Evaluation of Antimicrobial Activity of Root Canal Sealers Used in Primary Teeth Against E. faecalis

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Abstract

Aim: The aim of this study was to assess the antimicrobial activity of seven different root canal sealers used in primary teeth against Enterococcus faecalis.

Material and Method: The study was conducted using the agar diffusion inhibitory test. The sealers used were Sure-paste, Metapex, Tg Pex, Calciure, Endoflas FS, Sealapex, Ca(OH)₂ mixed with 2% Chlorhexidine Glucinate solution and one control group material (Klorhex Oral Gel). The injectable form sealers and the freshly-mixed sealers were placed into the prepared wells on the agar plates which were inoculated with the Enterococcus faecalis. After incubation periods of 24 hours, 48 hours, and 72 hours, the zones of the growth inhibition were observed and measured. The average of 8 measurements for each material was taken. The data were compared using one-way analysis of variance (ANOVA) (p<0.05).

Results: Even though Sure-paste, Calciure and Ca(OH)₂ powder mixed with Klorhex irrigation solution have shown antimicrobial activity against E. faecalis, Metapex, Tg pex, Endoflas FS and Sealapex have not. Calciure and Ca(OH)₂ powder mixed with Klorhex irrigation solution was more effective than Sure-paste (p<0.05). The largest zone of inhibition was found with Klorhex oral gel. Calciure and Ca(OH)₂ powder mixed with Klorhex irrigation solution. There were changes in the antimicrobial activity of Sealapex from the 24-h period through the 48-h period and 24-h period through the 72-h period.

Conclusion: Based on the results of this study it can be concluded that Ca(OH)₂/Chlorhexidine glucocan and injectable Ca(OH)₂ pastes more effective than Ca(OH)₂, iodoform and zinc oxide eugenol mixtures.

Key Words: Agar diffusion, antimicrobial, root canal sealer, primary teeth, Enterococcus faecalis.

Özet

Amaç: Süt dişlerinde kullanılan 7 farklı kök kanal dolgu materyalin Enterococcus faecalis’e karşı antimikrobiyal etkinliklerini in vitro ortamda değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Agar difuzyon yöntemi kullanılarak yapılmış bu çalışmada Sure-paste, Metapex, Tg Pex, Calciure, Endoflas FS, Sealapex, %2 lik Klorheksidin Glukonat solüşyonu ile Ca(OH)₂ (Kalsiyum hidroksit) tozundan hazırlanan pat ve kontrol grubu materyalı (Klorhex Oral Gel) kullanılmıştır. Enjektable formda bulunan ve karıştırılarak hazırlanılan patlar Enterococcus faecalis mikroorganizmasının inoküle edildiği agar petriplerinde hazırlanan çukurlara yerleştirilmiştir. 24, 48 ve 72 saat inhibisyon süresinden sonra, oluşan inhibisyon zonları gözlandi ve ölçüldü. Her bir materyal için elde edilen 8 ölçümün ortalama değerleri alındı. Veriler tek yönlü varyans analizi (ANOVA) ile karşılaştırıldı. (p<0.05)

Bulgular: Çalışmada kullanılan materyallerden Sure-paste, Calciure ve Ca(OH)₂ tozu/Klorhex irrigasyon solüşyonu karşıması E. faecalis suşları üzerinde antibakteriyel etkinlik göstermiştir. Metapex, Tg pex, Endoflas FS ve Sealapex ise herhangi bir aktivite göstermemiştir. Calciure ve Ca(OH)₂ tozu/Klorhex irrigasyon solüşyonu karşıması Sure-paste’den daha etkili bulunmaktadır. Fakat, en geniş inhibisyon zonu Klorhex oral gel (kontrol grubu) ile bulundu. Fakat, Klorhex oral gel, Calciure ve Ca(OH)₂ tozu/Klorhex irrigasyon solüşyonu karşıması arasında anlamlı bir fark yoktur. 24-saat süresinden 48-saat süresinde ve 24-saat süresinden 72-saat süresine kadar geçen zamanda Sealapex’in antibakteriyel etkinliğinde değişiklik olmuştur.

Sonuç: Ca(OH)₂ tozu/Klorhex irrigasyon solüşyonu karşıması ve enjektabl Ca(OH)₂ patlarının, Ca(OH)₂ iyoform ve çinko oksit ojenel karşımlardan ve Ca(OH)₂ bazlı bir kök kanal dolgu materyali olan Sealapex’den daha fazla antimikrobiyal etkinlikleri vardır.

