Efficacy of various intracanal medicaments against Enterococcus faecalis in primary teeth: An *in vivo* study

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The aim of this study was to evaluate the in vivo efficacy of three intracanal medicaments ($Ca(OH)_2$, 1% chlorhexidine gel and 1% chlorhexidine gel with $Ca(OH)_2$ against Enterococcus faecalis *in necrotic primary teeth.*

As a conclusion, chlorhexidine gel with or without $Ca(OH)_2$ was more effective than $Ca(OH)_2$ alone against Enterococcus faecalis.

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INTRODUCTION

Bacteria are the main causative factors in pulpal and periapical infections.[1] Anaerobes, especially black-pigmented gram-negative anaerobes are implicated in the development of acute periradicular inflammation, involving signs and symptoms such as pain, swelling, tenderness and exudation.[2,3] The microorganisms found in the root canals of primary teeth are similar to those in the root canals of permanent teeth.[4,5] One of the most important objectives of the root canal treatment is the elimination of microorganisms from the root canal system.[6] Although, chemomechanical preparation has an important cleaning effect, it cannot eliminate all the bacteria from the root canal system. These remaining bacteria may multiply during the period between appointments often reaching the same pathogenic level found at the beginning of the previous session, in cases where the canal is not dressed with a disinfectant between visits.[7] Therefore, the use of intracanal dressing with antibacterial properties may help to prevent this occurrence.[8]

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Calcium hydroxide $Ca(OH)_2$ is commonly used in endodontics for this purpose. When used as an intracanal medicament, $Ca(OH)_2$ has been shown to be effective in eliminating bacteria from the root canal space.[9,10] The antimicrobial 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 with consequent changes in the organic components and in nutrient transport.[11] However, specific microbes, such as *Enterococcus faecalis* (*E. faecalis*) are resistant to $Ca(OH)_2$.[12] Therefore, alternative medicaments should be sought that would maximize microbial eradication when used as an intracanal dressing.

Many studies demonstrated that chlorhexidine (CHX) was more effective than $Ca(OH)_2$ in eliminating *E. faecalis* infection inside dentinal tubules.[13-17] Especially CHX in gel form acts by adsorbing on to the microorganism cell wall and causing intracellular component leakage. CHX has been widely used in dentistry because of its broad-spectrum antimicrobial activity and substantivity.[18] Its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the CHX molecule to penetrate the bacteria with intracellular toxic effects.[19] The combination of $Ca(OH)_2$ and CHX gel was also found to be effective against *E. faecalis*.[16,20-22] However, in most of the studies, it has been reported that CHX gel alone was more effective than CHX gel with $Ca(OH)_2$ against *E. faecalis*.[5,22]

Studies on root canal treatments in primary teeth are insufficient. Therefore, the aim of the present study was to assess the effect of three different intracanal medicaments against *E. faecalis* in necrotic primary teeth in *in vivo* conditions.

MATERIALS AND METHODS

Canals of one hundred and twenty eight single-rooted maxillary primary teeth, with pulp necrosis and radiographically visible chronic apical infections in 56 children (4 to 6 years old) of both sexes were included. The patients did not report the use of antibiotics for at least 3 months before the treatment and were healthy. Ethics approval from the local commitee of Ege University Medical Faculty and written consents from the parents of all patients were obtained.

