EGYPTIAN DENTAL JOURNAL

VOL. 68, 1867:1874, APRIL, 2022

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

Submit Date : 2022-01-01 • Accept Date : 2022-03-02 • Available online: 2022-03-30 • DOI : 10.21608/edj.2022.113988.1934

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF IBUPROFEN, TRIPLE ANTIBIOTIC PASTE AND CALCIUM HYDROXIDE AS INTRACANAL MEDICAMENTS AGAINST ENTEROCOCCUS FAECALIS IN SINGLE ROOTED TEETH. A COMPARATIVE INVITRO STUDY

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ABSTRACT

Aim: To evaluate the antibacterial activity of Ibuprofen, Triple antibiotic paste (TAP) and Calcium hydroxide ($CaOH_2$) used as intracanal medicaments against *Enterococcus faecalis* (*E. faecalis*) in single rooted teeth.

Materials and methods: Seventy-five extracted single rooted teeth were decoronated, mechanically prepared up to F4 using Protaper universal rotary files, autoclaved, contaminated with *E. faecalis* and incubated at 37°C for 14 days. The teeth were then divided into 3 groups (25 each) according to the intracanal dressing used; Group 1: TAP, Group 2: Ibuprofen and a control group: $Ca(OH)_2$. TAP and Ibuprofen powders were mixed with distilled water (1:1w/v), placed inside the canals while $Ca(OH)_2$ was injected directly into the canals. The canals were then sealed and incubated at 37°C for 7 days. After 7 days the intracanal dressings were removed by irrigation. Bacterial samples were obtained from the canals using paper points before the application of the medicaments and the number of colony forming units (CFU) was calculated and recorded as (CFU1) then the same procedures were done after the intracanal medicaments application and removal to record (CFU2). The antibacterial action and the percentage of bacterial reduction for the tested medicaments were assessed.

Results: Ibuprofen, TAP and Calcium hydroxide showed a significant antibacterial activity against *E. faecalis* ($P<0.001$). However, there was no statistically significant difference in the percentage of bacterial reduction between the Ibuprofen, the TAP and the calcium hydroxide groups against ($P=0.270$).

Conclusion: Ibuprofen possess an antibacterial activity against *Enterococcus faecalis* which is comparable to that of TAP and calcium hydroxide.

KEYWORDS: Antibacterial, *Enterococcus faecalis,* Ibuprofen, Triple antibiotic paste, Calcium hydroxide.

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INTRODUCTION

Virulent microorganisms were shown to be the major cause of pulpal and periapical diseases, therefore, the primary goal of endodontic treatment is disinfection augmented by irrigation and intracanal medication[1].

The most commonly used intracanal medication is the calcium hydroxide owing to its power to dissolve necrotic tissue, alkaline pH, antibacterial effect and good biocompatibility[2]. It releases hydroxyl ions which is responsible for its antimicrobial action where these hydroxyl ions causes damage to the cytoplasm of the bacteria leading to the denaturation of the bacterial protein and the damage of the bacterial DNA[3,4]. Furthermore, it was shown that eradication of microorganisms in the root canal can be achieved more reliably with calcium hydroxide than with other medicaments. However, in some infections calcium hydroxide has limited effectiveness in eliminating some pathogens such as enterococci and yeasts from root canals as both could tolerate an alkaline environment in the canals[3,5-8].

Triple antibiotic paste (TAP); a mixture of metronidazole, ciprofloxacin, and minocycline, has been reported to be a successful intracanal dressing for controlling root canal pathogens[9]. However, concerns were raised regarding the possibility of antibiotic resistance and the risk of allergic reactions, in addition, minocycline was shown to cause teeth discoloration[10-13].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of commonly used drugs for the management of pain and inflammation. It was demonstrated that NSAIDs may have many different properties other than being analgesics such as antimicrobial activity through the inhibition of the bacterial DNA synthesis and the impairment of its membrane activity. They can also alter the bacterial cell envelope as well as their genes encoding, down regulate the efflux pumps and prevent the bacteria from colonizing the root canal and form resistant biofilms by hindering its quorum sensing along with its anti-plasmid activity[1,14-18]. Therefore, the possible use of non-antibiotics such as ibuprofen as intracanal medication could be a safe alternative to TAP.

To our knowledge, the comparative antibacterial effect of calcium hydroxide, TAP and ibuprofen against *Enterococcus faecalis* has not been studied so far. Therefore, the present study was carried out to evaluate the antibacterial effect of calcium hydroxide, TAP and ibuprofen against *Enterococcus faecalis* as a resistant endodontic bacterium.

