Comparison of anti-bacterial efficiency of ibuprofen, diclofenac, and calcium hydroxide against Enterococcus faecalis in an endodontic model: An in vitro study

Sherin Jose Chockattu, B. S. Deepak1, K. Mallikarjun Goud
Department of Conservative Dentistry and Endodontics, Bapuji Dental College and Hospital, Davangere, Karnataka, ‘Department of Conservative Dentistry and Endodontics, Dental College, RIMS, Imphal, Manipur, India

Abstract
Context: One of the important goals of root canal therapy is disinfection aided by irrigation and intracanal medicaments. Commonly used nonsteroidal anti-inflammatory agents have shown anti-bacterial activity, thus, when used as intracanal medicaments, nonantibiotic agents have the potential to provide anti-inflammatory, local analgesic, and anti-bacterial activity.

Aim: The aim of this study is to evaluate and compare the anti-bacterial efficiency of anti-inflammatory nonantibiotics ibuprofen and diclofenac, and routinely used intracanal dressing calcium hydroxide (Ca(OH)2), against Enterococcus faecalis, in an endodontic model.

Materials and Methods: A total of 76 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: ibuprofen, Group-2: diclofenac, Group-3: Ca(OH)2) were mixed with distilled water (1:1 w/v), placed into root canals, temporarily sealed, and incubated (37°C; 7 days). Group-4 received no medicament (control). 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, anti-inflammatory nonantibiotics (ibuprofen; diclofenac) were shown to have anti-bacterial effect against E. faecalis.

Conclusion: Since nonsteroidal anti-inflammatory drugs (NSAIDs) have an anti-bacterial effect, it is possible to replace Ca(OH)2 with NSAIDs, or even combine them to form a cocktail of local disinfectants to optimize canal disinfection.

Keywords: Anti-bacterial agents; anti-inflammatory nonantibiotics; calcium hydroxide; diclofenac; ibuprofen

INTRODUCTION

The most commonly used inter-appointment intracanal dressing is calcium hydroxide (Ca(OH)2), due to its ability to dissolve necrotic tissue, promotion of drying of weeping periapical lesions, and good biocompatibility. However, this medicament has limited effectiveness in eliminating bacteria from root canals.

The conventionally used class of analgesics- nonsteroidal anti-inflammatory drugs (NSAIDs)– may possess additional therapeutic properties such as anti-bacterial efficacy through

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inhibition of bacterial DNA synthesis and/or impairment of membrane activity; prevention of bacterial colonization and biofilm formation by interfering with quorum sensing of bacteria; and antiplasmid activity. This finding is merely one in the long line of research investigating the antibacterial effects of “nonantibiotic agents,” commencing with Ehrlich’s (1854–1915) discovery of the antibacterial effects of the Phenothiazine compound, methylene blue dye. The term “nonantibiotics” was coined by Kristiansen and Amaral (1997) to refer to drugs developed to treat noninfectious diseases but have been shown to exhibit anti-microbial activity.

Other anti-inflammatory agents such as corticosteroids (Ledermix paste) are already in use as inter-appointment intracanal medicaments. They have shown efficacy in reducing post-operative pain compared to Ca(OH)₂, but they may compromise host immune response. Local antibiotics in the root canal are currently not routinely recommended since there is no antibiotic that is efficient for all types of microorganisms occurring in infected root canals.

Incorporating commonly prescribed NSAIDs such as Diclofenac or Ibuprofen as a component in inter-appointment dressing has the potential to utilize beneficial effects of Ibuprofen (anti-inflammatory action, local analgesia, and potential anti-bacterial action), and also to improve on the properties of Ca(OH)₂ (limited anti-bacterial action).

Microbiota difficult to eradicate from root canals include Enterococcus faecalis. They may colonize root canals in single infections, especially in unsuccessfully treated root canals. Their ability to penetrate dentinal tubules sometimes to a deep extent, and ability to form biofilms, can enable their escape from the action of endodontic instrumentation and irrigation. It is also resistant to Ca(OH)₂ due to its ability to resist high pH values. E. faecalis may also acquire virulence traits such as antibiotic resistance through plasmid transfer.

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

MATERIALS AND METHODS

This study was conducted to evaluate total viable bacterial count from root canals of extracted single-rooted mandibular premolar teeth after the use of conventional intracanal medicament (Ca(OH)₂) and test intracanal medicaments (ibuprofen, diclofenac). Seventy-six single-rooted mandibular premolar teeth with completely formed apices, single root canal, and minimum root length of 14 mm were selected. Teeth with resorption defects, root caries, cracks and fractures, and previous endodontic treatment were excluded from the study.