Periapical radiographs were taken using a long-cone technique and Kodak ultraspeed (22mm x 35mm) film with a film holder. Teeth with external or internal resorption, having excessive mobility were not included. After isolation with a rubber dam, the operative field was disinfected with 30% hydrogen peroxide and 10% iodine tincture as suggested by Moller.[23] Inactivation of these medicaments was performed by using 5% sodium thiosulfate solution. Firstly, a sample was taken to check the sterility of the operation field. The pulp chambers were opened under aseptic conditions with sterile water cooled high speed diamond round burs. After the root canal orifice was detected, a sterile size 20 paper point was introduced into the length of the canals as determined by an electronic apex locator (ROOT ZX - J. Morita MFG. Corp. Kyoto, Japan) for the initial microbiological sampling from the root canals. The paper point was kept in place for 60 seconds(s) and then immediately transferred to a test tube containing transport medium-VMGA III gel.[23] Instrumentation was performed starting one mm above the apices with Hedstroem files (FKG Dentaire, La Chaux de Fonds, Swistzerland) up to size 50. Only sterile distilled water was used for the irrigation of the root canals. After the mechanical preparation, a second microbiological sampling was carried out with a sterile paper point as decribed above and transferred to an another test tube containing VMGA III gel. Both first and second microbiological samples were taken to the microbiology laboratory for processing within 2 hours (h).

After the second sampling, the root canals (n=128) were irrigated with sterile distilled water and dried with sterile paper points. All root canals were randomly divided into three experimental and a control groups as described below:

1. (n: 32) $Ca(OH)_2$ powder mixed with distilled water (Aktu Tic Ltd, Izmir, Turkey)
2 (n: 32) 1% CHX gluconate gel (Drogsan, Ankara, Turkey)
3 (n:32) 1% CHX gluconate gel (Drogsan, Ankara, Turkey) with $Ca(OH)_2$ powder (Aktu Tic Ltd, Izmir, Turkey)
4 (n: 32) Empty, negative control group

All medicaments were applied with a syringe and a 26-gauge needle. In negative control group, no medication was applied. Subsequently, a sterile cotton pellet was placed at the entrance and the cavities were temporarily sealed with glass ionomer cement (Ketac-Fil, 3M ESPE, Germany).

For microbiological procedures, the test tubes containing samples in VMGA III were preincubated for 30 minutes (min) at 37ºC, and shaken vigorously in a vortex mixer (Vortex, Scientific Industries, Inc. Springfield, MA) for 60 s. Serial 10-fold dilutions were made up to $1:10^6$ in 1% sterile peptone water (Bacto peptone, Difco, Detroit MI, USA). From the serial dilutions, 0.1 ml was transferred and plated on Brucella (BBL, Becton Dickinson Microbiological Systems, Cockeysville, Md) blood agar plates. The plates were incubated in an anaerobic chamber for 48 hours and the *E. faecalis* counts were determined as CFU/ml. The purity of the cultures was confirmed by Gram staining, catalase production, colony morphology on blood agar and using a biochemical identification kit (API 20 Strep, bio-Merieux: Marcy-I'Etoile, France).

In order to evaluate the effect of the medicaments against *E. faecalis*, the patients were recalled after 48 hours and third microbiological sampling was carried out with sterile paper points from the root canals as described above. Before the sampling, the root canal was instrumented with Hedstroem file with size 50 and irrigated with 2 ml sterile distilled water to remove all medicaments. To neutralize CHX, 0.5% Tween 80 + 0.07% lecithin was used.[24] All microbiological procedures were done as described above.

After the last microbiological sampling, all root canals were filled with $Ca(OH)_2$, restored with glass ionomer cement (GC Fuji IX GP, Tokyo, Japan) and stainless steel crowns (3M Dental products, St. Paul MN 55144, Canada, USA) were cemented on all teeth. All patients were rescheduled for recall visits every three months.

Statistical analysis were carried out using the SPSS 13.0 software program (SPSS Inc., Chicago, IL, USA) with One way Anova, Wilcoxon and t-tests.

RESULTS

128 necrotic primary teeth were studied bacteriologically with special emphasis focused on the presence of *E. faecalis* in the root canals. *E. faecalis* was isolated from eighty of these 128 teeth (63%). Only the results of these eighty necrotic single-rooted maxillary primary teeth were evaluated in this study.

