ORIGINAL RESEARCH PAPER

Volume - 10 | Issue - 05 | May - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTI-BACTERIAL EVALUATION OF IBUPROFEN, DICLOFENAC, TRIPLE ANTIBIOTIC PASTE AND CALCIUM HYDROXIDE AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS IN AN ENDODONTIC MODEL: AN IN VITRO STUDY

Dental Science

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ABSTRACT

Aim: This study aimed at using Ibuprofen, Diclofenac, Triple Antibiotic Paste and Calcium Hydroxide as Intracanal Medicament against Enterococcus faecalis in an Endodontic model. **Materials and Methods :** A total of 50 single-rooted mandibular premolar teeth were decoronated and instrumented up to F4-ProTaper rotary. Apical foramen was sealed with composite resin, and all external surfaces made impermeable with nail varnish, except for coronal access. Roots were autoclaved (121°C for 20 min), placed in Eppendorf tubes, and contaminated with E. faecalis for 14 days. Colony-forming unit (CFU) counts were taken before (CFU-1), and after intracanal medication (CFU-2) by paper point sampling. Test medicaments (Group-1: Diclofenac , Group-2: Ibuprofen, Group-3: Triple antibiotic paste and Group-4: Ca[OH]2) were mixed with distilled water (1:1 w/v), placed into root canals, temporarily sealed, and incubated (37°C; 7 days). Kruskal–Wallis ANOVA was used to compare the four groups and Mann–Whitney U-test for pair-wise comparisons. **Results :** Within the limitations of the study, Diclofenac was shown to have superior anti-bacterial effect against E. faecalis. **Conclusion :** Therefore, in clinical situations usage of combinations rather than single medicament should be encouraged to better combat endodontic biofilm combinations. The usage of non-antibiotics as intracanal medicaments eliminates the risk of any systemic complications and preventing antibiotic resistance.

KEYWORDS

INTRODUCTION

The main goal of an endodontic treatment is complete removal of bacteria, their byproducts, and pulpal remnants from infected root canals and the complete disinfection of root canals. Intracanal medicaments should be used for root canal disinfection as part of controlled asepsis in infected root canals, in modern endodontics their role is secondary to cleaning and shaping of the root canal. An intracanal medicament is used to: (i) eliminate any remaining bacteria after canal instrumentation; (ii) reduce inflammation of periapical tissues and pulp remnants; (iii) render canal contents inert and neutralize tissue debris; (iv) act as a barrier against leakage from the temporary filling; (v) help to dry persistently wet canals. The root canal infection occurs as a result of multiple microorganisms, of which, E. faecalis dominates along with polymicrobial anaerobic species. Calcium hydroxide most commonly used intracanal medicament as it is stable for long periods, harmless to the body, and bactericidal in a limited area. [1]

A single antibiotic is insufficient to eradicate all polymicrobial flora; hence, triple antibiotic paste (TAP) is used to achieve complete disinfection. The combination that appears to be most promising consists of metronidazole, ciprofloxacin, and minocycline. A significant reduction was observed in microbial growth following the irrigation protocol and antibiotic paste application. However, development of resistant bacterial strains and tooth discoloration are the possible drawbacks of using TAP. [2]

Incorporating commonly prescribed NSAIDs such as Diclofenac or Ibuprofen as a component in inter-appointment dressing has the potential beneficial effects. [3]

Diclofenac sodium is a potent anti-inflammatory, analgesic, antipyretic and antibacterial. Several Studies reported its efficacy in reducing post operative pain. It has exhibited profound antibacterial effect against gram positive or gram negative bacteria. It has shown synergism with other antibiotics. [4]

Ibuprofen is the first member of propionic acid derivatives. It has potent anti-inflammatory, analgesic and antibacterial effects. Studies has reported inhibition of bacterial DNA synthesis or impairment of membrane activity as possible underlying mechanism for its antibacterial action. [5]

Thus, the aim of this study is to evaluate the anti-bacterial efficiency of anti-inflammatory nonantibiotics ibuprofen and diclofenac and routinely used intracanal dressing Ca(OH)2 and triple antibiotic paste against E. faecalis, using an endodontic model. The research hypothesis is that there is a difference in antibacterial efficiency of the three medicaments.

