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Research Article

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Antimicrobial Susceptibility of *Enterococcus faecalis* Isolated From Root Canal: An In Vitro Study

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Abstract

Background: One of the major causes of failure in root canal treatment is microorganisms like *Enterococcus faecalis*.

Methods: This study aimed to examine the effectiveness of triple antibiotic paste (TAP) containing ciprofloxacin, metronidazole, and minocycline in comparison with modified TAP (MTAP) containing metronidazole, ciprofloxacin, and clindamycin and calcium hydroxide on *Enterococcus* strains isolated from root canal of tooth associated with pre-apical lesion. Moreover, we evaluated the *E. faecalis* resistance and antibiotic susceptibility. To do so, 25 patients with previous failure of endodontic treatment were enrolled in this study.

Results: The antimicrobial effect of MTAP10%, MTAP1%, TAP10%, TAP1%, and CaOH was significantly better than the positive control group (P <0.05). There was no significant difference between antimicrobial effect of TAP1% and MTAP1%, and CaOH was the least effective one. The antimicrobial effect of TAP10% was significantly better than MTAP10% (P = 0.007). Out of 11 *E. faecalis* samples, 1 sample was resistant to MTAP1% and MTAP10%, though it was sensitive to other drugs; meanwhile, it showed the highest sensitivity to TAP10%.

Conclusion: TAP10% was the most effective choice followed by MTAP10%, but calcium hydroxide was not effective compared to other choices. Thus, MTAP might be a good choice for root canal medicament instead of TAP.

Keywords: Drug susceptibility, *Enterococcus faecalis*, Modified triple antibiotic paste, Triple antibiotic paste

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Introduction

Survival of microorganisms in the apical portion of the root-filled tooth is known as the major cause of root canal therapy failure (1). *Enterococcus faecalis*, which is a gram-positive coccus, is commonly identified as the most prevalent microorganism in the root canal therapy (2). As eliminating microorganisms in root canal leads to a higher success rate of endodontic treatments, the primary aim of endodontic treatment is removing microorganisms in root canal system of the tooth. A successful endodontic treatment requires a thorough mechanical and chemical disinfection of the root canal system. If a suitable and sufficient cleansing process of the root canal is not performed, it can result in a resistant periapical lesion (3). The common methods of root canal cleansing enable the rapist to reduce the bacterial load in the canal; but difficulties like anatomical diversity (such as accessory canals), imperfect root juncture, and end of canal deltas make the mentioned methods inaccessible and lead to an imperfect and unsatisfactory cleaning (4).

One of the ways of reducing bacterial load in root canal is utilizing in-root treatment procedures, such as using antibiotics in the root canal (5). In 2006, the American Association of Endodontics (AAE) published an article on several different antibiotics used for controlling root canal microorganisms which appear to possess a key role in pathogenesis and progression of the pulp and periapical apoptosis (6). In order to cure active and acute infections and dental diseases, various types of antibiotics and detergent formulas are used for disease prevention and prophylaxis.

Calcium hydroxide is known to be one of the most

widely used medications. Due to its easy application and antibacterial features, calcium hydroxide is used extensively in root treatment (7). This medicine possesses antimicrobial features and is able to destroy cellular membranes, protein structures, and DNA of germs in alkaline PH. But its antibacterial activity relies on the direct exposure of the substance with bacteria. However, calcium hydroxide has not proved to be successful in removing bacteria from dental tubules and is not able to thoroughly remove *E. faecalis*. Moreover, appropriate pH for calcium hydroxide activity is potentially toxic for root canal and might destroy dental soft tissues. In its high concentration, this can lead to chronic inflammation and cellular necrosis in clinical application (8).

Due to the mentioned problems and also several reports on the ineffectiveness of calcium hydroxide in treating resistant microorganisms, several other alternative drugs have been introduced. Recently, an antibiotics combination called triple antibiotic paste (TAP) has been introduced to be used in root canal. The compound is a combination of ciprofloxacin, metronidazole, and minocycline (9). As the germs inside the canal are necessarily anaerobic, the main ingredients are 3 different antibiotics. Previous studies have shown that low concentrations of TAP can eradicate *E. faecalis* with fewer side effects. At the same time, it has been assumed that this medicament possesses regenerative properties.

