The Antimicrobial Effect of Biopure MTAD on Eight Strains of *Enterococcus faecalis*: An In Vitro Investigation

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Abstract

The purpose of this investigation was to determine the antimicrobial effect of MTAD as a final irrigant on eight strains of *Enterococcus faecalis* (*E. faecalis*) and to measure the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MLC) of MTAD. The roots of 240 extracted human teeth were instrumented using 1.3% NaOCl and 17% EDTA. The roots were divided into eight groups and contaminated with one of eight strains of *E. faecalis*. After irrigating with 1.3% NaOCl, the root canal and the external surfaces were exposed to MTAD for 5 minutes. Roots or dentin shavings were cultured to determine the growth of *E. faecalis*. The results showed that this treatment regimen was effective in completely eliminating growth in seven of eight strains of *E. faecalis*. The MIC/MLC tests showed that MTAD inhibited most strains of *E. faecalis* growth when diluted 1:8192 times and killed most strains of *E. faecalis* when diluted 1:512 times. *(J Endod 2007;33: 1352–1354)*

Key Words

Antimicrobial, *Enterococcus faecalis*, extracted teeth, MTAD

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Bacteria are the primary etiology for the development of pulpal and periapical pathosis (1). In endodontically treated teeth with persistent periradicular infections, only one or a few bacterial species are present (2). These bacteria are primarily facultative anaerobic gram-positive species, mainly *Enterococcus faecalis* (3). *E. faecalis* is extremely resistant to current treatment modalities in endodontics (2, 4, 5). NaOCl does not remove the smear layer (6, 7) and does not kill all bacteria present in infected root canals (2, 8, 9). *E. faecalis* is resistant to intracanal medications such as calcium hydroxide (2, 5, 10) and chlorhexidine (11-13).

BioPure MTAD (a mixture of a tetracycline isomer [doxycycline], an acid [citric acid], and a detergent [Tween 80]; Dentsply Tulsa Dental, Tulsa, OK) can safely remove the smear layer (7) and kill a strain (ATCC 4082) of *E. faecalis* (14). Numerous strains of *E. faecalis* have been isolated from the oral cavity (15). During its evaluation as an antimicrobial root canal irrigant, it is important to determine whether the efficacy of MTAD is unique with a single strain of *E. faecalis* or whether similar findings can be observed with other strains of this species of bacteria. The purpose of this investigation was to examine the antimicrobial effect of BioPure MTAD as a final irrigant in conjunction with 1.3% NaOCl on eight strains of *E. faecalis* in contaminated root canals of extracted human teeth. The minimum inhibitory concentration and the minimum lethal concentration of MTAD were measured on the same strains.

Methods

Methods were a modification of those previously described by Shabahang and Torabinejad (14). Two hundred forty single-rooted human teeth were used in this study. After instrumentation and removal of the smear layer with EDTA, each root was autoclaved and immediately infected.

An overnight culture of eight strains of *E. faecalis* (ATCC 4082, 4083, 49532, 49383, 49452, 49477, 10541, and 19433) in brain heart infusion (BHI) broth was used to prepare eight tubes, one for each strain. The roots were randomly assigned to 8 groups of 30 teeth and immersed in 1 of the 8 inocula for 4 weeks.

The canals of 40 instrumented roots, 5 from each strain, were irrigated with distilled water and served as positive controls. The canals of another 40 instrumented roots, 5 from each strain, were irrigated with distilled water, autoclaved and served as negative controls. The remaining 160 roots were divided into 8 groups of 20 roots each. The root canals were irrigated with 5 mL of 1.3% NaOCl over a period of 15 minutes and then rinsed with 4 to 5 mL of BioPure MTAD. Each root was then placed in 5 mL of BioPure MTAD solution for 5 minutes and then individually placed in 4 mL of BHI and vortexed for 20 seconds to remove any residual irrigant. Each root was then transferred into a tube containing 4 mL of fresh BHI broth and incubated for 1 week to observe the growth of *E. faecalis*.

To determine whether viable bacteria remained in the dentinal tubules, dentin shavings were collected. A #4 sterile carbide round bur in a slow-speed handpiece was used to prepare three samples of dentin shavings initiating from the external surface and moving inward to the lumen of the root canal stopping just before penetration into the canal. All shavings were collected on BHI agar plates and incubated. If the morphology and gram-staining did not rule out the growth as a contaminant, the experiment was repeated. Kho and Baumgartner (16) and Baumgartner et al (17) used similar procedures to identify growth of bacteria without the use of histology or scanning electron microscopy.