Anahtar Kelimeler: Kök kanal dolgu patı, kalsiyum hidroksit, klorheksidin glukonat, süt diş, enterococcus faecalis.

Introduction

Maintenance of the integrity of the primary dentition until the normal exfoliation period is vital for proper development and maturation of the child, maintaining arch length, arch symmetry, normal occlusion, function and esthetics. Nonvital primary teeth with and without periapical pathosis should be retained until their normal exfoliation for preventing undesirable aberrations in the future dentition. So, endodontic treatment of these teeth has been a successful method. The success of endodontic treatment depends on the clinical and radiological state of the treated teeth, achieved through chemo-mechanical preparation of root canals elimination of infecting microorganisms and obturation of the root canal space with a good root canal sealer. However, even after careful
cleaning and shaping of the root canal system, microorganisms might still remain in the tubules or lateral canals\(^7\). Therefore, to avoid the growth of these microorganisms, endodontic filling materials should have an antibacterial effect.

Root canals provide an ideal culture media for bacterial growth which harbor different types of microorganisms\(^5\). Tomie-Karovic and Jelinek\(^6\), Edward and Nord\(^9\) demonstrated that root canal infections of primary teeth are usually polymicrobial in nature. It is known that the predominant pathogens in the root canal are especially anaerobes\(^10,11\). E. faecalis was the most common facultative anaerobic bacteria isolated from the root canals with pulpal and periapical infections\(^12,14\). In addition to this, E. faecalis was used in various studies which evaluate antibacterial activity of root canal sealers\(^1,4,6,13,14\).

The major goal of root canal treatment is the inhibition of microorganisms from root canal system and prevention of subsequent reinfection\(^15\). For this reason, to avoid the growth of the microorganisms, root canal sealers should have antibacterial effect.

The purpose of this study was to compare the antibacterial effect of different root canal sealers used in primary teeth.

**Materials and Methods**

The sealers and one control group material (Klorhex Oral Gel) tested for comparing antibacterial activity against E. faecalis in this study were; Sure-paste (Sure-endo, Sure Dent Co., Korea), Metapex (Meta Biomed Co., Korea), Tg Pex (Technical & General Ltd., London), Calcicure (Voco, Germany), Endoflas FS (Sanlor Laboratories, USA), Sealapex (Kerr, USA), Ca(OH)\(_2\) (Calcium Hydroxide U.S.P, Sultan Healthcare Inc., USA) mixed with Klorhex irrigation solution (\%2 Chlorhexidine Gluconate, Drogzan, Ankara, Turkey). The compositions of the sealers are shown in Table 1. Non-injectable materials as Sealapex and Endoflas FS were mixed according to manufacturers’ instructions. Ca(OH)\(_2\) powder and \%2 chlorhexidine mixed the specified proportions (2gr calcium hydroxide powder per ml of \%2 chlorhexidine) on the sterilized glass with spatula. After the pastes were prepared with toothpaste consistence, each freshly mixed dental material was back loaded into sterile 2ml syringes and kept ready.

