Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.912285

Original research

IN VITRO COMPARISON OF THE ANTIMICROBIAL EFFECTS OF DIFFERENT ROOT CANAL MEDICAMENTS ON *ENTEROCOCCUS FAECALIS* AND *CANDIDA ALBICANS*

*Burçin Arıcan[1]
Hesna Sazak Öveçoğlu[2]

ABSTRACT

Objectives: To evaluate the antimicrobial effects of experimental root canal medicaments on *Enterococcus faecalis* and *Candida albicans* on day 3 and day 7 using quantitative polymerase chain reaction (Q-PCR) analyses.

Materials and Methods: 240 single-rooted, single-canalled human teeth were used. Root canals were shaped mechanically and embedded in acrylic blocks, then sterilized in an autoclave. The samples were divided into two groups and infected with *E. faecalis* and *C. albicans*, and then divided into subgroups (n=10); calcium hydroxide ($Ca(OH)_2$), triple paste (TAP), double paste (DP), modified double paste (MDP), lactoferrin (Lf), negative and positive groups. At the end of 3 and 7 days, paper points containing the root canal samples were placed in empty Eppendorf tubes, and DNA was isolated. Real-time Q-PCR was applied and the data were analyzed statistically.

Results: The antimicrobial effects of each medicament increased from days 3 to 7. $Ca(OH)_2$ and TAP groups showed the similar eradication rates for *E. faecalis* and *C. albicans* on day 3 and 7 ($P>0.05$). There was no significant difference between DP, MDP and Lf for the eradication of microorganisms at both experimental days ($P>0.05$), except the amount of eradicated *E. faecalis* by DP at day 7 in which DP caught the similar percentages with TAP and $Ca(OH)_2$ ($P<0.05$).

Conclusions: Experimental medicaments demonstrated antimicrobial efficiancy similar to those used routinely in endodontic clinic. It is promising that lactoferrin which is a very biocompatible material can be used in different combinations as an intracanal medicament.

Key words: Antibiotic paste, *Candida albicans*, *Enterococcus faecalis*, Lactoferrin, Root canal medicament.

ORCID IDs of the authors:
B.A. 0000-0001-5757-0571
H.S.Ö. 0000-0003-4709-422X

[1] İstanbul Okan University, Faculty of Dentistry, Department of Endodontics, Turkey.
[2] Marmara University, Faculty of Dentistry, Department of Endodontics, Turkey.

Received : 09.04.2021
Accepted : 05.08.2021

How to Cite Arıcan B, Sazak Öveçoğlu H. In Vitro Comparison of the Antimicrobial Effects of Different Root Canal Medicaments on Enterococcus Faecalis and Candida Albicans. Cumhuriyet Dent J 2021;24:3:256-265.
***Corresponding Author:**
Prof. Dr. Necmettin Erbakan St. No: 2 Istanbul Okan University, Faculty of Dentistry, Department of Endodontics, Tuzla/Turkey.
Phone: + 90 532 604 89 76 **E-mail:** dr.burcinarican@gmail.com/burcin.arican@okan.edu.tr

INTRODUCTION

The central aim of root canal treatment is to eliminate bacteria from the infected root canal and prevent a subsequent reinfection.[1] Microorganisms and their byproducts are the main etiological factors in dentinal, pulpal, and periapical pathosis. *Enterococcus faecalis* and *Candida albicans* are the most common microorganisms isolated during root canal treatments that can best adapt easily to environmental changes.[2] Intracanal medicaments should be used to eliminate these factors following mechanical preparation.

Several root canal medicaments are in use to successfully eliminate microorganisms and infected tissues. $Ca(OH)_2$ is commonly used as an effective intracanal agent; however, some recent studies have questioned the ability of $Ca(OH)_2$ to kill some resistant bacterial species in root canals.[3,4] The combination of metronidazole, ciprofloxacin, and minocycline (TAP) has been developed to disinfect oral lesions.[5] However, this combination of medicaments has some disadvantages, such as the inability to remove the antibiotics from the root canal[6] and crown discoloration.[7] Thus, new medicaments and antibiotic combinations should be developed.

In 1979, *Streptococcus faecalis* was reported to be resistant to aminoglycosides. However, using the aminoglycosides with penicillin caused a synergistic effect against the same microorganism.[8] This combination has been advised for use as a treatment option against infective endocarditis by the European Society of Cardiology since 2009.[9] Accordingly, it may show antimicrobial effects against persistent microorganisms isolated from the root canal and may be used as a new generation intracanal medicaments in endodontics.