Preparation of the endodontic model

All 76 single-rooted mandibular premolar teeth were decoronated to standardize root length to 14 mm. Working length was established by inserting #10 K-file (MANI, Japan) into 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 F4-ProTaper (Dentsply-Maillefer, Switzerland) and 2.5% NaOCl (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India) irrigation after each instrument, and final irrigation with 2 mL of 17% EDTA (DEOR Deo Smear-Off, India), 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 (Single Bond Universal Adhesive) (3M, India) and light-cure composite resin. All external surfaces were made impermeable with nail varnish, except for coronal access. Teeth were then autoclaved (Confident Dental Equipments Pvt. Ltd., India) (121°C for 20 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 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 (DENTSPLY, India) 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 76 teeth were randomly allocated into 4 groups (n = 19), depending on medicament used:
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- Group 1 – Ibuprofen (≥98% pure powder) (Sigma–Aldrich, India) + distilled water (1:1 w/v)
- Group 2 – Diclofenac sodium salt (Sigma–Aldrich, India) + distilled water (1:1 w/v)
- Group 3 – Ca(OH)\(_2\) powder (Deepashree Products, Rathnagiri, India) + distilled water (1:1 w/v)
- Control: No medicament.

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 (Cavit, 3M ESPE, Germany), and kept in incubator (Confident Dental Equipments Pvt. Ltd., India) at 37°C for 7 days.

**Microbiological analysis after treatment with test medicaments**

After 7 days, each tooth was irrigated with 5 mL saline, including the control group (no medicament). 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, along with % change, and inter-group comparison are given in Table 1, and graphically represented in Figures 1 and 2.

**DISCUSSION**

Since the time of Ehrlich, antimicrobial activity of synthetic, nonchemothapeutic compounds, such as Methylene blue dye, has been known. Kristiansen and Amaral (1997) were monumental to the inception of research into antibacterial activity of “nonantibiotics,” i.e., not only drugs developed to treat noninfectious diseases but also exhibit anti-microbial activity.\(^{[9]}\)

Diclofenac has shown substantial reduction of postendodontic pain when administered preoperatively in a single oral dose.\(^{[14]}\) Sharma et al. evaluated pain-relief postextraction in patients receiving oral ibuprofen. Pain-relief was attributed to the rapid absorption of granule formulation and/or a local action of ibuprofen in solution in the mouth.\(^{[19]}\) Therefore, potential advantages that may be acquired by the use of NSAIDs as intracanal medicament are: anti-inflammatory action, local analgesia, and possible anti-bacterial action.

To the best of our knowledge, Domenico et al. conducted the first research into the anti-bacterial efficacy of NSAIDs, with sodium salicylate exhibiting activity against *Klebsiella pneumoniae*.\(^{[16]}\) In vitro and in vivo animal studies by Annadurai et al.,\(^{[4]}\) Dastidar et al.,\(^{[3]}\) and Dutta et al.\(^{[5,6]}\) revealed the anti-bacterial efficacy of diclofenac against *Salmonella typhimurium*, *Mycobacterium tuberculosis* and *Listeria monocytogens*. Shirin et al. demonstrated efficacy of ibuprofen against *Helicobacter pylori*.\(^{[17]}\)

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

**Table 1: Comparison between colony forming units counts at baseline (colony forming units-1) and after 7 days (colony forming units-2) in the 4 groups, along with percentage change, and inter-group comparison**

| Groups | CFU count | Mean (SD) | Percentage change | Pair-wise comparisons (Mann-Whitney U-test) |
|--------|-----------|-----------|------------------|-------------------------------------------|
| Group 1 | CFU-1     | 20.58 (19.53) | −98.98            | 0.0012* 1 versus 2  P=0.2549  No statistically significant difference |
|        | CFU-2     | 00.21 (00.42) |                   | 1 versus 3  P=0.9767  No statistically significant difference |
|        |           |           |                   | 2 versus 3  P=0.0076*  Very strong evidence (P<0.001) against null hypothesis |
|        |           |           |                   | 2 versus 4  P=0.2429  No statistically significant difference |
| Group 2 | CFU-1     | 14.26 (21.14) | −97.05            | 0.0093* 2 versus 3  P=0.0076* Very strong evidence (P<0.001) against null hypothesis |
|        | CFU-2     | 00.42 (01.84) |                   | 2 versus 4  P=0.2429  No statistically significant difference |
| Group 3 | CFU-1     | 21.79 (23.74) | −98.55            | 0.0033* 3 versus 4  P=0.0500* Strong evidence (P=0.001-0.01) against null hypothesis |
|        | CFU-2     | 00.32 (01.38) |                   |               |
| Group 4 | CFU-1     | 22.63 (21.17) | +112.09           | 0.1841 1 versus 4  P=0.0106* Strong evidence (P=0.001-0.01) against null hypothesis |
|        | CFU-2     | 48.00 (58.11) |                   |               |

*P<0.05. SD: Standard deviation, CFU: Colony-forming units
null hypothesis failed at the same. Group-3 showed strong evidence against the null hypothesis (other than NSAIDs) that have shown antibacterial activity against E. faecalis; whereas Ca(OH)₂ failed at the same. An in vitro study by Salem-Milani et al. was the first to comparatively assess anti-bacterial efficiency of ibuprofen, diclofenac and Ca(OH)₂, and also to recommend 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.

