ANTIBACTERIAL EFFECT OF STANNOUS FLUORIDE 0.63% AS AN INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

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ABSTRACT

Aim: The most purpose of using the intracanal drugs is to remove infection and bacteria from the canal and the dentinal wall. The aim of this study was to investigate the antibacterial properties of stannous fluoride (0.63%) against enterococcus faecalis and compare it with chlorhexidine gel and calcium hydroxide.

Materials & Method: Twenty-five plates containing blood agar were prepared and then cultured with Enterococcus faecalis. In each plate, 5 holes (6×3) were created and creamy mixture of calcium hydroxide, chlorhexidine gel and stannous fluoride gel 0.63% were inserted into the holes (25 holes for each material). 25 holes with ampicillin disks (positive control) and 25 holes with distilled water as negative controls were used, respectively. Plates were incubated for 24 h and then the diameter of microbial Zone of inhibition was measured. The differences between groups were analyzed by Kruskal-Wallis and Mann-Whitney U tests. A p-value < 0.05 was considered statistically significant.

Results: The mean diameter of ZOI for 2% chlorhexidine gel and stannous fluoride was 18.88 and 12.88 mm, respectively. The lowest mean diameter of the growth inhibitory region was related to calcium hydroxide with an average of 9.32 mm.

Conclusion: The 2% chlorhexidine gel has the most antibacterial effects against enterococcus faecalis species. While stannous fluoride showed an antibacterial effect comparable to that of chlorhexidine gel, it had the lowest antibacterial effects related to calcium hydroxide.

Introduction

Most pulpal and periapical diseases are directly or indirectly associated with microorganisms. Therefore, complete debridement and reduction of root canal microbial infection are necessary for long-term success of root canal treatment.[1,2] Clinicians in endodontic therapy are trying to control infection and bacteria in the canal and dentinal wall with canal cleaning and rinsing mechanically and chemically. According to the previous investigations, the success rate of removing root canal microbial agents can be as low as 50%, even with the help of canal cleaning techniques. In addition, anatomical variations and inaccessible areas, such as accessory canals, decrease the success rate of treatment. These sites, which continue to have necrotic materials and bacteria even after cleansing, can affect the treatment outcomes. Therefore, the use of intracanal medicaments is recommended due to the complex canal anatomy and the presence of certain areas inaccessible during the mechanical-chemical canal preparation.[3,4] The origin of endodontic infections is microorganisms that enter into periapical and sterile tissues.

Enterococcus faecalis is a non-motile, facultative aerobic and gram-positive spherical bacterium[5] that resists many antibiotics such as aminoglycoside, aztreonam, cephalosporin, clindamycin and penicillin.[6,7] This bacterium is one of the opportunistic oral pathogens that can be associated with root canal infections and periradicular abscess. *E. faecalis* is more resistant to chronic and asymptomatic secondry endodontic infections than primary ones, so that the treated root canals contain *E. faecalis* about 9 times more likely than the cases of primary infections. This bacterium has features allowing survival in treated canals, including resistance to intracanal medicaments, the ability of biofilm formation, invasion to dentinal tubules, and long-term survival if deprived of food.[8] Many studies have shown that *E. faecalis* cannot be effectively removed by calcium hydroxide.[5,9]

The calcium hydroxide is the most common intracanal medication in the endodontic therapy. The mechanism of antibacterial activity of this substance is attributed to rapid decomposition to calcium and hydroxyl ions and to create a high pH environment, inhibiting the enzymatic activity essential for microbial survive, i.e., metabolism, growth and cell division. These enzymes are located in the cytoplasmic membrane of microorganisms, whose deactivation by ions released from calcium hydroxide renders chemical changes in organic components and the transfer of nutrients and ultimately toxic effects on microorganisms.[10]