Another potential medicament could be lactoferrin (Lf), which is a multifunctional iron glycoprotein in human secretions, such as milk, amniotic fluid, vaginal mucus, synovial fluid, and seminal plasma.[10] Lf exhibits non-iron-dependent antibacterial, antioxidant, antifungal, antiviral, antitumor, anti-inflammatory, and immunoregulatory activities.[11,12] Lf inhibits the initial stage of infection by destroying the cell membrane structure or blocking adhesion to the host cell.[13] Moreover, it was reported that Lf severely prevent the formation of bacterial biofilm.[14]

Although there are several studies about the antimicrobial activity of $Ca(OH)_2$ and TAP against endodontic pathogens[3,7], to the best of authors' knowledge there are no studies in the literature regarding antimicrobial effect of the mentioned novel antibiotic combinations and lactoferrin. Therefore, in the present study, it was aimed to compare the antimicrobial activities of these new age medicaments and traditional agents towards *E. faecalis* and *C. albicans* using quantitative polymerase chain reaction (Q-PCR) analyses.

MATERIAL AND METHODS

All patients signed informed consent to permit use of their extracted teeth in this study. A total of 240 intact teeth which were freshly extracted for periodontal or orthodontic reasons were selected for study. The inclusion criterion was single-rooted and single-canalled teeth. The exclusion criteria were teeth from patients who received antibiotic therapy in the previous 3 months, teeth with a root fracture, previous endodontic therapy, oval shaped canal, curved canal, and carious lesions.

Preparation of root canals

A total of 240 roots from freshly extracted human teeth were used. The bone, calculus, and periodontal tissues on the root surface were gently removed with periodontal curettes. The teeth remained in saline solution until they were used. Each tooth was decoronated horizontally at the cement to enamel junction, and root lengths were standardized to 15 mm. After preparing the access cavity, root canal patency was defined with a 15 K file. The Protaper Universal System (Dentsply Maillefer, Ballaigues, Switzerland) was used as rotary instrumentation. An SX file was used followed by S1 and S2 files in the coronal part of the canal. The canals were finished using F1, F2, and F3 files to full working length. After using each instrument, 5.25% NaOCl was used for irrigation for 1 minutes. A final rinse with 10 mL of 5.25% NaOCl was used followed by 10 mL irrigation with 17% EDTA to remove the dentin particles.[7] The apical foramen was closed with composite resin and the outer surfaces of the specimens were covered with nail varnish to prevent possible

contamination from the external surface. The roots were embedded in acrylic blocks and sterilized by autoclaving for 30 min at 120°C.

Sample contamination

Ten teeth from each microorganism group were used as a negative control (Figure 1).

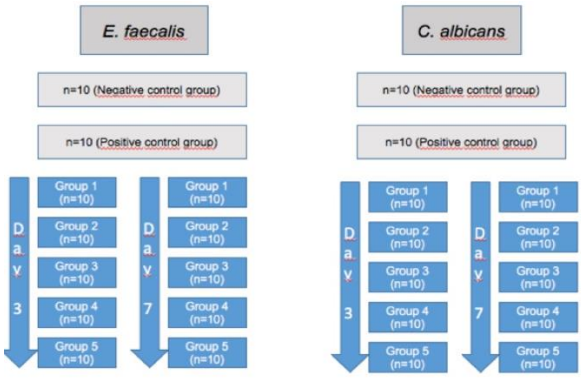

Figure 1: Distribution of the 240 extracted teeth according to the groups

The 220 teeth were randomly divided into two groups; 110 teeth were contaminated with freshly subcultured (24 h) *E. faecalis* ATCC 29212, which was grown in brain heart infusion (BHI) agar and BHI broth. The other 110 teeth, contaminated with freshly subcultured (24 h) *C. albicans* ATCC 10231, were grown in Sabouraud dextrose (SD) agar and SD broth. The cell suspensions were adjusted to 0.5 McFarland units. Each tooth was inoculated with microorganisms using a micropipette and agitated with a #15 K file. This procedure was repeated every 24 h for 2 days using freshly prepared 24 h cultures. The root canal orifices were sealed with temporary filling material each time. The teeth were always kept in a humid environment at 37°C.

Dressing of the canals

At the end of the 48h inoculation period, the temporary filling material was removed and the root canals were irrigated with 10 mL saline solution. Ten teeth from each microorganism group were used as a control to check for contamination of the canal. Three sterile paper points were placed in the canal and kept in the place for 60 s to sample the microbes. These paper points were placed in sterile Eppendorf tubes.

In total, 100 teeth from each microorganism group were divided into five groups, according to the intracanal medicaments used, as follows:

- Group 1 (n: 10 + 10): Calcium hydroxide ($Ca(OH)_2$) (Vision, Frankfurt, Germany) was mixed with sterile saline in a ratio of 2:1 (liquid/power) to obtain a paste-like consistency.
- Group 2 (n: 10 + 10) (TAP): Equal weights (200 mg) of metronidazole (Flagyl, Eczacıbaşı, Turkey), ciprofloxacin (Cipro, Biofarma, Turkey), and minocycline (Minocin, Teofarma, Italy) (1:1:1) were mixed with sterile saline[5] to a final concentration of 0.1 mg/mL. A paste-like consistency was delivered to canal using a lentulo.
- Group 3 (n: 10 + 10) (DP): Ampicillin (Alfasilin, Actavis, Istanbul, Turkey) (200 mg) was mixed with gentamicin (Genta, İE Ulagay, Istanbul, Turkey) (125 µL) to obtain a paste-like consistency.
- Group 4 (n: 10 + 10) (MDP) Ampicillin (200 mg) was mixed with gentamicin (125 µL) and 2% LF suspension (50 µL) to obtain a paste-like consistency,
- Group 5 (n: 10 + 10): A 2% suspension of Lf (Sigma Aldrich, USA) in sterile saline was prepared in an Eppendorf tube. The tube was covered with aluminum foil to design a dark medium. F3 paper points soaked in this suspension were placed into the root canal.