On a related note, NSAIDs other than the aforementioned, may serve the role of a nonantibiotic, namely, indomethacin and ketoprofen. Nonantibiotic agents (other than NSAIDs) that have shown antibacterial activity against E. faecalis are: chlorpromazine (anti-psychotic/anti-emetic), amiloride-HCl (potassium-sparing diuretic), and lignocaine (local anesthetic/anti-arrhythmic).

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. Blanscet et al. showed anti-bacterial activity of Ca(OH)₂ at 400 and 600 µg/ml. These recommendations cannot be practically applied to the clinical situation. In the present study, powder forms of the medicaments were mixed in 1:1 w/v ratio (approximately) with distilled water, similar to the preparation of triple-antibiotic paste. 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. This model represents a clinical situation where retreatment is indicated. E. faecalis was the test bacterium because it has been associated with resistant endodontic infections. Using planktonic bacteria may not accurately reflect the environment inside root canal system where normally biofilms form. To obtain growth for root canal sampling, incubation time must not be <2 weeks.

Endodontic model also gives consideration to dentin buffering action, i.e., ability of dentin to inactivate medicaments placed in the root canals. Nutrient supply was also restricted by placement of a coronal seal, in comparison to culture plate methods.

A total of 6/19 (Group 1), 10/19 (Group 2), 8/19 (Group 3), and 5/19 (Group 4) teeth revealed no growth at the 1st sampling. This does not necessarily mean a failure of root canal contamination, as demonstrated by CFU counts detected at the subsequent sampling. This finding most likely reveals a drawback of paper point sampling—the biofilm was not dislodged sufficiently to be adsorbed onto the paper point. If dentin shavings were to be utilized as sampling method, it represents a confounding factor in standardization of this in vitro study—since a part of inner dentin is removed, it ineffect enlarges the root canal preparation. Furthermore, the baseline reading requires dentin shavings obtained through canal preparation; this removes a portion of the biofilm, thus allowing the medicament improved access to dentinal tubules.

Microbiological analysis was performed both before and after medication, to rule out false positive results. For the control group (Group 4), a coronal seal was placed, thus restricting the nutrients available to the bacteria under study. In spite of this, there was a nearly two-fold increase in their numbers after incubation. This finding is in agreement with the fact that E. faecalis can adapt and survive in the presence of restricted nutrients.

Change in CFU counts, which is a measure of the anti-bacterial efficiency, revealed that all 3 medicament groups (Groups-1, 2, and 3) were significant in their anti-bacterial effect, but not significantly different from each other. This is partly in agreement with the agar plate study by Salem-Milani et al. where diclofenac and ibuprofen showed greater anti-bacterial action than Ca(OH)₂. This variation may be attributed to the dentin buffering effect of root canals in the present study.

Comparing the medicaments with the control (Group-4), ibuprofen (Group-1) showed very strong evidence against the null hypothesis (P < 0.001), and diclofenac (Group-2) and Ca(OH)₂ (Group-3) showed strong evidence against the null hypothesis (P = 0.001–0.01). Thus, the null hypothesis was rejected.

