

Antibacterial Effect of Diclofenac Sodium on *Enterococcus faecalis*

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Abstract

Objective: Non-steroidal anti-inflammatory drugs (NSAIDs) have shown antibacterial activity in some recent studies. The aim of this study was to evaluate the antibacterial effect of diclofenac against *Enterococcus faecalis* (*E. faecalis*) as a resistant endodontic bacterium in comparison with ibuprofen, calcium hydroxide and amoxicillin.

Materials and Methods: The antibacterial activity of materials was evaluated using agar diffusion test and tube dilution method. Mixtures of 400 mg/ml of materials were prepared. The bacteria were seeded on 10 Muller-Hinton agar culture plates. Thirty microliter of each test material was placed in each well punched in agar plates. After incubation, the zone of bacterial inhibition was measured. Minimum inhibitory concentration (MIC) of the test materials was determined by agar dilution method. One-way Analysis of Variance (ANOVA) followed by Sidak post hoc test was used to compare the mean zone of microbial growth in the groups.

Results: There were significant differences between the two groups ($p < 0.05$). Results of the agar diffusion test showed that antibiotics (amoxicillin, gentamycin) had the greatest antibacterial activity followed by NSAIDs (ibuprofen, diclofenac). Ca(OH)_2 failed to show antibacterial activity. Diclofenac and ibuprofen showed distinct antibacterial activity against *E. faecalis* in 50 $\mu\text{g/ml}$ and above concentrations.

Conclusion: Within the limitations of this in vitro study, it is concluded that diclofenac and ibuprofen have significantly more pronounced antibacterial activity against *E. faecalis* in comparison with Ca(OH)_2 .

Key Words: Amoxicillin; Anti-bacterial Agents; Calcium Hydroxide; Diclofenac; Ibuprofen

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INTRODUCTION

Micro-organisms play a central role in the development of pulp and periapical diseases [1].

Therefore, local or systemic applications of various antibacterial agents have been used in the management of these pathoses. Calcium

hydroxide (Ca(OH)₂) is commonly used as an effective intracanal agent [2]; however, some recent studies have questioned the ability of Ca(OH)₂ in killing some resistant bacterial species in root canals [3, 4]. Therefore, many antibacterial agents have been proposed as a substitute for Ca(OH)₂.

Antibiotics have also been used in the form of systemic or intracanal application. However, the extensive and irrational use of antibiotics has caused the problem of antibiotic resistance [5]. One solution for this problem is to search for non-antibiotic compounds that exert antibacterial activity through different mechanisms [5]. Recent studies have shown that some medicines have antibacterial activity in addition to their main function. They include some antihistamines, antipsychotics, tranquilizers, anti-hypertensives and even local anesthetics [6-8]. All of these drugs with moderate to powerful anti-microbial properties have been classified under the common term "non-antibiotics" [9]. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used medicines for the management of pain and inflammation in dentistry. Studies have demonstrated that some NSAIDs have antibacterial action [10, 11]. This property has been more extensively studied about diclofenac sodium in comparison with other NSAIDs.

Diclofenac sodium is a potent anti-inflammatory, analgesic and anti-pyretic agent with less gastrointestinal side effects [12]. Several studies have shown its efficacy in reducing post-operative complications following the removal of an impacted mandibular third molar [13-15]. Intracanal or systemic application of diclofenac sodium has also shown to reduce post-operative endodontic pain [16, 17]. Diclofenac has exhibited profound antibacterial effect against both gram-positive and gram-negative bacteria [5, 18-22]. It has also shown synergism with other antibiotics [21, 22]. These studies have suggested diclofenac as a potent non-antibiotic antibacterial agent. The question is whether diclofenac sodium is a

suitable antibacterial agent in systemic or intracanal usage in endodontics with simultaneous anti-inflammatory and pain management effects. The antibacterial effect of diclofenac on endodontic pathogens has not been studied so far. Therefore, the present study was carried out to evaluate the antibacterial effect of diclofenac sodium against *Enterococcus faecalis* (*E. faecalis*) as a resistant endodontic bacterium in comparison with common intracanal and systemic antibacterial agents.