Antibacterial activities of the sealers were evaluated against the E. faecalis (ATCC 29212) for Agar Diffusion Test. The strain was obtained from the Department of Microbiology, Faculty of Medicine of Kirikkale University. 8 petri dishes previously sterilized were prepared containing Mueller-Hinton agar, in the necessary volume to obtain 4 mm of thickness. Inocula from a 24 hour growth of the test organisms were added in sterile saline, incubated at 37 °C, and allowed to grow to obtain a turbidity equivalent to the 0.5 McFarland Standard. Using a sterile swab the entire surface of the agar plate was swabbed 3 times to ensure even distribution of the inoculum. 8 wells (6mm diameter, 4mm depth) were made by removal of agar at equidistant points using sterile pipette tip, and these were filled immediately with sealers. Klorhex Oral Gel was used as a positive control. After the sealers setting, the agar plates were incubated at 37 °C for 24 hours to allow the microorganisms to grow and reagents to diffuse through the culture medium. After incubation, the inhibition zones (mm) formed around the wells containing sealers were measured with a millimeter ruler and recorded at 24 hour, 48 hour and 72 hour intervals. The test was performed in 8 replicas, and the average reading with a standard deviation was calculated for each sealer tested (Table 2). The data were compared using one-way analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>MATERIAL NAME</th>
<th>MANUFACTURERS</th>
<th>COMPOSITIONS</th>
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</thead>
<tbody>
<tr>
<td>Klorhex Oral Gel</td>
<td>Drogzan, Ankara, Turkey</td>
<td>%1 Chlorhexidine Gluconat</td>
</tr>
<tr>
<td>Sure-paste</td>
<td>Sure Dent Co., Korea</td>
<td>Calcium hydroxide, Barium sulfate, Thickening agent, Glycol derivatives, Purified water</td>
</tr>
<tr>
<td>Metapex</td>
<td>Meta Biomed Co., Korea</td>
<td>Iodoform, Calcium hydroxide, Silicone oil</td>
</tr>
<tr>
<td>Tg Pex</td>
<td>Technical &amp; General Ltd., London</td>
<td>Calcium hydroxide</td>
</tr>
<tr>
<td>Calcicure</td>
<td>Voco, Germany</td>
<td>Water, Cellulose derivatives, Barium sulfate, Barium sulfate, Zinc oxide, Eugenol, Iodoform, Calcium hydroxide, Zinc acetate</td>
</tr>
<tr>
<td>Endoflas FS</td>
<td>Sanlor Laboratories, USA</td>
<td>Calcium oxide, Bismuth trioxide, Zinc oxide, Sub-Micron silica, Titanium dioxide, Zinc stearate, Tricalcium Phosphate, Ethyl toluene sulfonamide, Methylene methyl salicylate, Isobutyl salicylate, Pigment</td>
</tr>
<tr>
<td>Sealapex</td>
<td>Kerr, USA</td>
<td>Calcium hydroxide, %2 Chlorhexidine Gluconat</td>
</tr>
<tr>
<td>Klorhex irrigation solution</td>
<td>Drogzan, Ankara, Türkiye</td>
<td>Ca(OH)(_2) powder</td>
</tr>
<tr>
<td>Calcium Hydroxide U.S.P.</td>
<td>Sultan Healthcare Inc., USA</td>
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Wound edges were closed with primary interrupted sutures (Fig 3.c,3.d). Histopathological examination revealed cystic lesion with an squamous epithelial lining with prominent granular layer and cyst content of lamellar keratin (Fig.4).

Results

The mean diameter of inhibition haloes (mm) around the agar wells and standard deviations of the antibacterial activity for freshly mixed, 24 hour old, 48 hour old and 72 hour old endodontic sealers used in primary teeth are shown in Table 2. However, wells filled with Klorhex oral gel called positive control group showed zone of growth inhibition in all intervals. Even though Sure-paste, Calcicure and Ca(OH)₂ powder mixed with Klorhex irrigation solution have shown antibacterial activity against E.faecalis, Metapex, Tgpex, Endoflas FS and Sealapex have not. Calcicure and Ca(OH)₂ powder mixed with Klorhex irrigation solution was more effective than Sure-paste (p<0.05).

Discussion

However, even after instrumentation, irrigation and intracanal dressing procedures in endodontic treatment, total elimination of microorganisms from the root canal system is not possible because of the complexity of the root canal system. Therefore, both intracanal medication and root canal filling materials should have an antibacterial activity.

Generally, three in vitro techniques have been used for evaluating antibacterial activities of materials which get infection under control: the dilution method, which yields a quantitative result for the amount of antimicrobial agent that is required; the agar diffusion method, which gives an inhibition zone around the wells containing the agent; and the direct exposure method, which relies on direct and close contact between the test microorganism and the tested material. All of these methods have advantages and disadvantages. The disadvantage of the agar diffusion test is that the result of this method depends on the solubility and diffusibility of the tested material. For example, a material which diffuses more easily than others, may have larger zones of microbial growth inhibition. The agar diffusion test used in this study is one of the most frequently used methods for evaluating the antimicrobial activity of root canal sealers and it permits to compare the root canal sealers against the test microorganisms directly. The contribution of microorganisms to indication and development of pulpal and periapical infections are mixed and polimicrobial, with predominance of obligate anaerobes, facultative anaerobes and rarely aerobes. E.faecalis, a facultative anaerobic microorganism, has been used in various studies of antibacterial properties as used in this study, because of it is the most resistant species to eliminate from root canal and can survive in filled root canals. It is frequently isolated failure of the endodontic treatments. E.faecalis has been reported to survive in an alkaline environment and has been resistant to antibacterial agents. In addition to all these features, E.faecalis isolation...
and identification is relatively easy, so it was used in various antibacterial efficiency studies\textsuperscript{21,22,24-27}.