The chlorhexidine as an intracanal medicament is another substitute for calcium hydroxide, which has broad-spectrum antibacterial properties, penetrates into the bacteria due to positive molecular charge and damages them. It has been shown that the chlorhexidine is more effective against *E. faecalis* than the calcium hydroxide. The special properties of chlorhexidine result from its attachment to hydroxyapatite, causing a more stable effect.[11] The chlorhexidine is harmful at high concentrations, but safe at lower concentrations such as mouthwash and contact lens solutions though some studies reported some complications even at low concentrations.[12,13] The main disadvantage of chlorhexidine is failure to effect on the smear layer and has a fixative property.[14] On the other hand, it has been determined that excess use of chlorhexidine 2.0% can reduce the success rate of dental root treatment.[15] At present, the use of

appropriate aseptic techniques and mixing chlorhexidine 2% with sodium hypochlorite is the most effective method for managing this bacterium.[5]

In recent years, other compounds have been exploiting due to the presentation of reports on the ineffectiveness of calcium hydroxide on resistant microorganisms such as *E. faecalis* and *Candida albicans*, as well as the introduction of these microorganisms as the main contributing agents to the failure of endodontic treatments.[16]

Fluoride and relevant products have been used locally in oral cavity for years to enhance the tooth decay resistance. Bibby and Van Kesteren in 1940 for the first time showed that the mixture of 1 ppm of F^- (NaF) reduces the bacteria and their acidic products.[17]

Stannous fluoride has shown significant antibacterial activity against oral bacteria in comparison to Naf in the in vivo and in vitro conditions.[18,19] The stannous fluoride effects are reportedly anti-plaque/gingivitis, anti-sensitivity and anti-cavity. The stannous fluoride is the only source of fluoride that applies these triple effects in the oral cavity.

Mickle *et al*., in an in vitro study, showed that the stannous fluoride inhibited *E. faecalis* growth more than calcium hydroxide and the combination of both.[20]

Due to the antibacterial benefits of the stannous fluoride and limited studies about its effect on *E. faecalis*, this study was conducted to investigate the effect of stannous fluoride 0.63% on *E. faecalis* using agar diffusion test and to compare the antibacterial properties of calcium hydroxide and chlorhexidine gels.

Materials & Method

This in vitro study was conducted to compare antibacterial activity of 3 intracanal medicaments, including calcium hydroxide (Golchadent Co., Iran), chlorhexidine 2% gel (Ultra dent Co., USA), stannous fluoride 0.63% (Kimia Co., Iran). The standard strain of *E. faecalis* ATCC 1394 (Asr-e-Enghelab Co., Iran) was prepared to perform testing. The bacteria were cultivated in broth culture suspensions were prepared and adjusted to No. 0.5 McFarland standard (approximately 1.5 × 108 cells/mL). Aliquots of the suspension containing E. faecalis were spread on Petri dishes containing Blood Agar medium (Merck, Darmstadt, Germany). In each section of each plate, a well 6 mm in diameter was created with a sterile stainless steel cylinder. In each plate, 5 holes (6 × 3) were created and creamy mixture of calcium hydroxide, chlorhexidine gel and stannous fluoride gel 0.63% were inserted into the holes (25 holes for each material). Ampicillin disks (positive control) and distilled water as negative controls were used; respectively The inoculated plates were dried for 15 minutes at 37°C. A sample of each freshly mixed dental material was placed into wells in each section of the 25 plates. All plates were incubated for 24 hours at 37°C under aerobic conditions, after that time, shortest distance from the outer margin of the wells to the initial point of bacterial growth was measured in mm as the ZOI. The differences between groups were analyzed by Kruskal-Wallis and Mann-Whitney U tests. A p-value < 0.05 was considered statistically significant.

Results

The mean ZOI diameter was 0 in negative control and 19.8 in positive control and had a significant difference with all three medicaments.

Table 1 shows the mean and standard deviation for each group. The chlorhexidine 2% gel with the mean ZOI diameter of 18.88 mm had the highest antibacterial activity, respectively followed by the stannous fluoride 0.63% with the mean ZOI diameter of 12.88 mm and lastly the calcium hydroxide with the mean ZOI diameter of 9.32 mm.