A total of 12 pilot studies were conducted to determine the proportions of the experimental root canal medicaments. Of these pilot studies, 2 are on the agar diffusion method, 8 on the culture method and 2 on the testing of different PCR methods.

The prepared medicaments were placed in the root canals by using lentulo until the excess medicaments were seen from the coronal access. Then F3 paper point was inserted into the root canal 2mm beyond the working length and thus it was aimed to ensure the contact of the medicament with the dentin wall. Then this space was filled with the medicament again. The root canal orifices were sealed with temporary filling material after applying the intracanal medicaments, which was day 0. All root canals were kept in a humid environment at 37°C. At the end of day 3, the temporary filling material from 50 specimens (10 specimen from each group) was removed. Excess medicament was removed with a #30 K file, and each root canal was irrigated with sterile saline

solution. Microbiological samples were collected using three sterile paper points which were held in the root canal for 1 min and stored in sterile Eppendorf tubes. The same procedure was applied for the other 50 specimens (10 specimen from each group) at the end of day 7.

DNA isolation

Dead cells were removed from the samples via a DNase-I treatment.[15] A 200 µL aliquot of 0.1 U/mL DNase-I was added to the samples and incubated for 15 min at 37°C. The samples were homogenized at 3,000 rpm for 1 min. A 400 µL aliquot of binding buffer (6 M guanidine thiocyanate, 20 mM Tris-HCl, pH 8) was combined with the samples and incubated at 98°C for 10 min. The extracted DNA was combined with 400 µL 2-propanol and captured on a silica column. The column was washed twice with a buffer containing 20 mM NaCl and 2 mM Tris-HCl, pH 7.5; 80% v/v ethanol. The DNA was eluted in 100 mM Tris-HCl pH 8.0 and stored at –20°C.

Quantitative real-time PCR

The Biospeedy™ Real-Time PCR EvaGreen Master Mix (Bioeksen Ltd., Co., Istanbul, Turkey) was used for all reactions. *E. faecalis* cells were quantified using the BactF 5'-AGA GTT TGA TCC TGG CTC AG-3' and BactR 5'-AAG GAG GTG ATC CAG CCG CA-3' primers that target bacterial DNA coding 16S Rrna.[16] *C. albicans* cells were quantified using the FungF 5'- TCC TCC GCT TAT TGA TAT GC-3' and FungR 5'-GGA AGT AAA AGT CGT AAC AAG G-3' primers that target the fungal internal transcribed spacer region.[17] The reaction mixtures contained 25 mg template DNA, 6 mg/mL bovine serum albumin, 5 mg/mL PEG 400, 0.25% Tween 20, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 U Proof Reading Hot-Start DNA Polymerase, 1× EvaGreen and 200 nM of each primer. The following thermocycling program was applied: 98°C, 3 min; 35 cycles of 10 s at 95°C, 5 s at 52°C and 20 s at 72°C. Melting curve analyses were performed from 65°C to 95°C to determine if only one amplified product was generated during Q-PCR. The Q-PCR runs were analyzed using CFX Manager Software 3.0. The $2^{-\Delta Ct}$ method was used for relative quantification of the targets in the different samples.[18]

Statistical analyses

Eradication of *E. faecalis* and *C. albicans* were reported as a number (percentage) and the differences were detected using the two-tailed *t*-test. MINITAB 17 software (Minitab Ltd., London, England) was used for all calculations. Degrees of freedom (df) and alpha level (p) were 18 and 0.05, respectively. All the tests and analyses were executed with p=0.05.

RESULTS

Relative eradication (%) of microorganisms compared to day 0 was represented in Table 1.

Table 1. Relative eradication (%) of microorganisms compared to day 0. Values for the groups marked with different superscript letters in the same column were significantly different ($P < 0.05$).