The distribution of these 80 primary root canals was:

1 **n: 23** (29%) $Ca(OH)_2$ powder mixed with distilled water
2 **n: 19** (24%) 1% CHX gluconate gel
3 **n: 20** (25%) 1% CHX gluconate gel
4 **n: 18** (22%) Empty, negative control group

According to the results of the initial microbiological sampling, no significant difference was found in the mean *E. faecalis* counts (CFU/ml) between all groups ($p>0.05$). In the second microbiological sampling, a statistically significant decrease was found in the mean counts of *E. faecalis* (CFU/ml) compared to the initial sampling in all groups (70.9%) ($p<0.05$) (Table-I). However, a statistically significant difference was not found between all groups ($p>0.05$). (Table 1)

Table 1. The mean (SD) E. faecalis counts (CFU/ml) of the samples

	The mean (SD) *E.faecalis* counts before instrumentation (CFU/mL)	**The mean (SD) *E.faecalis* counts after instrumentation** (CFU/mL)	**The mean (SD) *E.faecalis* counts 48 h after medication** (CFU/mL)
Group I ($Ca(OH)_2$)	1.9×10^5 (5.6×10^5)	$3.7x10^3$ ($2.8x10^2$)▽	$8.6x10^2$ ($3.7x10^1$)▼
Group II (1%CHX gel)	3.1×10^5 (5.2×10^5)	$5.2x10^3$ ($3.1x10^2$)▽	$3.7x10^1$ ($1.1x10^1$)
GroupIII (1% CHX gell with $Ca(OH)_2$)	8.9×10^5 (8.1×10^5)	$5.9x10^3$ ($5.3x10^2$)▽	$1.2x10^1$ ($1.0x10^1$)
Group IV (Positive control group)	8.4×10^5 (9.6×10^4)	$2.7x10^3$ ($6.9x10^3$)▽	$3.4x10^4$ ($8.6x10^3$)□

SD: Standard deviation

▽ Statistically significant decrease was found after mechanical preparation with the iriigation of only sterile distilled water when to the initial E.faecalis counts (CFU/ml) ($p<0.05$).

▼ According to the 48 h results, the mean E. faecalis counts (CFU/ml) in the Ca(OH)2 group (Group-I) were significantly higher than the 1% CHX gel (Group-II) and 1% CHX gel with Ca(OH)2 groups (Group-III) ($p<0.05$).

□ According to the 48 h results, the mean E. faecalis counts (CFU/ml) in control group (Group-IV), were significantly higher than the experimental medicament groups (Group I-III) ($p<0.05$).

At the third sampling which carried out 48 hours after the medication, the mean counts of *E. faecalis* (CFU/ml) in all experimental medicament groups (Group I-III) were significantly lower than the negative control group (Group-IV) ($p<0.05$). Nevertheless, the efficacy of $Ca(OH)_2$ against *E. faecalis* was significantly lower than CHX gel with or without $Ca(OH)_2$ at 48 hours results ($p<0.05$). There was no statistically significant difference between the efficacy of CHX gel and CHX gel with $Ca(OH)_2$ against *E. faecalis* at 48 hour results ($p>0.05$).

DISCUSSION

Chemomechanical preparation is effective in reducing the number of bacteria in the root canals. However, bacteria may remain viable after root canal preparation, multiplying in the period between treatment. [25,26] Gomes et al.[27] showed that whereas chemomechanical preparation generally reduced the frequency of isolation different bacterial species in subsequent appointments a limited number of species, especially *E. faecalis* was isolated more frequently in later appointments. Thus, intracanal medication may be a valuable adjunct to chemomechanical preparation in the disinfection of the root canal system. In accordance with recent studies, it was observed that *E. faecalis* counts were decreased in 70.9% of all samples immediately after mechanical instrumentation (at the second sampling) with the irrigation of only sterile distilled water in this study. Nevertheless, 48 h after the medication (at the third sampling), the mean *E. faecalis* counts in the negative control group (Group-IV) were significantly higher than the experimental groups (Group I-III). This was pointed out the recurrence of the infection in the root canals when intracanal medication had not been used which was concordant with most of the studies.[26,28,29-31]