MATERIALS AND METHODS

Seventy-five human extracted single rooted single canaled mature teeth were collected from the dental clinic of the national diabetes and endocrinology institute, Cairo - Egypt, and from the clinic of oral surgery department at the faculty of dentistry, Cairo University. The teeth were examined under microscope to exclude and cracks, fractures or decays and were radiographed to exclude any resorptive lesions, calcifications and to verify the presence of a single canal. The teeth were cleaned thoroughly from any attached tissue debris and blood and were disinfected by immersing for 5 minutes in a 5% Sodium hypochlorite solution (Clorox, Household Cleaning Products, Egypt) and then they were scraped to remove any residual periodontal ligament fibers and any calculus deposits from the root surface. The teeth were then randomly assigned into 2 intervention groups and 1 control group as follows: Intervention group 1: Ibuprofen used as an intracanal medication (n=25), Intervention group 2: Triple antibiotic paste used as an intracanal medication (n=25) and the control group: Calcium hydroxide used as an intracanal medication (n=25).

After that, teeth were decoronated to a standardized length of 14 mm. Working length was

determined by inserting a #10 K file (MANI, Japan) into the root canal until it could be seen at the apical foramen then taking the length measurement and subtracting 1 mm from it. Mechanical preparation was done using Protaper universal rotary files up to file size F4 (Dentsply Maillefer, Switzerland). The canals were irrigated with 2.5% sodium hypochlorite (NaOCl) between each two successive files then at the end of the preparation, they were irrigated with 2 mL of 17% EDTA (METABIOMED CO.LTD,Korea) in order to remove the smear layer followed by a final flush of saline then the canals were dried using Protaper paper points corresponding to a size 40. The apical foramina were closed using self-cure acrylic resin (Acrostone Manufacturing and Import Co., Egypt) and the external root surfaces were painted with a layer of nail varnish (YOLO, YOLO cosmetics, France) in order to create an impermeable surface. Finally, the teeth were sterilized by autoclaving at 121°C for 20 minutes and after that, they were placed in a 1.5 mL Eppendorf tubes kept upright in an Eppendorf holder rack.

Contamination protocol

The bacterial strain *E. faecalis* (ATCC 29212) was revived by plating it on blood agar. five µL of *E. faecalis* suspension; which was adjusted to 0.5 McFarland standard, were inoculated into the teeth using a sterile micropipette. The previous procedure was repeated every 72 hours for 14 days during which the teeth were kept in an incubator (FISHER ISOTEP* INCUBATOR. WTC Binder, TUTTLINGEN/ GERMANY) at 37°C.

The first sampling (premedication sample)

The first sample was obtained from the canals before intracanal medication application using paper point sampling technique then the paper points were placed in Wasserman tubes containing 1 ml of phosphate buffered saline (PBS) in order to remove the loosely attached bacteria. After that the paper points were transferred to another Wasserman tube containing 1 ml of Brain heart infusion broth (BHI) and the tubes were vortexed to resuspend the remaining viable bacteria on the paper points. Serial 10-fold dilutions of the bacterial suspensions were made (10^1 to 10^5). A volume of 20 microliter was taken from each dilution and plated on bile esculin plates which were incubated at 37°C aerobically for 24 hours. After 24 hours, the approximate number of colony forming units (CFU) per ml was calculated and recorded as Colony forming unit (CFU1).

Medications preparation and insertion

Intervention group 1 (Ibuprofen)

The powder of Ibuprofen was extracted from its tablets (≥98%)(Brufen, Kahira pharmaceuticals and industries company under the license from Abbott Laboratories Limited, USA), where ibuprofen 600 mg tablets were ground into powder and extracted with acetone then filtered with Whatman cellulose filter paper to obtain ibuprofen pure solution in acetone. The purity of the solution was confirmed using Thin Layer Chromatography (TLC) then it was completely evaporated under reduced pressure using Rotavapor to give an off-white crystalline powder of ibuprofen. At the time of application, the powder was mixed with distilled water to obtain a homogenous paste. It was placed inside the canasl using an endodontic plugger.

Intervention group 2 (Triple antibiotic paste)

Equal weight proportions of Ciprofloxacin (Ciprobay, Hikma Pharma, Egypt), Metronidazole (Flagyl, Sanofi-Aventis, Egypt), and Minocycline (Minocin, TEOFARMA, Italy) were crushed to form a powder which was mixed with equal proportions of 1:1:1 ratio with a concentration of 1 mg/ml. At the time of application, the powder was mixed with distilled water in an equal ratio of (1:1 w/v) to obtain a homogenous paste. It was placed inside the canals using an endodontic plugger.