		E. faecalis		***C. albicans***	
		day 3	**day 7**	**day 3**	**day 7**
Group 1	Mean	97.618^{d}	98.389^{D}	99.849^{a}	99.986^{A}
	Std dev.	2.033	1.854	0.182	0.004
Group 2	Mean	96.947^{d}	96.918^{D}	99.915^{ab}	99.988^{AB}
	Std dev.	2.082	2.157	0.023	0.003
Group 3	Mean	91.673^{e}	96.798^{DF}	99.892^{a}	99.988^{A}
	Std dev.	6.121	2.111	0.056	0.004
Group 4	Mean	89.505^{e}	94.062^{EF}	99.898^{ac}	99.986^{AC}
	Std dev.	8.880	4.114	0.020	0.003
Group 5	Mean	89.943^{e}	92.509^{E}	99.900^{a}	99.986^{A}
	Std dev.	7.794	4.674	0.016	0.003

(Std dev: Standart deviation)

E. faecalis and *C. albicans* present in the root canals decreased in all study groups on days 3 and 7. All medicaments eliminated at least 99.849% of *C. albicans* and 89.505% of *E. faecalis* on day 3 compared to day 0. At least 99.986% of the *C. albicans* and 92.509% of the *E. faecalis* were eradicated on day 7 compared to day 0 (Table 1). $Ca(OH)_2$ and TAP groups showed similar the eradication rates for *E. faecalis* and *C. albicans* on day 3 and 7 (P>0.05). There was no significant difference between DP, MDP and Lf for the eradication of microorganisms at both experimental days (P> 0.05), except the amount of eradicated *E. faecalis* by DP at day 7 in which DP caught the similar percentages with TAP and $Ca(OH)_2$ (P<0.05).Beside, adding Lf to DP did not change its efficiancy against the tested pathogens (P>0.05).

Differences in eradication rates on days 3 to 7 was shown in Table 2. The antimicrobial effects of each medicament increased from days 3 to 7. However, no significant difference was observed between the antimicrobial effects of the medicaments (P>0.05) (Table 2).

Table 2. Differences in eradication rates on day 3 to day 7.

	E. faecalis	***C. albicans***
Group 1	0.38717406*	0.04071924**
Group 2	0.97614066*	0.00000335**
Group 3	0.02914106**	0.00042520**
Group 4	0.16525757*	0.00000017**
Group 5	0.39266484*	0.00000002**
P value	>0.05	>0.05

P > 0.05 indicates *, P < 0.05 indicates **

Q-PCR DNA amplification and melt peak of *E. faecalis* (Figure 2) and *C. albicans* (Figure 3) on experimental days were also shown. The same melting peaks revealed both the accuracy of the application of the method and no contamination.

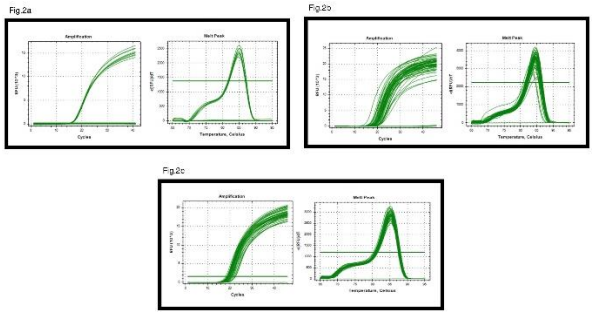

Figure 2: Q-PCR DNA Amplification and Melt peak of Enterococcus faecalis on day 0 (A), day 3 (B) and day 7 (C)

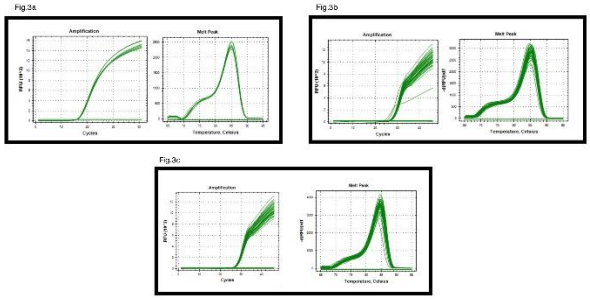

Figure 3: Q-PCR DNA Amplification and Melt peak of Candida albicans on day 0 (A), day 3 (B) and day 7 (C).

DISCUSSION

Adequate elimination of microorganisms from root canals is the golden key of a successful endodontic treatment. Although calcium hydroxide is routinely used in endodontics, it is insufficient for this purpose in some cases. Therefore, the creation of new drugs with better antimicrobial activity and more biocompatible than calcium hydroxide could open a new era in endodontic therapy. From this point of view, it was aimed to test new intracanal medicament combinations against endodontic pathogens in this study.

The agar diffusion test, direct contact test, culture and molecular methods have been widely used to determine the effectiveness of removing microorganisms from root canals.[4,19-22] Because of being an extensive investigation, we performed several pilot studies. We used agar diffusion test, culture methods, Reverse transcription polymerase chain reaction (RT-PCR) and Q-PCR method. When compared with DNA-based methods, RT-PCR is an excellent indicator of cell viability. By using this method, we also succeeded to obtain double-chained DNA. However, we did not prefer

to use this method because of being very complex, sensitive, time consuming and not reproducible.[23]

