In vitro antimicrobial activity of maleic acid and ethylenediaminetetraacetic acid on endodontic pathogens

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Objective. The aim of this study was to evaluate the antimicrobial efficacy of 7% maleic acid (MA) and 17% ethylenediaminetetraacetic acid (EDTA) in elimination of Enterococcus faecalis, Candida albicans, and Staphylococcus aureus at different time intervals.

Study design. Transfer culture of microbial strains were used for inoculum preparation and determination of time-kill assay. The viability counts of 7% MA and 17% EDTA suspensions were performed at 0, 2, 4, 6, 12, and 24 hours. Assay results were analyzed by determining number of strains that yielded log10 CFU/mL of -1 compared with counts at 0 hours, for test medicaments at time intervals. Medicaments were considered to be microbicidal at a minimum inhibitory concentration that reduced original inoculum by >3 log10 CFU/mL (99.9%) and microbistatic if inoculum was reduced by <3 log10 CFU/mL. Statistical analysis was performed using chi-square and Fisher exact tests as well as Friedman test for comparison of the time interval within the MA and EDTA groups.

Results. At all time intervals, there was no significant difference between MA and EDTA for all of the organisms (P > .05). However, within the MA and EDTA groups at various time intervals, there were significant differences (P < .001).

Conclusions. Equivalent antimicrobial activity was observed by MA and EDTA against all of the organisms tested at various periods. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:696-700)

Complete debridement and effective disinfection of the root canal space are considered to be essential for predictable long-term success of endodontic treatment.1 Studies have shown that endodontic infections are polymicrobial with a predominance of anaerobes.2,3 Once microorganisms are established within the root canal system, they cannot be eliminated by host defense mechanism and therefore should be treated by chemomechanical procedure.4 Although mechanical instrumentation of the root canal system removes large amount of microbes, complete debridement is not achieved, owing to its morphologic complexity.5 Therefore, root canal irrigation is essential for disinfection of the root canals.6-8

Mechanical instrumentation of the root canal system produces smear layer.9 This is an amorphous layer consisting of odontoblastic fragments, microorganisms, dentin debris, and necrotic materials.10 A final flush of combined application of ethylenediaminetetraacetic acid (EDTA) followed by sodium hypochlorite (NaOCl) is commonly used for the effective removal of smear layer from the instrumented root canal system.11-13 Recently, 7% maleic acid (MA) was found to be better than 17% EDTA in removal of the smear layer from the instrumented root canal system.14 It was found to be less cytotoxic than EDTA.15

Enterococcus faecalis, Candida albicans, and Staphylococcus aureus are considered to be the most resistant species in infected root canals, and they are often associated with failed endodontic treatment.16-18 Antimicrobial activity of EDTA against these microorganisms has been previously demonstrated.5,19,20 Recently, MA was found to be more effective against E. faecalis compared with EDTA.20 However, the antimicrobial activity of MA against C. albicans and S. aureus has not been subjected to adequate scientific scrutiny.

The purpose of this investigation was to evaluate in vitro the antimicrobial efficacy of 7% MA and 17% EDTA in the elimination of E. faecalis, C. albicans, and S. aureus at different time intervals.

MATERIAL AND METHODS

Microbial strains
Transfer culture of bacterial strains S. aureus (ATCC 29,212) and E. faecalis (ATCC 29,212) and fungal strain C. albicans (ATCC 24,433) from the stock col-
lection were carried out on Muller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates, respectively. The cultures were passaged aseptically thrice to ensure purity.

Inoculum suspension preparation and determination of microbial population

Two to three well separated colonies were inoculated into 5 mL Muller-Hinton Broth (MHB; Himedia Laboratories, Mumbai, India). The tubes were incubated at 37°C for 4-6 hours with intermittent shaking at 150 rpm in a water bath to ensure the logarithmic phase cultures. The suspensions thus obtained were adjusted spectrophotometrically to 1.8 nm using buffered phosphate diluent to match the turbidity equivalent of 0.5 McFarland standard with final cell concentration of $1.5 \times 10^5$ colony-forming units (cfu)/mL. In case of inoculum preparation for C. albicans, MHB was replaced with RPMI medium and MOPS [(3-N-morpholino)propanesulfonic acid] buffer at pH 7.0.

Time-kill assay

An inoculum with a sensitivity threshold of $5 \times 10^6$ cfu/mL was set up to determine the 99.9% killing. This was prepared by diluting a 16-hour broth culture with 5 mL MHB broth (Himedia Laboratories, Mumbai, India) for S. aureus and E. faecalis and RPMI broth (Difco, Lawrence, KS, USA) for C. albicans to obtain the inoculum density of $5 \times 10^6$ cfu/mL. One milliliter of the standardized microbial suspension was added to the equivalent amount of each test medicament at their respective concentrations. After incubation for the respective time duration and brief vortexing, 1 mL of each diluted inoculum was delivered by pipette and plated for viability counts (0 h). Only tubes with an initial inoculum within the range $5 \times 10^6$ cfu/mL were acceptable for the assay. Test medicaments 7% MA and 17% EDTA were chosen to comprise dilutions at the minimum inhibitory concentration (MIC) range.

The viability counts of test medicaments 7% MA and 17% EDTA suspensions were performed at 0, 2, 4, 6, 12, and 24 hours by plating 0.1-mL aliquots of inoculum of each test microorganism onto trypticase soy agar (TSA) (Himedia Laboratories, Mumbai, India) for S. aureus and E. faecalis and SDA (Himedia Laboratories) for C. albicans. The plates were incubated for up to 48 hours, and colony counts were noted for the recovery, yielding 30-300 colonies. The lower limit of sensitivity of colony counts was 250 cfu/mL. Three tubes were used for each organism at each time interval, and the entire experiment was repeated thrice.