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CONCLUSION

Within the limitations of this study, anti-inflammatory nonantibiotics (ibuprofen and diclofenac) were shown to have anti-bacterial effect against *E. faecalis*. Since NSAIDs act by a different mechanism of action, it is possible that in the future, we can replace Ca(OH)₂ with NSAIDs, or even combine NSAIDs with Ca(OH)₂ to form a cocktail of local disinfectants that can be used to optimize canal disinfection. Future in vitro research is needed to determine any possible interactions between NSAIDs and Ca(OH)₂, and in vivo research to determine the magnitude of the local analgesic actions of NSAIDs when used as an intracanal medicament.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Haapasalo M, Qian W. Irrigants and intracanal medicaments. In: Ingle JI, Bakland LK, Baumgartner JC, editors. Ingle’s Endodontics. 6th ed. Hamilton: B.C. Decker Inc.; 2008. p. 1009-10.
2. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: A systematic review and meta-analysis. Int Endod J 2007;40:2-10.
3. Dastidar SG, Ganguly K, Chaudhuri K, Chakrabarty AN. The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int J Antimicrob Agents 2000;14:249-51.
4. Annadurai S, Basu S, Ray S, Dastidar SG, Chakrabarty AN. Antibacterial activity of the antiinflammatory agent diclofenac sodium. Indian J Exp Biol 1998;36:86-90.
5. Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, et al. Potential management of resistant microbial infections with a novel non-antibiotic: The anti-inflammatory drug diclofenac sodium. Int J Antimicrob Agents 2007;30:242-9.
6. Dutta NK, Mazumdar K, Dastidar SG, Park JH. Activity of diclofenac used alone and in combination with streptomycin against *Mycobacterium tuberculosis* in mice. Int J Antimicrob Agents 2007;30:336-40.
7. Ulusoy S, Bosgelmez-Tinaz G. Nonsteroidal anti-inflammatory drugs reduce the production of quorum sensing regulated virulence factors and swarm in motility in human pathogen *Pseudomonas aeruginosa* [corrected]. Drug Res (Stuttg) 2013;63:409-13.
8. Mazumdar K, Dastidar SG, Park JH, Dutta NK. The anti-inflammatory non-antibiotic helper compound diclofenac: An antibacterial drug target. Eur J Clin Microbiol Infect Dis 2009;28:881-91.
9. Kristiansen JE, Amaral L. The potential management of resistant non-infections. J Antimicrob Chemother 1997;40:319-27.
10. Ehrmann EH, Messer HH, Adams GG. The relationship of intracanal medicaments to postoperative pain in endodontics. Int Endod J 2003;36:868-75.
11. Zehnder M. Root canal irritants. J Endod 2006;32:389-98.
12. Pozzi A, Gallelli L. Pain management for dentists: The role of ibuprofen. Ann Stomatol (Roma) 2011;2:3-24.
13. Baumgartner JC, Siqueira JF Jr., Sedgley CM, Kishen A. Microbiology of endodontic disease. In: Hargreaves KM, Cohen P, editors. Cohen’s Pathways of the Pulp. 10th ed. Missouri: Mosby/Elsevier; 2011. p. 252.
14. Metri M, Hegde S, Bhandi S. Effect of pretreatment diclofenac sodium on postendodontic pain: A randomised controlled trial. J Conserv Dent 2016;19:7-10.
15. Sharma NK, Kindelan JD, Hutchinson D, Lancaster L. A study to compare ibuprofen effervescent granules with ibuprofen tablets in the treatment of acute dental pain. Prim Dent Care 1994;1:5-8.
16. Domenico P, Schwartz S, Gunha BA. Reduction of capsular polysaccharide production in *Klebsiella pneumoniae* by sodium salicylate. Infect Immun 1989;57:3778-82.
17. Shirin H, Moss SF, Kancherla S, Kancherla K, Holt PR, Weinstein IB, et al. Non-steroidal anti-inflammatory drugs have bacteriostatic and bactericidal activity against *Helicobacter pylori*. J Gastroenterol Hepatol 2006;21:1388-93.
18. Riordan JT, Dupre JM, Cantore-Matyi SA, Kumar-Singh A, Song Y, Zaman S, et al. Alterations in the transcriptome and antibiotic susceptibility of *Staphylococcus aureus* grown in the presence of diclofenac. Ann Clin Microbiol Antimicrob 2011;10:30.
19. Salem-Milani A, Balaee-Gajan E, Rahimi S, Moosavi Z, Abdollahi A, Zakeri-Milani P, et al. Antibacterial effect of diclofenac sodium on *Enterococcus faecalis*. J Dent (Tehran) 2013;10:16-22.
20. Jayasimha Raj U, Myleswaran S. The effect of 4% lignocaine gel, 5% salicylate. Infect Immun 1989;57:3778-82.
21. Blanscet ML, Tordik PA, Goodell GG. An agar diffusion comparison of the antimicrobial effect of calcium hydroxide at five different concentrations on *Pseudomonas aeruginosa* – The role of ibuprofen. Drug Res (Stuttg) 2013;63:409-13.
22. Sponchiado EC, Pereira JV, Marques AA, Garcia Lda F, França SC. In vitro assessment of antimicrobial activity of *Pothomorphum umbellata* extracts against *Enterococcus faecalis*. Indian J Dent Res 2014;25:64-8.
23. Potterier I, Wattimo TM, Haapasalo M. *Enterococcus faecalis* – The root canal survivor and star in post-treatment disease. Endod Top 2003;8:135-59.
24. Haapasalo HK, Sirén EK, Wattimo TM, Orstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine: An in vitro study. Int Endod J 2000;33:126-31.

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