Under harsh conditions, such as insufficient nutrient supply and environmental stress including extremes in temperature, pH, UV irradiation, and fluctuating levels of toxic chemicals and oxygen concentrations, microorganisms enter a viable but non-cultivable (VBNC) state.[21] When optimum conditions are realized, these microorganisms recover from this phase.[22] Molecular methods are useful for determining which microorganisms enter the VBNC phase. Q-PCR is the sensitive and reliable method. However, Song reported several major practical problems that yield false results, including inadequate removal of PCR inhibitors from the sample, ineffective release of microbial DNA from the cells, poor DNA recovery after the purification step, presence of compounds derived from extracted substrates that inhibit the reaction, and contamination of the assay by background DNA leading to false results.[19]

In light of these studies and our pilot studies, samples were taken from the canals using three paper points to increase sample volume. The samples were placed in empty sterile Eppendorf tubes to avoid any excess substrates that could inhibit the PCR reaction, and DNA was isolated immediately. DNA-based methods detect total amounts of DNA but cannot differentiate living or dead cells.[13] Thus, DNA of viable cells was distinguished from dead microorganisms using DNase I followed by the real-time Q-PCR. The same melting peaks revealed no contamination except for the sample with a melting peak of 81.5°C, number 9 in group 5 on day 7, which was removed from the study.

According to previous studies, $Ca(OH)_2$ and TAP are the most popular medicaments used in microbiota studies.[3,4,23] However, the evidence for these medicaments is paradoxical. Chua *et al*.[24] evaluated the antimicrobial effects of several medicaments on *C. albicans*, and no difference was detected between TAP and the $Ca(OH)_2$ groups, which were prepared in saline solution. The antimicrobial effects of $Ca(OH)_2$ and TAP were evaluated at the end of a 7-day period in another study in which root blocks were contaminated with *E. faecalis*. According to that study, TAP was more effective than $Ca(OH)_2$ for eliminating the microorganisms from the root canal wall.[3] These results are in accordance with Mozayeni *et al*.[4] In our study, no significant difference between the groups was found. The vehicle could have been a cause for this difference. Polyethylene glycol, which has an antimicrobial effect[27], was used as the liquid vehicle in those investigations. Saline solution was chosen as the vehicle in the present study to evaluate the antimicrobial effects of newly designed medicaments. Saline solution has no antimicrobial effect, so using this liquid may have decreased the efficacy of the paste. In addition, the extracted teeth were mechanically shaped instead of using dentine blocks to mimic routine root canal therapy. Furthermore, a Glidden drill was used to take samples from the root canals. In light of pilot studies, paper points, which were used by Siqueira and Roças, were used to take samples from the canal.[28]

E. *faecalis* has high capacity to form biofilm.[29] Several studies reported that 100% E. *faecalis* strains had the ability to form biofilm.[30,31] However, it is an open-secret that creating biofilm is not an easy procedure in *in-vitro* conditions. Several factors such as surface energy of the substrate, temperature, pH, flow rate of the fluid passing over the surface, virulance factors, contact time, surface hydrophobicity, and nutrient availability affect the biofilm formation.[32] Although genetic structure of the biofilm formation is unclear, virulance factor such as enterococcal surface protesin (esp), surface aggregating protein (asa), cytolysin A (cylA), aggregation subtance (agg) were also shown to be associated with weak, medium, or strong biofilm formation of E. *faecalis*.[29,33] Beside this, the adhering capacity of E. *faecalis* in VBNC state reduces. For this reason, they are unable to form measurable biofilms, but still have the ability to form polymeric matrix and maintain its pathogenicity.[34]

In our pilot studies, we obtained variable and contradictory data using culture methods because of the VBNC phase of the *E. faecalis*. Therefore, our aim in this study was to examine the eradication of these microorganisms which are

capable of living in any environment changes such as VBNC state in which microorganism still have the ability to synthesize polymetric matrix. For this purpose, the root canals and the lumen were infected with experimental microorganisms for 2 days rather than creating strong and mature biofilms.

Shen *et al.* evaluated the susceptibility of biofilm formation at different growth phases for time periods ranging from 2 days to 12 weeks. They showed that although biofilm formation could occur even at 2 days with the average thickness of 57 μm, mature biofilms at 3 weeks were more resistant to chlorhexidine solution.[35] Kristich *et al.*[33] studied the esp-independent biofilm formation by several E. *faecalis* strains and reported a dense biofilm within 24 h of growth. Zheng *et al.*[29] also used 1 day biofilm formation in their study in which they analyzed the association between virulance factor and biofilm formation of E. *faecalis* strains by using RT-QPCR. In the present study, each tooth was infected with microorganisms every 24 h for 2 days using freshly prepared 24 h cultures. According to our results, all medicaments eradicated at least 90% microorganisms from the root canal at both day 3 and day 7. The possible reason for this good result could be the creation of young and fresh biofilm on the root surface.