MATERIALS AND METHODOLOGY

This study was conducted to evaluate total viable bacterial count of E. faecalis (ATCC 29212) of from root canals of extracted single-rooted mandibular premolar teeth after the use of conventional intracanal medicament ($Ca[OH]_2$ Triple antibiotic paste) and test intracanal medicaments (ibuprofen, diclofenac sodium). Fifty single-rooted mandibular premolar teeth with completely formed apices extracted for periodontal reasons were selected for the study. They were stored in normal saline at room temperature immediately after extraction until use. Inclusion criteria were single-canal roots, straight roots, absence of root decay, absence of previous endodontic treatment were selected. Teeth with resorption defects, root caries and cracks were excluded from the study.

PREPARATION OF THE ENDODONTIC MODEL

All 50 single-rooted mandibular premolar teeth were decoronated to standardize root length to 12 mm. Working length was established by inserting #10 K-file (MANI, Japan) into to each root canal until it is only visible at the apical foramen, then subtracting 1 mm from this point. The roots were subjected to standardized instrumentation using

rotary upto F3-ProTaper (Dentsply-Maillefer,) and 2 ml of 3% NaOCl irrigation after each instrument, and final irrigation with 2 mL of 17% EDTA, allowing it to remain for 1 min, followed by rinsing with 2 mL of saline. After drying with size 40 absorbent paper points, apical foramen was sealed with bonding agent and light-cure composite resin. All external surfaces were made impermeable with nail varnish, except for coronal access. Teeth were then autoclaved (121°C for 15 min). To simplify manipulation during contamination and medicament placement, specimens were placed in 1.5 mL Eppendorf tubes.

Root Canal Contamination With Enterococcus Faecalis

The bacterial strain of *E. faecalis* (ATCC 29212) from the stock were revived by plating on blood agar medium. Isolated colonies were transferred to sterile brain heart infusion broth and once again incubated overnight. With the aid of a syringe, 5 µL of *E. faecalis* microbial suspension adjusted to the McFarland standard no. 1 was inoculated into the previously autoclaved teeth. This procedure was repeated every 72 h for 14 days. During this period, the teeth were kept in an hot air oven at 37°C.

Confirmation of tooth contamination

After 14 days, each tooth was irrigated with 100 µL of sterile saline and then a size-40 sterile absorbent paper point was inserted into the root canal and left for 5 min. After this, the paper points were transferred to a test tube containing 1 mL of saline solution, from which four serial dilutions were made. Aliquots of 25 µL of each dilution were plated onto Mueller–Hinton agar plates. Colony forming units (CFU-1) were counted after 24 h incubation.

Application Of Test Intracanal Medicaments

The samples were randomly allocated into 4 groups (n = 10), depending on medicament used:

- Group-1 -Diclofenac sodium Ibuprofen + distilled water (1:1 w/v)
- Group-2 -Ibuprofen + distilled water (1:1 w/v)
- Group-3 - Triple Antibiotic Paste + distilled water (1:1 w/v)
- Group -4 - $Ca(OH)_2$powder + distilled water (1:1 w/v)

Intracanal medicaments were prepared on sterile glass slab by mixing test material with distilled water to obtain a creamy mix. Test medicaments were then placed into root canals with lentulo spiral placed up to working length. Root canal orifices (including control group) were plugged with cotton pellet, sealed with temporary restoration, and kept in incubator at 37°C for 7 days.

Microbiological analysis after treatment with test medicaments

After 7 days, each tooth was irrigated with 5 mL saline, the technique used to collect the first set of CFU counts were used to obtain the second set of counts (CFU-2).

Statistical Analysis

Kruskal–Wallis ANOVA test was used to compare the four groups. Mann–Whitney U-test was used for pair-wise comparison, and Wilcoxon matched pair test for comparison within the group.

RESULTS

Comparison between CFU counts at baseline (CFU-1) and after 7 days (CFU-2) in the four groups and inter-group comparison are given in Table 1, and graphically represented in Figures 1.

TABLE 1

GROUPS		MEAN	PERCENTAGE CHANGE	P VALUE
DICLOFENAC	CFU 1 CFU2	22.80(21.16) 00.49(01.84)	-97.98	0.0085 0.2559
IBUPROFEN	CFU1 CFU2	20.62(19.58) 00.41(00.42)	-98.25	0.2049 0.7767
TRIPLE ANTIBIOTIC PASTE	CFU 1 CFU2	21.80(23.85) 00.39(01.39)	-98.11	0.0900
CA(OH)2	CFU1 CFU2	20.41(23.75) 00.35(01.49)	-111.02	0.0106

FIGURE 1

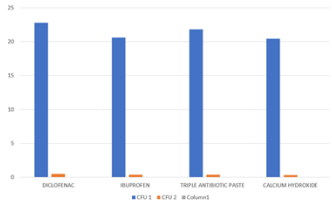

Table 1 and Figure 1 indicates change in CFU counts, which is a measure of the anti-bacterial efficiency, revealed that all 4 medicament groups (Groups-1, 2, 3 and 4) were significant in their anti-bacterial effect, but not significantly different from each other.