Group	N	Mean	SD	P-value
Ampicillin	25	19.8	1.68	<0.001
CHX	25	18.8	1.42	
CaOH	25	9.3	2.07	
SF	25	12.8	1.61	

Table 1: Mean and standard deviation (SD), of microbial zone of inhibition (ZOI) in mm

As shown in Figure 1 the non-parametric Kruskal-Wallis test showed a significant difference in the mean ZOI diameter for *E. faecalis* following the use of intracanal medicaments (p-value = 0.00).

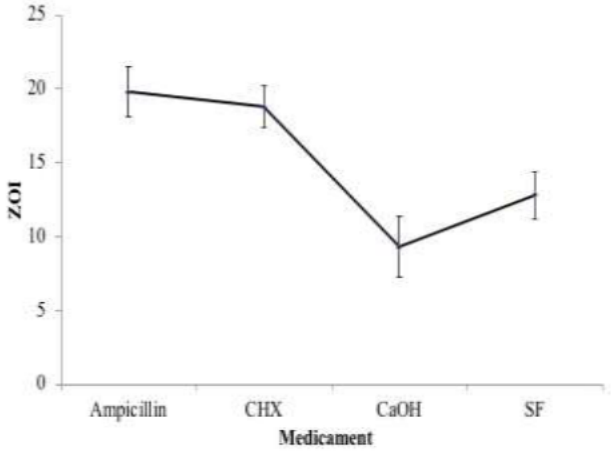

Figure 1: Comparison of microbial zone of inhibition (ZOI) in groups.

The Mann-Whitney test showed a significant difference between chlorhexidine 2% gel and calcium hydroxide (p-value =0), chlorhexidine 2% gel and stannous fluoride 0.63% (p-value =0), calcium hydroxide and stannous fluoride 0.63% (p-value =0).

In summary, the intracanal medicaments in the root canals were classified according to their ability to control *E. faecalis* strain as follows:

chlorhexidine 2% gel > stannous fluoride 0.63% > calcium hydroxide.

Discussion

The present in vitro study showed that stannous fluoride 0.63% can deactivate E. faecalis better than calcium hydroxide.

The severity of the disease and inflammation in the pulpal and periapical tissues is directly related to the microbial count in the root canal system; therefore, the basis of successful root canal treatment is the removal of root canal bacteria and their products.[21] The intracanal medicaments can act as a physical and chemical barrier or as disinfectants in the root canal. For this reason, in the context of chemical cleaning, the use of various intracanal medicaments in the root canal has been proposed between therapeutic sessions.[22]

E. faecalis is commonly extracted from the canal in most root infections and most of the endodontic treatment failures. Studies have shown that this bacterium can even exist as monoinfection inside the canal.[5] This bacterium can survive for a long time with the help of genetic polymorphisms and tolerate long-term non-nutritional conditions as well. The bacteria, when exposed in adverse environmental conditions, change their cell wall that can be protected. Sometimes it can also form biofilms with the help of virulence factors such as gelatinase or adhesins. This microorganism adheres firmly to collagen and resists against common canal rinsing solutions.[23] For this reason, this bacterium was selected in this study.

Since many studies have employed ampicillin as a positive control in evaluating the antibacterial properties of different agents against *E. faecalis*, such as Tabrizi Zadeh *et al* and Mickle *et al*., so the ampicillin disc was also considered as the positive control in this study.[1,20]

The calcium hydroxide is a drug widely used by dentists during the intersession intervals. The antibacterial properties of calcium hydroxide depend on its alkali nature and ability to destroy cytoplasmic membrane, denaturation of bacterial proteins, and damage to bacterial DNA. Despite the frequent demonstration of the poor anti-bacterial properties of calcium hydroxide against *E. faecalis*, this drug also has a good biological effect, which is effective in neutralizing bacterial lipopolysaccharides, has an anti-erosion activity and contributes to the formation of hard tissue. These encourage the dentists to apply widely the calcium hydroxide.[24] In the present study, the calcium hydroxide also had a small effect on the elimination of *E. faecalis*.