New-generation root canal medicaments should be developed because of the poor antimicrobial effects of routinely used medicaments. Several antibiotics and their combinations have been used against resistant microorganisms, such as *E. faecalis* in *in vivo* and *in vitro* studies. Pinheiro *et al*.[36] evaluated the root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. All species isolated from these failed canals were susceptible to penicillin-type antibiotics. The results of that study were compatible with Aksoy's study.[19] Aslangül *et al.*[37] proposed the use of a combination of penicillin and glycopeptide against *E. faecalis*. Wang *et al*.[38] also evaluated drug resistance in outpatients over 8 years and showed that the combination of ampicillin and gentamicin was very effective against enterococcal infections. The synergistic effects of a penicillin type of ampicillin and a kind of glycopeptide, gentamicin, was an option in microbiological studies in the dental sciences and clinical practice. In our *in-vitro* study, groups 3 and 4 showed considerable antimicrobial activities, which tended to increase from days 3 to 7. Further investigations should be conducted to consider the timing interval.

Dental studies based on Lf are generally conducted on treating oral dryness[39], anti-biofilm efficacy[40], toothpaste content[28], effects on cariogenic microorganisms[41], and irrigation solutions.[42] Gudipaneni *et al.*[28] showed that the tooth pastes containing Lf, lysozyme and lactoperoxidase reduced the salivary levels of *Streptococcus mutans* and L*actobacillus acidophilus* in children with severe early childhood caries. Mizuhashi *et al.*[39] reported that the oral dryness was related with the level and flow rate of Lf in the saliva. Alves *et al.* used lactoferrin as a root canal irrigant in different concentrations and compared its effect with xylitol, farnesol and salicylic acid on the biomass of bacterial biofilms. They reported that the combination of farnesol, xylitol and lactoferrin was the most effective against *E. faecalis* MB35 and this combination also reduced the biomass of *Staphylococcus epidermis* biofilms.[42] Surprisingly, no study has used Lf or the aminoglycoside-penicillin combination as intracanal medicaments despite their antimicrobial activities. In the present study, Lf showed antimicrobial affect for both experimental microorganisms when used in alone or combination with antibiotics. The antimicrobial effect of Lf against both *E. faecalis* and *C. albicans* had tendency to increase from day 3 to day 7 and almost caught the performance of other experimental groups. When taking into consideration the results of this study, Lf could be used in several forms as intracanal medicament by means of its biocompatibility. However, new product should be tested not only about their antimicrobial activity but also their effect on dentinal tissue[43] and its microhardness for various time intervals.[44] Further studies conducted with different forms of Lf and focused on dentinal

effects of it could make a new epoch in endodontics.

CONCLUSIONS

Under the limitation of this study, all intracanal medicaments resulted in significant reductions in *E. faecalis* and *C. albicans* compared to day 0. Although no significant differences were observed between medicaments in both microorganism groups, DP and MDP displayed increases from days 3 to 7. As a result of all these results, the use of new experimental combinations in the endodontics clinic is promising. However, it is not clear how many days the use of these new generation intracanal medicaments will give better results in the clinic. For this reason, further investigations using different time intervals and concentrations are needed.

FUNDING

This study was supported by the Marmara University Scientific Research Projects Committee (Research No: SAG-C-DRP 150513–0154).

ETHICAL APPROVAL

The Ethics Committee of the Marmara University Institute of Health Science approved the protocol. (Number of approval: 05-04-2013-9)

CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.

Farklı Kök Kanal Medikamentlerinin Enterococcus Faecalis ve Candida Albicans Üzerine Antimikrobiyal Etkilerinin in Vitro Olarak Karşılaştırılması

ÖZ

Amaç: *Bu çalışmanın amacı deneysel kök kanal medikamentlerinin 3 ve 7 günlük süreçlerde Candida albicans ve Enterococcus faecalis üzerindeki antimikrobiyal etkisini gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (PZR) ile araştırmaktır.* ***Gereç ve yöntem:*** *Çalışmamızda 240 adet tek kök tek kanallı insan dişi kullanıldı. Kök kanalları şekillendirildikten ve akrilik bloklara gömüldükten sonra otoklavda steril edildi. Örnekler iki gruba ayrılarak birinci grubun kök kanallarına E. faecalis diğerine ise C. albicans süspansiyonu inoküle edilmiş ve alt gruplara ayrıldı; kalsiyum hidroksit ($Ca(OH)_2$), üçlü antibiyotik patı (TAP), ikili antibiyotik patı (DP), modifiye üçlü pat (MDP), laktoferrin (Lf), negatif ve pozitif kontrol grupları. Üçüncü ve yedinci gün sonunda kök kanallarından paper point ile alınan örnekler boş eppendorf tüplere alınıp bir seri işlemden geçirildikten sonra DNA izolasyonu gerçekleştirildi. Daha sonra da Gerçek Zamanlı PZR işlemi uygulandı. Veriler istatistiksel olarak analiz edilmişti.* ***Bulgular:*** *Tüm medikamentlerin antimikrobiyal etkinliği 3. günden 7. güne yükseldi. $Ca(OH)_2$ ve TAP grupları, 3. ve 7. günlerde E. faecalis ve C. albicans için benzer eradikasyon oranlarını gösterdi ($P>0,05$). DP'nin yedince günde E. faecalis'i eredike etme oranının TAP ve $Ca(OH)_2$ ile benzerlik göstermesinin dışında, DP, MDP ve Lf arasında her iki deney gününde de mikroorganizmaların yok edilmesi için anlamlı bir farklılık bulunmadı ($P<0,05$).* ***Sonuçlar:*** *Bu çalışmanın sonuçlarına göre deneysel olarak kullanılan kök kanal medikamentleri endodonti kliniği rutininde olarak kullanılanlan CH'ye benzer antimikrobiyal etkinlik göstermiştir. Biyouyumlu bir malzeme olan laktoferrinin kanal içi bir ilaç olarak farklı kombinasyonlarda kullanılabileceği umut vericidir.* ***Anahtar kelimeler:*** *Antibiyotik patı, C. albicans, E. faecalis, Kök kanal medikamenti, Laktoferrin.*