TABLE 1. Presence of Turbidity in Tube Containing Root and 4 mL of Fresh BH1 Broth After 1 Week of Incubation

	Presence of Turbidity		
ATCC	Positive Control	Negative Control	Experimental
4082	5/5	0/5	0/20
49532	5/5	0/5	0/20
4083	5/5	0/5	0/20
49383	5/5	0/5	0/20
49452	5/5	0/5	0/20
49477(a)	5/5	0/5	0/20
49477(b)	5/5	0/5	0/20
10541	5/5	0/5	0/20
19433	5/5	0/5	0/20

49477 experiment was repeated (a & b).

To control for the possibility of carryover, an experiment was designed to determine whether enough BioPure MTAD remained on the test tooth to kill or inhibit the growth of bacteria in a culture. Root canals of 10 single-rooted teeth were cleaned, shaped and irrigated as previously described. The roots were placed in a culture of *E. faecalis* (ATCC 49532) to determine whether there was sufficient residual BioPure MTAD remaining on the root to kill or inhibit the growth of the bacteria in the media.

MIC and MLC were calculated for each strain of *E. faecalis* using the broth tube dilution method. Serial dilutions of BioPure MTAD ranging from 1:2 to 1:8192 were prepared using sterile BHI broth. The lowest concentration of BioPure MTAD preventing the appearance of turbidity was considered the MIC. The MLC was determined by subculturing MIC tubes and examining for bacterial growth.

Results

In all positive control samples from the eight strains, there was turbidity at 1 week and growth observed from the dentin shavings (Table 1). All Gram stains showed gram-positive cocci (Table 2). In all negative control samples, there was an absence of turbidity at 1 week (Table 1). There was no growth observed from the dentin shavings, with the exception of one sample from ATCC 4083 (Table 2). The Gram stain showed presence of gram-negative cocci. This finding was ruled out as a contaminant.

In all experimental samples, there was no turbidity at 1 week (Table 1). For ATCC 4082, no experimental samples exhibited any growth (Table 2). For ATCC 49532, one large colony was found in one sample (Table 2). The staining showed the presence of gram-negative rods; therefore, the colony was ruled out as a contaminant. For ATCC 4083, one large colony was found on one sample. The staining showed gram-positive rods and was therefore ruled out as a contaminant. For ATCC 49383 and 49452, no experimental samples exhibited any growth. For ATCC 49477, two positive findings were observed. Staining showed the presence of gram-positive cocci; however, the colonies were medium to large and did not resemble the small pin-point colonies that are characteristic of *E. faecalis*. This experiment was repeated, and no growth colonies were observed. For ATCC 10541, no experimental samples exhibited any growth. For ATCC 19433, two positive samples were found. The Gram stain showed gram-positive cocci, and the colony size resembled *E. faecalis*. These findings were identified as *E. faecalis*.

TABLE 3. Summary of MIC and MLC

	MIC/MLC Summary	
ATCC	MIC	MLC
49532	≥8192	512
19433	≥8192	1024
4083	≥8192	512
49452	≥8192	512
49477	≥8192	512
10541	≥8192	512
4082	2048	256
49383	2048	128

In all MIC tests, there was no turbidity after 24 hours in dilutions up to 1:8192 except ATCC 4082 and 49383 (Table 3). All dilutions were plated to determine the MLC. The majority of the strains tested were bactericidal at a 1:512 dilution. ATCC 49532, 4083, 49452, 49477, and 10541 showed MLC at 1:512. ATCC 19433 had only 7 cfu at 1:1024 dilution. ATCC 4082 had <10 cfu at 256 dilution, and 49383 had <10 cfu at 128 dilution.

In the experiment designed to test the potential carryover effect, there was turbidity at 24 hours and 1 week. All Gram stains showed the presence of gram-positive cocci consistent with *E. faecalis* colony morphology. Continued turbidity confirmed that carryover did not occur and *E. faecalis* continued to grow.

Discussion

Sources of *E. faecalis* in this experiment include the root canal of a pulpless tooth (ATCC number 4082 and 4083), blood (ATCC number 49532 and 49383), and other clinical isolates (ATCC number 49477). It was hypothesized that *E. faecalis* strains may vary in resistance to BioPure MTAD.

