Antimicrobial Properties of Endodontic Biomaterial with Chlorhexidine

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Abstract

Introduction: In recent years, a new endodontic cement (Calcium Enriched Mixture or CEM) has been introduced, with clinical applications similar to those of MTA. It has been shown that CEM has antibacterial activity better than that of MTA. On the other hand, use of chlorhexidine to promote the antibacterial activity of different dental materials is increasing. The aim of the present study was to evaluate the effect of adding CHX to CEM on its antibacterial activity.

Materials and methods: The antibacterial activities of the materials under study [(CEM cement+CEM solution+2%CHX) and (CEM cement+CEM solution)] against P. aeroginosa, E. faecalis, S. aureus and E. coli were evaluated using agar diffusion technique, followed by determination of the diameter of microbial zone of inhibition around the materials by 3 independent observers after 72 hours. Data were analyzed by Mann-Whitney U test. Statistical significance was defined at P<0.05.

Results: The mean diameters of zones of inhibition in the CEM+CEM solution and CEM+CEM solution+CHX groups against P. aeroginosa, E. faecalis, S. aureus and E. coli were (13.2 and 9), (21.10 and 6), (20.2 and 9) and (17 and 9.75) millimeters, respectively, with larger diameters in the CEM+CEM solution+CHX group compared to CEM+CEM solution group with all the microorganisms (P<0.05).

Conclusion: Incorporation of CHX into CEM resulted in an increase in antimicrobial activity of CEM.

Key words: CEM cement, chlorhexidine, antibacterial.

1. Introduction

Mechanical pulp exposure and exposures due to caries in teeth with immature apices, without the symptoms and signs of irreversible pulpitis, should be sealed in order to preserve pulp vitality and prevent pathologic changes in periradicular tissues (Reyhani et al., 2015b). In addition, communication pathways between the root canal and the periodontium, including perforations, should be sealed with restorative materials to prevent bacterial leakage (Reyhani et al., 2015a). Since these materials are at close contact with vital tissues they should be biocompatible and induce regeneration of the affected tissues and restore the conditions before their involvement. CEM cement is a new dental material with applications similar to those of MTA. The main ingredients of MTA are calcium oxide, sulfur tricalcium, phosphate oxide, silicon oxide, aluminum oxide, sodium oxide, manganese oxide and chlorine, which are mixed with a water-based liquid to yield bioactive calcium and phosphate (Asgary et al., 2008b). The results of a recent study showed that CEM cement releases calcium and phosphate ions. CEM cement has a pH value similar to that of MTA; however, it has higher fluidity compared to MTA with shorter working time and less film thickness (Shahi et al., 2015). A study by Asgary et al. showed that CEM has antimicrobial activity against pathogens, similar to that of calcium hydroxide and better than that of MTA. Antifungal effects of MTA and CEM against Candida albicans have been compared and it has been shown that both materials completely destroy the fungus in 24 hours (Asgary and Kamrani, 2008, Ayhan et al., 1999, Hasan Zarrabi et al., 2009).

Chlorhexidine has been initially introduced as an irrigation solution due to its broad-spectrum antibacterial activity. Studies have shown that CHX is effective against bacterial species that are isolated from the infected root canals; these microorganisms include S. aureus, E. faecalis, S. salivarius, E. coli and C. albicans (Barrios et al., 2013, Gomes et al., 2003, Hernandez et al., 2005). Use of CHX to promote the antimicrobial properties of dental materials with the aim of improving prognosis is increasing. Studies have shown that adding 0.12%
CHX to MTA increases its antibacterial activity (Hernandez et al., 2005). A study by Bidar et al, using direct contact technique, showed that incorporation of CHX into CEM resulted in an increase in its antimicrobial activity; however, the effects of adding different concentrations of CHX to CEM were not significantly different from each other (Bidar et al., 2015).

The aim of the present study was to evaluate the antimicrobial activity of CEM on *P. aeroginosa*, *E. faecalis*, *S. aureus* and *E. coli* using agar diffusion technique.

2. Materials and Methods

The antimicrobial activities of the materials under study were evaluated against *P. aeroginosa*, *E. faecalis*, *S. aureus* and *E. coli* using the agar diffusion technique. Standard microbial strains were provided by the Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences. All the bacterial strains were grown in Mueller-Hinton Broth (MHB) for 24 hours at 37°C. Then a suspension was prepared from each bacterial strain at a concentration of $1.5 \times 10^8$ CFU/mL (turbidity equal to McFarland’s 0.5 standard solution). Each suspension was used to culture bacterial species on MHA using a sterile swab. The materials under study were placed on the basal layer in each plate in a well. The plates were incubated at 37°C for 24 hours. A total of 8 plates were used for each bacterial strain, i.e. on the whole 34 plates were used, which were randomly divided into 4 groups and two plates were used as positive and negative controls, containing solutions with and without microorganisms, respectively. Evaluations were carried out in sterile MHA culture media measuring 4 mm in depth in plates measuring 2×10 cm. A sterile punch was used to produce two identical holes measuring 4 mm in diameter at least 3 mm apart from each other in the basal layer of each plate. Each hole was filled separately with the materials under study, which consisted of the following: a mixture of 1 g of CEM cement powder +0.36 mL of CEM cement solution, and a mixture of 1 g of CEM cement powder +0.18 mL of CEM cement solution + 0.18 mL of 2% CHX (Conseppsis, Ultradent Products, South Jordan, Utah, USA). Finally, the diameter of zone of inhibition around each test material was measured using a ruler accurate to 0.5 mm, after 72 hours.
3. Results
With all the microorganisms under study the mean diameters of zones of inhibition in the CEM+CHX group were significantly greater than those in the CEM group (P<0.05). Table 1 shows the mean diameters of zones of inhibition in the study groups.