REFERENCES

1. Gomes BPFA, Souza SFC, Ferraz CCR, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J. 2003;36: 267–275.

2. Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis: The root canal survivor and 'star' in post treatment disease. Endod Topics. 2003;6:135–159.

3. Adl A, Hamedi S, Sedigh Shams M, Motamedifar M, Sobhnamayan F. The ability of triple antibiotic paste and calcium hydroxide in disinfection of dentinal tubules. Iran Endod J. 2014;9(2):123–126.

4. Mozayeni MA, Haeri A, Dianat O, Jafari AR. Antimicrobial effects of four intracanal medicaments on Enterococcus faecalis: An in vitro study. Iran Endod J. 2014;9(3):195-198.

5. Hoshino E, Takushige T. LSTR 3Mix-MP method: Better and efficient clinical procedure of lesion sterilization and tissue repair (LSTR) therapy. Dental Review. 1998;666:57–106.

6. Arslan H, Çapar ID, Saygılı G, Uysal B, Gok T, Ertaş H. Efficacy of various irrigation protocols on the

removal of triple antibiotic paste. Int Endod J. 2014;47(6) :594–599.

7. Akçay M, Arslan H, Yasa B, Kavrık F, Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. J Endod. 2014;40(6):845–848.

8. Horodniceanu T, Bougueleret L, El-Solh N, Bieth G, Delbos F. High-level, plasmid-borne resistance to gentamicin in Streptococcus faecalis subsp. zymogenes. Antimicrobial Agents Ch. 1979;16(5):686–689.

9. Habib G, Hoen B, Tornos P, et al. ESC Committee for Practice Guidelines. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): The Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J. 2009;30(19):2369–2413.

10. Levay PF, Viljoen M. Lactoferrin: A general review. Haematologica. 1995;80:252–67

11. Orsi N. The antimicrobial activity of lactoferrin: Current status and perspectives. Biometals. 2004;7(3):189–196.

12. Velliyagounder K, Kaplan JB, Furgang D, et al. One of two human lactoferrin variants exhibit increased antibacterial and transcriptional activation activities and is associated with localized juvenile periodontitis. Infect Immun. 2003;71(11):6141–6147.

13. Krupińska AM, Bogucki Z. Clinical aspects of the use of lactoferrin in dentistry. J Oral *Biosci.* 2021;Epub ahead of print.

14. Rosa L, Lepanto MS, Cutone A, Ianiro G, Pernarella S, Sangermano R, et al. Lactoferrin and oral pathologies: a therapeutic treatment. Biochem Cell Biol. 2021; 99(1): 81-90

15. Villarreal JV, Jungfer C, Obst U, Schwartz T. DNase I and Proteinase K eliminate DNA from injured or dead bacteria but not from living bacteria in microbial reference systems and natural drinking water biofilms for subsequent molecular biology analyses. J Microbiol Methods. 2013;94(3):161–169.

16. Lane DJ.16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M. (Eds.), Nucleic acid techniques in Bacterial systematics. Wiley, Chichester, England. 1991.

17. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci. 2012;109(16):6241-6246.

18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and and the $2^{-\Delta\Delta C_T}$ Method. Methods. 2001;25(4):402–408.

19. Aksoy SÇ. Isolation of Enterococcus faecalis from permanent tooth root canals and identification by cultural and molecular methods and detection of antibiotic susceptibility (Master thesis). Ege University, Science Institute, Biology Department. 2008

20. Available from: http://acikerisim.ege.edu.tr:8081/xmlui/handle/11454/3402?show=full

21. Siqueira JF Jr, Rôças IN. PCR methodology as a valuable tool for identification of endodontic pathogens. J Dent. 2003;31:333–339.

22. Guerreiro JCM, Ochoa-Rodrígez VM, Rodrigues EM, et al. Antibacterial activity, cytocompatibility and effect of Bio-C Temp bioceramic intracanal medicament on osteoblast biology. Int Endod J. 2021; Epub ahead of print.

23. Song Y. PCR-based diagnostics for anaerobic infections. Anaerobe. 2005;11:79–91.

24. Sheridan GEC, Szabo EA, Mackey BM. Effect of post-treatment holding conditions on detection of tufA mRNA in ethanol-treated Escherichia coli: implications for RT-PCR-based indirect viability tests. Lett Appl Microbiol. 1999;29**:**375–379.