The result from the positive control group showed that irrigation using distilled water is unable to render the root canal system free of bacteria and that the bacteria remained viable throughout the experiment. Contamination can occur and was thus controlled by including

TABLE 2. Presence of Growth of Dentin Shavings on BHI Agar Plates After 48 Hours of Incubation

	Presence of Growth					
ATCC	Positive Control	Observations	Negative Control	Observations	Experimental	Observations
4082	5/5	Gram-positive cocci	0/5		0/20	
49532	5/5	Gram-positive cocci	0/5		1/20	Gram-negative rods
4083	5/5	Gram-positive cocci	1/5	Gram-negative cocci	1/20	Gram-positive rods
49383	5/5	Gram-positive cocci	0/5		0/20	
49452	5/5	Gram-positive cocci	0/5		0/20	
49477(a)	5/5	Gram-positive cocci	0/5		2/20	Gram-positive cocci
49477(b)	5/5	Gram-positive cocci	0/5		0/20	
10541	5/5	Gram-positive cocci	0/5		0/20	
19433	5/5	Gram-positive cocci	0/5		2/20	Gram-positive cocci

49477 experiment was repeated (a & b).

negative controls in the experiment and testing all growth colonies with Gram staining to rule out contaminants. While Gram staining alone does not definitively rule out all contaminants (e.g., gram-positive cocci), this technique does rule out most contaminants. The samples from the negative control group showed that contamination generally did not occur during the culturing procedures. One negative control in ATCC 4083 showed growth. The colony was Gram stained and identified as gram-positive rods and therefore ruled out as a contaminant.

In Shabahang and Torabinejad's study (14), seven samples in the NaOCl groups that were initially free of growth showed the presence of bacteria after culturing the dentin shavings (29.2% increase in infected samples). Our study confirmed this finding. For ATCC 19433 and 49477, no turbidity was present in the tubes; however, growth was present after culturing the dentin shavings. Seven of eight strains of *E. faecalis* showed no gram-positive cocci in the tubules of the experimental samples. These findings suggest that BioPure MTAD is able to penetrate the dentinal tubules and eliminate *E. faecalis*. Although relevant strains of *E. faecalis* were selected, one limitation of this study was utilization of ATCC strains exclusively. These strains have typically undergone several passages and may have incorporated mutations with time. Future experiments should also determine differences in susceptibility of primary clinical isolates with that of ATCC strains. The results could have been different if other bacteria were used. Another study is currently underway determining the effect of NaOCl on these same strains of *E. faecalis*.

Previous in vitro studies have shown a high level of susceptibility of *E. faecalis* to MTAD, even when this solution is diluted 200×, whereas NaOCl loses its antibacterial activity against the same isolate beyond 32× dilution (14). Our MIC results revealed that MTAD effectively inhibits the growth of *E. faecalis* up to a dilution of 2048 in all strains. Most strains were inhibited when diluted 8192 times. Our MLC results indicated that BioPure MTAD can kill *E. faecalis* in as high as a 1024 dilution. Most strains of *E. faecalis* were eliminated at a dilution of 512 with only two strains effectively eliminated at lower dilutions (128 and 256). This finding is clinically significant because lower levels of the antimicrobial agent might reach bacteria in the dentinal tubules.

A recent publication stated that the superior bactericidal effect of MTAD may have been caused by a carryover effect of the doxycycline in the MTAD preparation (18). Although prerinsing the roots in 4 ml of BHI broth should address any residual solution on the roots (as shown by positive growth with the NaOCl groups in a previous investigation [14]), in this study, we further tested residual carryover in a separate experiment. BioPure MTAD–irrigated roots that were subjected to a wash with 4 mL of BHI broth were placed in an overnight culture of *E. faecalis* (ATCC 49532) in BHI broth. Continued presence of turbidity clearly showed that carryover of doxycycline did not occur in sufficient amounts to kill or inhibit the growth of the bacteria.

The results of the current investigation corroborated the findings of Shabahang and Torabinejad (14), Portenier et al (19), Royal et al (20), Ghoddusi et al (21), and Davis et al (22).

On the other hand, our results are in disagreement with those of Dunavant et al (23), Kho and Baumgartner (16), Baumgartner et al (17), Clegg et al (24), Ruff et al (25), and Krause et al (18). The inconsistency in the results may be caused by differences in methodology and variance in strains tested. For instance, failure to test MTAD in accordance to the manufacture's recommendations could impact the results of the study. Torabinejad's group recommend the use of 1.3% NaOCl for 15 to 20 minutes before the final rinse with MTAD (14). Some of these studies (18, 23, 25) did not use NaOCl at all or used it for a shorter time period before irrigating with MTAD.

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