Calcium hydroxide has also been used as a root filling material for primary teeth alone\textsuperscript{28,29} or mixed with iodoform; it is commercially available as Vitapex\textsuperscript{30}, Metapex\textsuperscript{31} and TgPex. Calcium hydroxide is frequently used intracanal medication because of its consistent antibacterial activity\textsuperscript{32,33}. The antibacterial effect of Ca(OH)\(_2\) is related to its ionic dissociation into calcium and hydroxyl ions and their toxic effects on bacteria, acts by inhibiting cytoplasmic membrane enzymes\textsuperscript{34}. However, specific microbes, such as E. faecalis are resistant to Ca(OH)\(_2\).\textsuperscript{35} But, there is respectable variation in the release of hydroxyl ions and therefore in the antibacterial activity for different composition of these materials\textsuperscript{36}.

Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent that has been reported to be an effective medicament in endodontic therapy\textsuperscript{37}. In addition, it is also effective against strains resistant to Ca(OH)\(_2\)\textsuperscript{38}. Depending on this information, Basrani et al.\textsuperscript{39} and Gomes et al.\textsuperscript{40} demonstrated that CHX was effective against E. faecalis, but Ca(OH)\(_2\) alone had no effect. Other studies showed that the antibacterial effect of Ca(OH)\(_2\) increased significantly when mixed with chlorhexidine\textsuperscript{41,42}. CHX gel or Ca(OH)\(_2\) powder mixed with CHX gel may have promise as an intracanal medicament in teeth with infected canals and may have use of a root canal cement, but further research is required\textsuperscript{43}.

In 1928 Walkoff\textsuperscript{44} introduced iodoform as a root canal filling material in primary teeth and proved to be a bactericidal, non-irritant radiopaque material. On the other hand, Ca(OH)\(_2\) + Iodoform exhibited no antibacterial activity against most pure cultures in agar diffusion tests\textsuperscript{45}. Nurko and Garcia\textsuperscript{46} reported that Vitapex (calcium hydroxide/iodoform paste) used as a root canal filling material for infected primary teeth, found clinically and radiographically successful. In the present study, the Vitapex and showed the worst antimicrobial results against E. faecalis as the previous studies\textsuperscript{1,44}. The weak activity may be partially explained by the fact that Ca(OH)\(_2\) an ingredient of these sealers has been demonstrated to interfere with the antisep tic capacity of dyadic combinations of endodontic medicaments (45). Endoflas is a resorbable paste which contains similar components as Vitapex, with the addition of zinc oxide and eugenol\textsuperscript{46}. In 2007, Reddy and Ramakrishna\textsuperscript{47} found weak inhibitory effect against Gram-positive bacteria as E. faecalis for ZOE. In this study, ZOE containing Endoﬂas showed no antibacterial effect.

Sealapex, is a calcium hydroxide-based sealer, used in root canal treatment for primary teeth. It showed the high success rate, the absence of any adverse response and the resorption of the extruded material\textsuperscript{47}. Sealapex, releases calcium hydroxide in the fresh state. A study that evaluate the antibacterial activity of five different endodontic sealers, using both Direct Contact Test (DCT) and Agar Diffusion Test (ADT). According to DCT results, Sealapex inhibit bacterial growth when freshly mixed but lose their activity over time\textsuperscript{48}. Depending to this data, absence of the antibacterial effect for Sealapex may be explained.

Based on the results of this in vitro study, it was concluded that Ca(OH)\(_2\)/Chlorhexidine gluconate mixture and injectable Ca(OH)\(_2\) pastes have more antimicrobial efficiency than Ca(OH)\(_2\), iodoform and zinc oxide eugenol mixtures and a Ca(OH)\(_2\) based root canal sealer called Sealapex.

References

47. Sari S, Okte Z. Success rate of Sealapex in root canal treatment for primary teeth: 3-year follow-up.

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