There are some disadvantages reported on minocycline constituent of TAP, including tooth discoloration, toxicity for preapical stem cells, and chelation with tooth's calcium; therefore, some studies suggest substitution of clindamycin with minocycline (10,11,12). Clindamycin is a broad-spectrum antibiotic which is active against aerobic and anaerobic microorganisms. Several studies have noted that this compound (antibiotic) possesses a good level of antimicrobial effect on peri radicular infections (13).

Accordingly, the present study aimed to evaluate the effect of above mentioned antimicrobial medicaments on tooth root-canal due to *E. faecalis*.

Materials and Methods

A 3% sodium hypochlorite compound and 30% hydrogen peroxide solutions were used to disinfect the teeth and their surrounding areas. The existing restorations were removed and pulp access cavity was fully and rapidly prepared by a sterile high speed carbide bur. Each tooth was isolated from the others using a rubber dam. In order to prevent debris and microorganism from entering root canal and pulp, the tooth and operatory field was disinfected again by the compounds, and finally the compound was neutralized by 5% sodium thiosulfate (14,15). In a sterile situation and without any chemicals gutta-percha in the coronal part of the canal and the materials in the apical part of the canal were removed by 2 and 3 Gates-Glidden drills (Mani Inc. Tokyo, Japan) and Hedstrom files (25 mm, Mani Inc. Tokyo, Japan). The contents of the canal were also transferred to the transport medium of lysogen broth (LB). A 20 Hedstrom file was inserted 1 mm shorter than the length of the canal, which was roughly measured by radiography, and removed after a brief filling operation. The cutting part of the file was separated from the file's handle with a sterile clipper and dropped into the LB medium. Then the canal was humidified by 0.9% normal saline and a 20-paper point (Dentsply-Maillefer, Ballaigues, Switzerland) was later inserted into the approximate length of canal. The paper point was removed from the canal after 1 minute and transferred to the previous transport tube medium. In the next step, the shaving of the canal walls was performed using the 25 Hedstrom file. The cutting part of the file was separated and transferred to the same transport tube medium. Finally, the canal medium was wetted by normal saline and a 25-paper point was inserted into and remained in the canal for 1 minute, and then dropped in the same medium (16,17).

In order to prepare a methylcellulose based MTAP, 1 mg of antibiotic powder made of 43% clindamycin, 14% ciprofloxacin, and 43% metronidazole were dissolved in 1 ml of sterile water. In order to create a homogeneous antibiotic gel, methylcellulose powder was added to the mixture and stirred for 2 hours at 45°C (18).

In order to create 1 mg/mL methylcellulose-based TAP, 1 mg of US Pharmacopoeia grade antibiotic powders, made of equal portions of metronidazole and ciprofloxacin, was used; the antibiotic-free placebo gel composed of sterile water and methylcellulose was also prepared in the same method. The viscosity of the prepared gels was selected based on pilot studies which had examined the viscosities of various methylcellulose–based gels (19).

After collecting the samples and moving them to tubes which contain LB broth (Merck, Germany), the samples were promptly moved to a microbiology lab. Gram staining was used to identify the microorganisms. Distinguishing the bacteria was done by their morphological colonies, including their size, color, and border. After colony recognition based on their morphological features, a loop of the sample existing in the LB broth vial was cultivated linearly on a bile-esculin agar in order to confirm the Enterococcus. This procedure was completed under laboratory hood and in sterile environment. The plates were moved to an incubator at 37°C for 24 hours to give time to colony growth.

Biogram Test

A 0.5 McFarland standard was prepared from the separated strains and cultivated on 2 Muller-Hilton Agar culture media by using a sterile swab. Each media was then divided into 3 parts and a well was created on each part so that the created compounds could be poured in each well. The medicament was put in each well and then plates were moved to an incubator at 37°C for 24 hours and diameters of the inhibition zones were tested for each medicament and recorded, respectively.

Statistical Analysis

For statistical analysis of data, we used the IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA). This data was encrypted and entered into the software. Only patients isolated from *E. faecalis* samples were included in the statistical analysis. Quantitative variables were expressed as mean ± standard deviation. We used Shapiro-Wilk test to evaluate the normality of data distribution and Wilcoxon test was used to compare the quantitative data. Significance level in this study was considered less than 0.05 (P <0.05).