Table 1. The mean diameters of zones of inhibition in the study groups

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Mean diameter of zones of inhibition</th>
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<tbody>
<tr>
<td></td>
<td>CEM+CHX</td>
</tr>
<tr>
<td>E. Faecalis</td>
<td>21.10</td>
</tr>
<tr>
<td>P. aeroginosa</td>
<td>13.20</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20.20</td>
</tr>
<tr>
<td>E. coli</td>
<td>17</td>
</tr>
</tbody>
</table>

4. Discussion
In the present study, the antibacterial activity of CEM mixed with chlorhexidine was evaluated against *P. aeroginosa*, *E. faecalis*, *S. aureus* and *E. coli*. The results showed the positive effect of adding CHX to CEM on its antimicrobial activity.

Treatment of teeth with immature apices and repair of perforations are two important procedures in the field of endodontics and MTA is the most commonly used material to this end (Asgary et al., 2008a). CEM was introduced by Asgari et al in recent years. The main constituents of CEM are calcium oxide, sulfur tricalcium, calcium phosphate, calcium carbonate, calcium silicate, calcium hydroxide and calcium chloride. CEM has dental applications similar to those of MTA (Asgary et al., 2008b). Studies comparing these two materials have shown that they have comparable sealing ability; however, the antibacterial activity of CEM is higher than that of MTA (Hasan Zarrabi et al., 2009). Since microorganisms are the main factors involved in the failure of endodontic treatment, the antimicrobial activity of materials used in endodontic treatment has always been of great significance. The bacterial species included in the present study are real endodontic pathogens, which are related to cases resistant to treatment (Shakouie et al., 2014). Although aerobic bacteria or the related microorganisms do not have a great role in initiating primary infections, they are found with a high frequency in root canal treatment failure cases. These bacteria can enter the root canal system before treatment, during treatment and after treatment to cause secondary infection (Kayaoglu et al., 2005). In the present study, agar diffusion technique was used, which is the most commonly used method to evaluate antibacterial activity and has been used by many researchers in a large number of studies. The results of the present study showed that adding 2% CHX to CEM solution results in a significant increase in its antibacterial activity, consistent with the results of a study carried out by Bidar et al, in which direct contact method and bacterial species other than those used in the present study were used (Bidar et al., 2015). The antibacterial
effect of CHX against all the microorganisms in the present study has already been shown. Studies have shown that CHX is effective against bacterial species found in infected root canals, including *S. aureus*, *E. faecalis*, *S. salivarius*, *E. coli* and *C. albicans* (Gomes et al., 2003, Holt et al., 2007, Stowe et al., 2004). Use of CHX is on the rise to increase the antibacterial activity of dental materials to improve prognosis.

CHX is a synthetic cationic bis-guanide, which consists of 2 similar circles of 4-chlorophenyl and two biguanide groups, which are connected to each other with a central chain of hexa-methylene (Stowe et al., 2004). CHX is a lipophilic and hydrophobic positively-charged molecule, which reacts with bacterial cell membrane phospholipids and lipopolysaccharides and enters the cell through a number of active and passive transport mechanisms. Its action is attributed to the reaction of its positive charge with the negative charge of phosphate groups on the cell membrane (Gomes et al., 2003). Therefore, the osmotic balance of the cell is disrupted, increasing cellular permeability and allowing CHX to enter the bacterial cell. CHX is a base and is stable like a salt. The most commonly used oral form of CHX is its gluconate form, which is soluble in water and at physiologic pH releases positively charged CHX. At low concentration of 0.2%, low-molecular-weight components such as potassium and phosphorus exit the cell. On the other hand, at concentrations higher than 2%, CHX results in cell death. Of course it should be kept in mind that adding CHX to WMTA results in cell death and decreases its compressive strength (Holt et al., 2007). In addition, a mixture of MTA and CHX gel did not set for the least 7 days. On the other hand, the solution and gel forms of CHX exert different effects on the setting time of MTA. Kogan et al mixed MTA powder with CHX gel in order to evaluate the compressive strength of this mixture; however, since the mixture did not set up to 7 days after mixing, it was not possible to measure its compressive strength (Kogan et al., 2006). Therefore, it is suggested that further studies be carried out on CEM to evaluate the effect of adding CHX on its physical properties, such as compressive strength and sealing ability.

References