The formulation of chlorhexidine gel is capable of removing *E. faecalis* within one minute. This material has been chosen for this study due to broad-spectrum antibacterial properties, high anti-biofilm effect, high intra-tubular penetration, high dentin bonding and low cytotoxicity.[25] In many of the reviewed studies, including Lakhani *et al*., Attia *et al*. and Mazayeni *et al*., the chlorhexidine had the most antimicrobial activity against *E. faecalis*. In addition, the results have repeatedly shown its superior antibacterial property against *E. faecalis* compared to the calcium hydroxide, in line with the findings of this study.[15,26,27]

The stannous fluoride 0.63% is used as mouthwash and gel for enamel reinforcement and decay control, but not yet as an intracanal medication. Given that several studies have been conducting to find the ideal intracanal medication, and since the stannous fluoride has antibacterial benefits.[20] We decided to select this substance for the present study. It should be noted that no comprehensive research has investigated the effect of stannous fluoride 0.63% on *E. faecalis* to date.

Various methods are available to study the antimicrobial properties, including dilution (broth dilution and agar dilution), agar diffusion test, and colony count.[28] Due to the fact that the antibacterial property of stannous fluoride 0.63% against *E. faecalis* has not been investigated up to now, we decided to use the simplest method to test the antibacterial properties of these materials, which is easy to use, does not need for complex devices, is cost-effective, and the its results can be exploited for more detailed examinations.[28]

One of the drawbacks of our research method is that the amount of material tested was based on the volume of the well, and that the density and weight of the material was not considered, affecting probably the test results. Therefore, it is better to be used more precise antimicrobial methods for comparison. It should be noted that the studied materials were tested with the same consistency used in the clinic.

In the present study, the chlorhexidine had the highest antibacterial effect, followed by the stannous fluoride with an effect close to chlorhexidine 2%. At last, the least antibacterial effect was related to the calcium hydroxide. Mickle *et al*. evaluated the antibacterial properties of calcium hydroxide and stannous fluoride both alone and in combination against *E. faecalis*. In their study, the combined calcium hydroxide and 0.3% stannous fluoride showed the highest effect and the calcium hydroxide alone had the least effect on *E. faecalis,*[20] which is in agreement with the present study.

Bellamy PG *et al* found that the anti-plaque effect of the stannous fluoride 0.454% is comparable with chlorhexidine 0.05%.[29]

Andres *et al* reported that the anti-bacterial effect of the stannous fluoride is due to tin and fluoride ion.[30]

Camosci and Tinanoff reported a unique property of SnF2 possibly the reactivity in an aqueous environment--may be responsible for its anti-bacterial properties.[31]

Fine D *et al* exhibited that the replacement of calcium ions by tin ions alters the bacterial enzyme activity and also the thiol group in the stannous fluoride makes the bacteria more vulnerable and reduces its enzyme activity.[32]

According to Hughes *et al*, the tin ions in stannous fluoride may be linked to the phosphate group of lipoteichoic acid (LTA) through electrostatic bond, thereby blocking

bacterial cell membrane instability and inhibiting its metabolism.[33]

The stannous fluoride 0.63% with antimicrobial effects close to chlorhexidine 2% gel has the benefits of increasing the oxygen dependent antibacterial activity in neutrophils,[34] preventing the cavity formation and potentiating the dental development via fluorohydroxyapatite.[35] The mechanism by which the stannous fluoride inhibits bacterial growth involves blocking the enzymatic activity of fructose-1,6-bisphosphate aldolase and triosephosphate dehydrogenase. Prevention of the activity of these two enzymes in the glycolytic pathway leads to a reduction in the bacterial metabolism and thus acid production.[36,37]

Conclusion

Stannous fluoride 0.63% had good antimicrobial effect against Enterococcus faecalis. Further studies are needed to confirm it.

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