Results

A total of 25 patients (13 males and 12 females) were included in this study. Of these, 11 subjects (7 males and 5 females) had a positive sample for *E. faecalis*. The mean age of patients was 43.27 ± 10.1 years. The antimicrobial effects of calcium hydroxide, TAP1%, TAP10%, MTAP1%, and MTAP10% were investigated for these samples. The results of the mean growth inhibition halo in different samples based on millimeters are presented in Figure 1.

One sample was resistant to MTAP1% and MTAP 10% and it did not create a growth inhibition halo (Figure 2), and one sample was only resistant to MTAP1%. For a more accurate and better judgment, the decision was made to remove the Outlier data.

Examination of the mean growth inhibition halo in the drug group compared to the control group showed that all drugs had significant antimicrobial effects compared to the control group; in addition, different concentrations of TAP and MTAP were significantly more effective than $Ca(OH)_2$ (P <0.05).

It was predictable that TAP10% had a better antimicrobial effect than TAP1% (P = 0.003) and MTAP10% (P= 0.007). In addition, the mean growth inhibition halo of MTAP10% was higher than MTAP1% (P = 0.007).

Comparing different concentrations of TAP and MTAP, we found that TAP1% and MTAP1% did not have a statistically significant difference in creating a growth inhibition halo (P = 0.24) and their antimicrobial efficacies on *E. faecalis* are comparable. MTAP10% was also significantly more effective than TAP1% (P = 0.011).

Discussion

A successful and thorough removal of pathologic microflora has always been one of the ultimate goals in endodontic treatment. An unsuccessful and insufficient disinfection of root canal may result in an unsuccessful root canal treatment and permanent pathology of the root (20). Numerous studies have shown that complete eradication of bacteria in the root canal system by modern root canal treatment techniques (such as mechanical debriman, chemical rinse, and calcium hydroxide therapy) is not always possible (21). Recently, a wide range of bacteria present in the root canal of an infected tooth (both primary and secondary) have been identified. However, there are still discrepancies regarding the main pathogens which can affect primary endodontic infections of the tooth. One of the reasons for such differences in different studies is the availability of different methods for identifying these pathogens (22).

Enterococcus faecalis is a gram-positive anaerobic bacterium and the most common microorganism isolated from teeth after unsuccessful endodontic treatment. It is widely believed that the application of intra-canal drugs between treatment sessions can help remove this microorganism from the dental canal (23). In the present survey, *E. faecalis* was isolated from the end of the root canal in 44% of patients who experienced an unsuccessful endodontic treatment.

Calcium hydroxide is known to be the most commonly used drug in the root canal, but due to the penetration effect of *E. faecalis* into the dentin tubules, this microorganism is relatively resistant to calcium hydroxide. Furthermore, when calcium hydroxide reaches the peripheral areas, its pH level reaches about 6-7.4, and this change may result in a severe damage for periodontal repairing cells. Due to the disadvantages and flaws of calcium hydroxide, application of other alternative drugs in the root canal has been suggested.

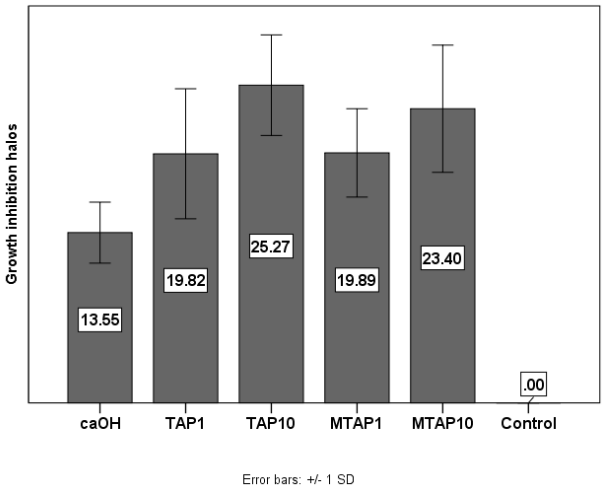

Figure 1. Mean Growth Inhibition Halo.