Time-kill assay results were analyzed by determining the numbers of strains which yielded $\log_{10} CFU/mL$ of $-1$ compared with counts at 0 hours, for test medicaments at different time intervals. Test medicaments were considered microbicidal at the MIC concentration that reduced the original inoculum by $\geq 3 \log_{10} CFU/mL$ (99.9%) and microbistatic if inoculum was reduced by $<3 \log_{10} CFU/mL$.

Statistical analysis

The data were analyzed using chi-square and Fisher exact tests for comparison of the efficacy between MA and EDTA for S. aureus, E. faecalis, and C. albicans. Friedman test was used for the comparison of the time interval within the MA and EDTA groups for S. aureus, E. faecalis, and C. albicans.

RESULTS

Figures 1-3 show the antimicrobial effect of MA and EDTA at various time periods against S. aureus, E. faecalis, and C. albicans, respectively. There was no significant difference between MA and EDTA for all of the microorganisms tested ($P > .05$) at various time intervals. But within the MA and EDTA groups at various time intervals there were significant differences ($P < .001$) for all of the microorganisms tested.

DISCUSSION

The purpose of endodontic treatment of teeth with pulpal and periapical pathosis is to eliminate the microorganisms from the root canal system and to prevent recontamination with an adequate obturation and rehabilitation of the teeth. Because E. faecalis, C. albicans, and S. aureus have been recovered in high quantities from failed root-filled teeth, these 3 microorganisms were chosen for the present study.

The results of this study demonstrated that EDTA and MA were equally effective against all 3 microorganisms tested at all time periods. This is in accordance with various other studies, which have shown that EDTA has an antimicrobial activity against E. faecalis, C. albicans, and S. aureus. EDTA, by combining with cations of the outer cell membrane of the gram-negative bacteria, may destabilize the cells, causing the release of lipopolysaccharides. However, it is not a powerful antimicrobial agent and has no effect on gram-positive species. Orstavik and Haapasalo reported that EDTA does not have disinfecting action in the root canals and on the dentinal tubules. Earlier studies have reported that the aqueous solution of 17% EDTA had no antibacterial activity even after a longer incubation. However, in the present study the antibacterial action of EDTA solution against E. faecalis and S. aureus was achieved after 15 minutes of direct contact with the solution. These differences in findings may be related to the methodology of the study used. Bulacio et al. reported $<5\%$ MIC for EDTA solution,
whereas other studies reported that 8.5% and 17% EDTA solutions were more effective than 0.5% NaOCl solution with the use of the agar diffusion test.\textsuperscript{7,29} Zehnder et al.,\textsuperscript{20} using a qualitative direct contact test, demonstrated that 17% EDTA solution inhibited the bacterial growth of \textit{E. faecalis} at dilutions of 1/1 and 1/10 but not at a dilution of 1/100. Yoshida et al.\textsuperscript{30} reported a significantly higher percentage of negative
culture with 15% of EDTA versus saline solution. It has been proposed that EDTA solution is not a chemical agent with active antimicrobial effect but rather reduces the amount of microbes in root canal by removing the intracanal smear layer. Even though the antibacterial properties of EDTA are very minimal compared with other endodontic irrigants, its antifungal property has been demonstrated well in various studies.23,18 Because calcium ions play a critical role in morphogenesis, adherence and growth of \textit{C. albicans},31,32 it has been reported that EDTA exerts its antifungal properties by chelating calcium ions both in the culture medium and cell wall.23 In addition to this, it has been reported that \textit{C. albicans} has a cell-associated collagenolytic activity originated from a pericellular metalloenzyme, and the action of that enzyme is completely inhibited by EDTA.33 In the present study, the antifungal effect of EDTA was achieved after 1 hour of direct contact with the solution.

MA with the concentration of 7% was used in this study because our previous study demonstrated 7% MA to be better than 17% EDTA in removal of the smear layer from the instrumented root canal system.14 In the present study, MA was equally effective as EDTA against all 3 microorganisms tested. There are hardly any studies in the literature evaluating the antimicrobial effect of MA on the 3 microorganisms tested in the present study. Recently, Ferrer-Luque et al.21 reported the antimicrobial activity of MA alone and in combination with cetrimide against \textit{E. faecalis} biofilm. They reported that MA eradicated the \textit{E. faecalis} biofilms at a concentration of 0.88% at 30 seconds compared with 15% EDTA or 15% citric acid which attained 100% bacterial kill at 1 minute of biofilm contact. However, in the present study, the antibacterial action of 7% MA against \textit{E. faecalis} was achieved at a 5-minute time interval.

The hypothesized antimicrobial action of MA is attributed to its being an organic acid. The inhibitory mechanism of organic acids is assumed to be due to the decrease of intracellular pH by the release of protons from undissociated molecules in the cytoplasm, thereby inactivating the essential enzymes causing the metabolic barricade.34 Time-kill assay was performed in the present study because it enables understanding the dynamics and kinetics of medicament action on microbial viability at the appropriate concentration to be chosen for treatment purposes.

In conclusion, an equivalent antimicrobial activity was observed by MA and EDTA against \textit{E. faecalis}, \textit{S. aureus}, and \textit{C. albicans} at all of the time periods tested. Because this was a preliminary study, further studies are in progress to confirm the findings using the biofilm model on the root canal system.

REFERENCES