Angeretti is a researcher, Department of Public Health and Microbiology, Turin University, Turin, Italy. Address requests for reprints to Dr. Elio Berutti, Via Susa 37, 10138, Torino, Italy.

References

- Schilder H. Cleaning and shaping the root canal. *Dent Clin North Am* 1974;18:269-96.
- Cohen S, Burns RC. Pathways of the pulp. 6th ed. St. Louis: CV Mosby, 1994:183-99.
- Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on necrotic tissue dissolution property of sodium hypochlorite. *J Endodon* 1978;4:60-8.
- Sundqvist G. Bacteriological Studies of Necrotic Dental Pulp. No. 7, Odontological Dissertations, Umea University, Department of Oral Microbiology, University of Umea, Sweden, 1976.
- Oguntobi BR. Dentine tubule infection and endodontic therapy implications. *Int Endod J* 1994;27:218-22.
- Akpata ES, Blechman H. Bacterial invasion of pulpal dentin wall in vitro. *J Dent Res* 1982;61:435-8.
- Ruddle CJ. Endodontic canal preparation: breakthrough cleaning and shaping strategies. *Dentistry Today* 1993;13:44-9.
- Scianamblo MJ. La preparazione della cavità endodontica. In: Castellucci A, ed. *Endodonzia*. Prato: Edizioni Odontoiatriche Il Tridente, 1993:374-403.
- Haapasalo M, Ranta H, Ranta KT. Facultative Gram-negative enteric rods in persistent periapical infections. *Acta Odontol Scand* 1983;41:19-22.
- 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.
- Gutierrez JH, Garcia J. Microscopic and macroscopic investigations on results of mechanical preparation of roots canals. *Oral Surg* 1971;31:108-16.
- Moodnik RM, Dom SO, Feldman MJ, Levey J, Borden BG. Efficacy of biomechanical instrumentation: a scanning electron microscopic study. *J Endodon* 1976;2:261-6.
- Shovelton DS. The presence and distribution of microorganisms within non-vital teeth. *Br Dent J* 1964;117:101-7.
- Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. *J Endod* 1975;1:127-35.
- Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. *J Dent Res* 1987;66:1375-9.
- Wayman BE, Kopp WM, Pinero GJ, Lazzari EP. Citric and lactic acids as root canal irrigants in vitro. *J Endodon* 1979;5:258-60.
- Fogel HM, Pashley DH. Dentin permeability: effects of endodontic procedures on root slabs. *J Endodon* 1990;16:442-45.
- Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. *J Endodon* 1987;13:147-57.
- 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.