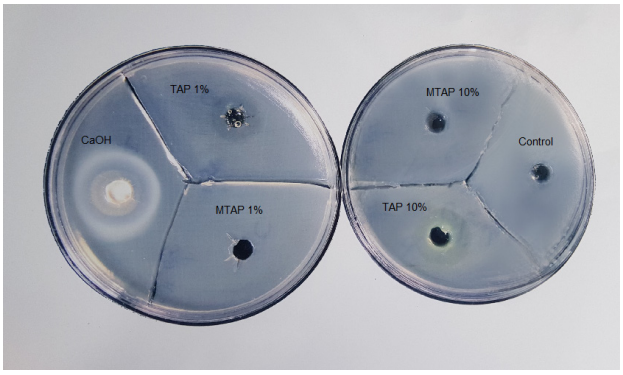

Figure 2. MTAP1% and MTAP10% Resistant Sample.

In the present survey, a paste of three antibiotics which were modified with clindamycin with concentrations of 1 mg/mL and 10 mg/mL with a mixing percentage of 43% clindamycin, 43% metronidazole, and 14% ciprofloxacin was used. The antimicrobial features of these two drugs were analyzed together with calcium hydroxide and paste of three common antibiotics at concentrations of 1 mg/mL and 10 mg/mL, respectively. Due to their water solubility characteristics, intra-canal drugs are very difficult to apply, and also they cannot be placed in the tooth canal. Hence, in order to promote the application of drugs and to place them in the canal between sessions, a carrier substance was applied. This substance must not interfere with the antibiotics' properties, antimicrobial effect, and also their penetration power, and it should be easily removed from the canal and washed thoroughly at the end of the treatment. Several studies suggested the use of methylcellulose (19,24). Methylcellulose is a cellulose compound which is not absorbed by the human body. It is widely used as a thickener in the food, pharmaceutical, and cosmetic industries in the world. Methylcellulose is a water-soluble compound and can be easily removed and cleaned from the canal space. In this study, methylcellulose was used in order to evaluate the effects of the drug. Algarni et al reported that the antibacterial properties of MTAP containing metronidazole, ciprofloxacin, and clindamycin were identical to DAP containing metronidazole and ciprofloxacin (18).

TAP, which is a blend of metronidazole, ciprofloxacin, and minocycline, has been proved to be effective in eradicating pathogens existing in the root canal. The first application of TAP was discussed by Sato et al (25). The effectiveness of this blend in eliminating common endodontic pathogens has been proven in many studies (3,10, 19).

There have been many studies on the antibiotics in TAP (and its depth of penetration) and on the antibacterial effects at different concentrations. In the early studies, a concentration of 1000 mg/ml was used; but some later surveys proved that this concentration has unfavorable effects, including toxicity to stem cells around the root, discoloration of tooth crown, and reduced tooth stiffness. Numerous studies have been done on lower concentrations of this medicament that lead to fewer side effects (3,10,19).

Due to some problems with the application of TAP (such as tooth discoloration and toxicity to periapical stem cells) and difficulty of access, in recent years, several alternative drugs have been suggested. Clindamycin was the antibiotic studied in this survey. It was applied as a substitute for minocycline in three antibiotic pastes. Several different concentrations of clindamycin have been used in studies. Studies on the clindamycin-modified TAP have used concentrations of 1 mg/ml and a 43% blend of clindamycin, 43% metronidazole, and 14% ciprofloxacin (18,26).

In our study, the antibacterial properties of 1 mg/mL and 10 mg/mL concentrations of TAP and MTAP drugs were not notably different from the studies performed on the samples, except for 2 cases. One of the studies had both MTAP concentrations of 1 mg/mL and 10 mg/mL, which showed resistance, and the other had only the concentration of 1 mg/mL MTAP. To further explore and ensure the results, these two samples and other three samples were re-cultured and laboratory steps were carried out on them. The achieved results were similar to the previous ones.

Conclusion

According to the results of the present study, TAP and MTAP drugs in concentrations of 1 mg/mL and 10 mg/mL (except for MTAP-resistant cases) have relatively similar properties. Owing to the problems of using TAP (e.g., tooth discoloration and toxicity to periapical stem cells) and also similar and adequate effects of lower concentrations of antibiotics, low-concentration MTAP could be used to replace high-concentration usage of TAP (if the results are confirmed in future studies). However, MTAP10% was not as effective as TAP10%. Therefore, it seems that MTAP can be used instead of TAP1%.

Conflict of Interests

The authors report no conflict of interests.

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