Control Group (Calcium hydroxide)

A ready made intracanal calcium hydroxide medication was used (Hydrocal, Cerkamed, Poland). It was injected into the canal starting apically while the syringe was being withdrawn gradually coronally.

All the teeth were sealed and incubated at 37°C for 7 days.

The second sampling (Post medication)

After 7 days, the teeth were irrigated with 5 mL saline each to remove the intracanal medication from the canal. A Second sample was taken by paper point sampling using the same technique of the first one (CFU2).

The antibacterial effect of each medication between CFU2 and CFU1 and the percentage of bacterial reduction were calculated and the results were compared with each other.

RESULTS

Statistical analysis was done with IBM® SPSS® (ver. 26. SPSS Inc., IBM Corporation, Armonk, NY, USA). Data were presented as mean, standard deviation (SD. Data were explored for normality using Shapiro-Wilk test. Data showed a non-normal distribution. Wilcoxon signed rank test was used to compare between before (CFU1) and after (CFU2) application of the medications. Kruskal Wallis test used to for comparison between the three tested materials. The significance level was set at ($p < 0.05$).

All the tested medications showed a significant reduction in the CFU numbers from the pre medication sample CFU1 to the post medication sample CFU2. However, there was no statistically significant difference in the antibacterial activity between the three tested medications group (Table 1, Fig 1).

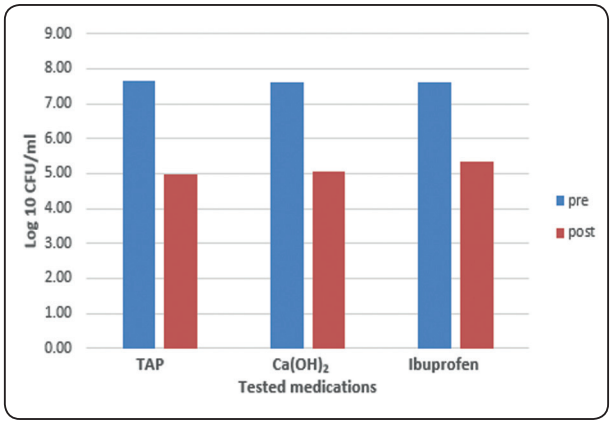

Fig. (1): A bar chart representing the antibacterial activity of the three tested medications against *E. faecalis*.

TABLE (1): Mean, standard deviation SD of log10 of *E. faecalis* colony forming unit counts and the percentage of bacterial reduction among the three tested medication groups.

Medication used	Pre medication		Post Medication		p-value	Percentage of bacterial reduction
	Mean	SD	Mean	SD		
TAP	7.65	0.07	4.97	2.55	<0.001*	35.01%
Ca $(OH)_2$	7.63	0.08	5.04	2.58	<0.001*	33.94%
Ibuprofen	7.62	0.09	5.33	2.39	<0.001*	30.08%
p-value	0.387 NS		0.491 NS			0.270 NS

**=significant, NS=Non-significant*

DISCUSSION

The use of an effective intracanal medication possessing antimicrobial properties in between appointments may reduce or eliminate the remaining bacteria in the root canal, hence significantly increases the success rate of root canal therapy[19].

The present study evaluated the antibacterial activity of Ibuprofen, Triple Antibiotic Paste and Calcium Hydroxide as intracanal medicaments against *Enterococcus faecalis* in Single Rooted Teeth.

In the present study, the choice of the tested medicaments was based on some factors; Calcium hydroxide is the most widely used intracanal medication and researchers have reported that it may be the best interappointment medicament available to reduce intracanal residual microbial flora[20,21]. Ibuprofen was chosen as it is one of the most commonly used NSAIDs for the management of endodontic pain where NSAIDS were shown to possess an antibacterial property in addition to its analgesic and anti-inflammatory effects[1,15-16,22]. TAP which is a combination of 3 antibiotics (minocycline, metronidazole, and ciprofloxacin) was chosen in this study because it was proven to highly efficient in eradicating endodontic pathogens in vivo, in vitro and in situ [10,18]. A previous research by Milani *et al*. 2013[18] was the first to assess the antibacterial efficacy of ibuprofen, diclofenac and Calcium hydroxide and they concluded that NSAIDS would be beneficial as intracanal medicaments.

In the present study, an endodontic model was used, where single rooted teeth were decoronated and mechanically prepared then were contaminated for two weeks with *E. faecalis*. This model is considered a presentation of the clinical situations and being single canaled, excluded the presence of anatomical variations and complexities. In addition, easy placement and removal of the intracanal medicaments could be achieved[1,23-24].