DISCUSSION

The leading cause of endodontic treatment failure is the persistence of microbial invasion of the root canal system and peri radicular tissue. The infection of the root canal system is polymicrobial, containing both anaerobic and aerobic bacteria. The treatment of a root canal is a procedure involving many steps like irrigation and mechanical instrumentation, which is aimed at making the root canals free of bacteria up to 50–70%. So the 30-50% of the root canal which are not bacteria-free will end in intracanal infection and, consequently, periapical infection, leading to root canal treatment failure. That is why intracanal medicaments represent an additional step to achieve complete bacterial eradication, especially EF Intracanal medicaments would include any agent with intended pharmacological action introduced into the root canal space. Calcium hydroxide is most commonly used intracanal medicament.[6,7]

EF has been selected for this study, as it is the primary and most dominant microorganism found in failed root canal treatment. They have importance because of their resistance to multiple antimicrobials. [7,8]

The prevalence of complex root canal system results in sheltering the micro organisms from the effect of medicaments. Single antibiotic paste is insufficient to eradicate polymicrobial flora. Hence TAP is used to achieve complete disinfection. But , TAP has limited usage has it causes tooth discoloration and development of resistant strains. To overcome these limitations, in the present study, Non- Antibiotics were used. [6,7,9]

Kristiansen and Amaral (1997) has done extensive research on antibacterial activity of “nonantibiotics,” i.e., not only drugs developed to treat noninfectious diseases but also exhibit anti-microbial activity. A variety of compounds which are employed in the management of pathological conditions of a non-infectious aetiology have also been shown to modify cell permeability and to exhibit broad-spectrum antimicrobial activity in vitro against bacteria and other microorganisms. Such compounds are here called 'non-antibiotics'. [7]

Domenico et al. conducted the first research into the anti-bacterial efficacy of NSAIDs and reported that it has potent antimicrobial activity.[10,11]

In the current study, an endodontic model was used, where single-rooted mandibular premolar teeth were cleaned and shaped, followed by contamination for 2 weeks with. E. faecalis. To obtain growth for root canal sampling, incubation time must not be <2 weeks.

In the present study, Change in CFU counts, which is a measure of the anti-bacterial efficiency, revealed that all 4 medicament groups (Groups-1, 2, 3 and 4) were significant in their anti-bacterial effect, but not significantly different from each other.

An in vitro study by Salem-Milani et al. was the first to comparatively assess antibacterial efficiency of ibuprofen, diclofenac and Ca(OH)2, and recommended the use of NSAIDs as intracanal medicaments. Utilizing the agar diffusion test, the authors demonstrated the anti-bacterial of NSAIDs (ibuprofen, diclofenac) against E. faecalis; whereas Ca(OH)2 failed at the same which are in accordance with the present study. [4,12,13]

Shojae et al concluded that Triple antibiotic paste has Minocycline which is the most effective component against Enterococcus faecalis and also have an appropriate effect on Enterococcus faecalis than calcium hydroxide.[14]

The exact mechanism of antibacterial activity of diclofenac and ibuprofen remains unclarified. Studies have proposed the following mechanism(s) of action:

- Inhibition of bacterial DNA synthesis
- Impairment of membrane activity
- Anti-plasmid activity
- Alteration in genes encoding transport/binding proteins, DNA synthesis and cell envelope
- Down-regulation of efflux pumps

- Reduced quorum sensing-controlled motility leading to reduced biofilm [12,14]

Regarding effective drug dose, Salem-Milani et al. demonstrated that diclofenac and ibuprofen have anti-bacterial activity against E. faecalis at 50 µg/ml and above. [4,12,13]

CONCLUSION

Within the limitations of the present study, antimicrobial effectiveness of diclofenac was found to be superior to other tested materials. Therefore, in clinical situations usage of combinations rather than single medicament should be encouraged to better combat endodontic biofilm combinations. The usage of non-antibiotics as intracanal medicaments than antibiotics eliminates the risk of any systemic complications and preventing antibiotic resistance.

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