E. faecalis was chosen as the test species because of its implication as a possible microbial factor in therapy-resistant apical infection. It is a non-fastidious microbe that is relatively easy to culture and has been shown *in vitro* to predictably penetrate deeply into human dentinal tubules.[32]

Culture studies have revealed that *enterococci* are not normally present or are present in very low numbers in primary root canal infection.[33] Its incidence in root filled permanent teeth with periradicular lesions can range from 30% to 40% of the cases.[34] Although it is occasionaly found in the initial root canal infections in permanent teeth, it was found to be present in 63% in necrotic primary teeth in the present study. This distinction might be because of the differences between the previous studies[33,34] and the present study. The canals of the primary necrotic single-rooted incisor teeth in this study were exposed to oral cavity due to the early childhood caries in all patients. The high frequency of *E. faecalis* counts at initial microbiological sampling might be due to this situation.

Choosing $Ca(OH)_2$ as an intracanal medicament in this study was due to its popularity as an intracanal medication in root canal treatments. Besides, there is some controversy about its efficacy against *E. faecalis*.[35,36] In contrast to these studies, Almyroudi et al.[20] indicated that $Ca(OH)_2$ was effective against *E. faecalis*. The present study also confirmed that $Ca(OH)_2$ was effective against *E. faecalis* 48 h after the medication placement. However, the efficacy of $Ca(OH)_2$ was significantly lower than the other tested medicaments containing CHX gel. This finding was in accordance with other studies.[13-17]

The results of the present study revealed that CHX gel with $Ca(OH)_2$ was the most effective against *E. faecalis* than the other tested treatments. Nevertheless, many studies demostrated that CHX gel with $Ca(OH)_2$ application compared to CHX gel alone had a decreased antibacterial effect between days 7-15.[16,20,37] Gomes et al.[16] explained the decreased antibacterial effect of $Ca(OH)_2$ on CHX possibly due to loss in its capacity to adhere to the bacterial cell wall. Other hypotheses point to the buffer effect that dentin exerts over $Ca(OH)_2$ reducing its antibacterial action on 1% CHX gel.[38] They indicated that the antibacterial activity

against *E. faecalis* of 2% CHX gel alone and/or 2% CHX gel with $Ca(OH)_2$ plus applied into the root canals was zero by day 30. The present study revealed that 1% CHX gel alone effectively reduces the *E. faecalis* counts in necrotic primary teeth after 48 hours. This result is in accordance with previous studies.[14,36,38-40] The combination of $Ca(OH)_2$ and 1% CHX was also found to be effective against *E. faecalis* (20) which has been corroborated by the results of the present study. In contrast, with most of the previous studies,[16,22,36,37] CHX gel with $Ca(OH)_2$ was found to be the most effective intracanal medication against *E. faecalis* in the present study. This difference might be due to the difference between the *in vivo* and *in vitro* conditions. We should understand that the benefit of the $Ca(OH)_2$ is to physically seal the canal for the duration of the 48 hours. On the other hand, only the sterile distilled water was used to irrigate the root canals during the mechanical preparation in the present study. This procedure was used in order to eliminate the residual and/or combined antimicrobal effects of the irrigants with intracanal medicaments.

The significance of this study derives from its being conducted under *in vivo* conditions where the use of an intracanal medication could disrupt established, nutritional interrelationships, eliminating some bacteria that might have been essential to the growth of others or leaving some bacteria whose presence would inhibit the growth of others. These microorganisms which were present but undetected in the preintervention sample depressed by the enterococci, might have been unaffected when the enterococci were challenged. It could be suggested that further *in vivo* investigations with other root canal microorganisms might be useful.

According to the best of our knowledge, this is the first *in vivo* study indicating the effect of the different intracanal medicaments against *E. faecalis* in necrotic primary root canals. It was demonstrated that CHX gel with or without $Ca(OH)_2$ was more effective against *E. faecalis* than $Ca(OH)_2$ within 48 hour period in necrotic primary root canals in *in vivo* conditions.

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