E. faecalis was selected as a test species for this study because it was shown to be associated with resistant endodontic infections and a major cause for endodontic flare ups and failures. *E. faecalis* has the ability to persist inside the dentinal tubules for at least 10 days without nutrient supply and it plays a critical part in persistent apical periodontitis cases[25-26]. Using planktonic bacteria as a study species may not accurately reflect the actual environment inside the root canals where normally biofilms form[25]. To form a mature bacterial biofilm, infected teeth were incubated aerobically for 14 days at 37°C[1,27].

The mechanical preparation of the teeth was performed using ProTaper universal rotary files up to size 40 to provide a standardized root canal preparation with the same taper and size and to ensure complete debridement and better penetration of the medicament to the apical part of the canal[28].

Irrigation was done using 2.5% NaOCl between each two successive files owing to its antimicrobial activity and tissue dissolving effect. Final irrigation was performed with 2 ml of 17% EDTA solution followed by 2 ml of 2.5% NaOCl with a saline wash in between. This was shown to be an effective method to remove the smear layer and superficial debris from the surface of instrumented root canals leaving patent dentinal tubules which decreases the time necessary to achieve the disinfecting effect of intracanal medications[29-31]. The medicaments were removed using a saline wash before bacterial sampling as it has no antimicrobial effect thus it won't affect the results of the study[32].

Sterilization of the teeth using autoclave was done after mechanical preparation to ensure that the canals were microorganisms free before incubation of the experimental microorganisms[33].

Bile esculin was selected as a culture media because it is a very characteristic media for culturing of *Enterococcus faecalis*[25]. The CFU counting method was adopted in this research because it

allows rapid and efficient calculation of the quantity of microorganisms within the root canals[34].

In the current study, the medications were applied for 1 week since previous studies reported that a negative culture could be achieved in the root canals after 7 days of intracanal medication application[1,35-36]. Microbiological analysis was done before and after medication application in order to rule out possible false positive results[37].

In the present study, analysis of the microbiological samples revealed a large statistically significant decrease of the CFU counts from the premedication sample to the post medication one in the three test groups ($P<0.01$). These results showed that the three intracanal medications has a potent antibacterial action against *E. faecalis*. However, there was no statistically significant difference between the antibacterial activity of the three medications when compared to each other ($P = 0.270$), where the highest percentage of bacterial reduction was found in the TAP group (35.01%) followed by the calcium hydroxide group (33.94%) and the least was found in the Ibuprofen group (30.08%).

The results were in accordance with Hersh *et al*. 1991 who demonstrated the antibacterial activity of ibuprofen against six common periodontal pathogens[38]where the actual mechanism of this antibacterial activity is unclear, however, studies have proposed that NSAIDS can cause the inhibition of bacterial DNA synthesis and the impairment of its membrane activity[14,39]. The results were also in agreement with Chockattu *et al* 2018[1] who found that anti-inflammatory nonantibiotics (ibuprofen and diclofenac) were proven to have an antibacterial activity against *E. faecalis* that was comparable to calcium hydroxide and they concluded that it could be possible to replace $Ca(OH)_2$ with NSAIDs, or even combine them in a synergistic relation to form a potent mixture of local disinfectants to optimize root canal disinfection. In addition, the results were in agreement with Tirukkolluru and Thakur 2019[40] who showed that the antimicrobial efficacy of TAP and calcium hydroxide were comparable when tested against *Streptococcus* spp. and *E. faecalis* in chronic apical periodontitis patients with Type II diabetes mellitus.

The results of the present study were in partial agreement with Milani *et al* 2013[18] who studied the antibacterial effect of ibuprofen, diclofenac and calcium hydroxide against *E. faecalis* and concluded that ibuprofen and diclofenac had greater antibacterial activity than calcium hydroxide.

On the other hand, the results were in disagreement with Sayoo *et al* 2021[4] who compared the antibacterial activity of TAP, calcium hydroxide and *Acacia nilotica* against *E.faecalis, Streptococcus mutans* and *Candida albicans* and concluded that TAP showed the best antimicrobial efficacy followed by *Acacia nilotica* and least was that of the Calcium hydroxide. This disagreement could be due to the difference in methodology as they assessed the antibacterial activity using the agar well diffusion method.

CONCLUSION

Within the limitations of this in vitro study, it is concluded that Ibuprofen possess an antibacterial activity against *Enterococcus faecalis* which is comparable to that of TAP and calcium hydroxide.

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