25. Trevors JT. Viable but non-culturable (VBNC) bacteria: Gene expression in planktonic and biofilm cells. J Microbiol Methods. 2011;86:266–273.

26. Lleo M, Bonato B, Tafi MC. Adhesion to medical device materials and biofilm formation capability of some species of enterococci in different physiological states. FEMS Microbiol Lett. 2007;274(2):232-237.

27. Chua EG, Parolia A, Ahlawat P, Pau A, Amalraj FD. Antifungal effectiveness of various intracanal medicaments against Candida albicans: An ex-vivo study. BMC Oral Health. 2014;14:53.

28. Carreira C de M, dos Santos SS, Jorge AO, Lage-Marques JL. Antimicrobial effect of intracanal substances. J Appl Oral Sci. 2007;15(5):453–458.

29. Gudipaneni, RK, Kumar RV, Jesudass G, Peddengatagari S, Duddu Y. Short-term comparative evaluation of antimicrobial efficacy of toothpaste

containing lactoferrin, lysozyme, lactoperoxidase in children with severe early childhood caries: A clinical study. J Clin Diagnostic Res. 2014;8(4):18–20.

30. Zheng JX, Wu Y, Lin ZW, Pu ZY, Yao WM, Chen Z, et al. Characteristics of and Virulence Factors Associated with Biofilm Formation in Clinical Enterococcus faecalis Isolates in China. Front Microbiol. 2017;8:2338.

31. Seno Y, Kariyama R, Mitsuhata R, Monden K, Kumon H. Clinical implications of biofilm formation by Enterococcus faecalis in the urinary tract. Act Med Okayama. 2005;5:79–87.

32. Sandoe JA, Witherden IR, Cove JH, Heritage J, Wilcox MH. Correlation between enterococcal biofilm formation in vitro and medical-device-related infection potential in vivo. J Med Microbiol. 2003;52:547–550.

33. Mohammadi Z, Palazzi F, Giardino L. Microbial Biofilms in Endodontic Infections: An Update Review. Biomed J. 2013;36:59-70.

34. Kristich CJ, Li YH, Cvitkovitch DG, Dunny GM. Esp-Independent Biofilm Formation by Enterococcus faecalis. J Bacteriol. 2004;186(1):154-163.

35. Lleo MM, Tafi MC, Canepari P. Nonculturable Enterococcus faecalis cells are metabolically active and capable of resuming active growth. Syst Applied Microbiol. 1998;21(3):333–339.

36. Shen Y, Stojicic S, Haapasalo M. Antimicrobial Efficacy of Chlorhexidine against Bacteria in Biofilms at Different Stages of Development. J Endod. 2011;37(5):657-661.

37. Pinheiro ET, Gomes PBFA, Ferraz CCR, Teixeira FB, Zai AA, Souza-Filho FJ. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. Oral Microbiol Immun. 2003;18:100–103.

38. Aslangul E, Ruimy R, Chau F, Garry L, Andremont A, Fantin B. Relationship between the level of acquired resistance to gentamicin and synergism with amoxicillin in Enterococcus faecalis. Antimicrob Agents Ch. 2005;49(10):4144-4148.

39. Wang JT, Chang SC, Wang HY, Chen PC, Shiau YR, Lauderdale TL. TSAR Hospitals. High rates of multidrug resistance in Enterococcus faecalis and E. faecium isolated from inpatients and outpatients in Taiwan. Diagn Micr Infect Dis. 2013;75(4):406–411.

40. Mizuhashi F, Koide K, Toya S, Takahashi M, Mizuhashi R, Shimomura H. Levels of the antimicrobial proteins lactoferrin and chromogranin in the saliva of individuals with oral dryness. J Prosthet Dent. 2015;113(1):35–38.

41. Ammons MCB, Ward LS, James GA. Anti-biofilm efficacy of a lactoferrin/xylitol wound hydrogel used in combination with silver wound dressings. Int Wound J. 2011; 8:268–273.

42. de Andrade FB, de Oliveira JC, Yoshie MT, Guimaraes BM, Gonçalves RB, Schwarcz WD. Antimicrobial activity and synergism of lactoferrin and lysozyme against cariogenic microorganisms. Braz Dent J. 2014,25(2):165–169.

43. Alves FR, Silva MG, Rôças IN, Siqueira Jr. JF. Biofilm biomass disruption by natural substances with potential for endodontic use. Braz Oral Res. 2013;27(1):20-25.

44. Cochrane S, Parashos P, Burrow MF. Effect on the mechanical properties of human and bovine dentine of intracanal medicaments and irrigants. Aust Dent J. 2019; 64:35–42.

45. Yilmaz S, Dumani A, Yoldas O. The effect of antibiotic pastes on microhardness of dentin. Dent Traumatol. 2016;*32*(1):27-31.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/PeoLrY