MATERIALS AND METHODS

In this study, the antibacterial activity of diclofenac sodium (Shasun Ltd, India) was evaluated and compared with ibuprofen (St. Louis, MO, USA), calcium hydroxide (Ariadent, Tehran, Iran) and amoxicillin (Sigma-Aldrich, Disenhofen, Germany). Gentamicin (Sigma-Aldrich, Disenhofen, Germany) was also used as the positive control. The raw materials were obtained from Dana pharmaceutical company (Tabriz, Iran). One ml of distilled water was placed in a vial and 400 mg powder of the test materials were incrementally added while mixing with a spatula. In this way, mixtures of 400 mg/ml concentration of test materials were prepared.

E. faecalis (American type culture collection [ATCC] 29212) was obtained and maintained in brain-heart infusion (BHI) broth. The density of inoculum was adjusted to the turbidity of 0.5 McFarland (1.5×10^8 bacteria/ml). The antibacterial activity of materials was evaluated using agar diffusion test and tube dilution method.

Agar diffusion method

Ten Muller-Hinton agar culture plates (Merck, Germany) were used in this study. Six wells with 4 mm diameter and 5 mm depth were punched in each agar plate. All the procedures were carried out under aseptic condition. The bacteria were seeded on agar plates. Cotton swabs were used to ensure an even distribution of bacteria. Each well was filled with 30 μ l of the test materials. One remaining well was left

empty to serve as the negative control. The plates were incubated aerobically at 37° C for 48 hours. After incubation, the zone of bacterial inhibition around each well was measured by a blind examiner as the shortest distance (mm) from the outer margin of the wells to the initial point of bacterial growth.

Tube dilution method

The minimum inhibitory concentration (MIC) of the test materials was determined by the tube dilution method. Test materials were prepared in 50 µg/ml concentration and serially diluted from 1:2 up to 1:2048 dilutions. One milliliter of Muller-Hinton broth (Merck, Germany) and the same amount of test preparations were mixed in tubes. One hundred microliters of *E. faecalis* inoculum was added to each test tube. The tests were carried out in triplicate. The turbidity of the tubes was evaluated by observation after 24 hours incubation at 37° C.

Statistical Analysis

All analyses were performed by STATA software version 10 (StataCorp, College Station, Texas). Normality of data was assessed and approved by Kolmogorov-Smirnov test. One-way Analysis of Variance (ANOVA) followed by Sidak post hoc test was used to compare the mean zone of microbial growth in the groups. P-values less than 0.05 were considered statistically significant.

RESULT

No zone of inhibition was observed adjacent to empty control wells. The means and standard deviations of the inhibition zones for test medicines are shown in Table 1. The results of ANOVA showed significant differences among the groups (F (5,50)= 232.4, P<0.001). Sidak post hoc test showed a significant difference between the two groups (p< 0.05) except for Ca(OH)₂ and the negative control (Table 1). Amoxicillin and gentamycin showed the lowest MIC (25 µg/ml) followed by diclofenac and ibuprofen (50 µg/ml). Ca(OH)₂ was

the least effective of the test materials and could not inhibit bacterial growth in any concentrations.

DISCUSSION

In the present study, the antibacterial activity of diclofenac against *E. faecalis* was compared with Ca(OH)₂ as the commonly used intracanal medication and amoxicillin as a choice antibiotic when conventional root canal treatment alone is not sufficient [23]. Ibuprofen was used as one of the most commonly used NSAIDs for the management of endodontic pain. Before commencement of the study, a pilot study was carried out to choose a potent antimicrobial agent against *E. faecalis* as the positive control. In this pilot study, the antibacterial effect of four antibiotics -gentamycin, rifampin, erythromycin and clindamycin- was compared using agar diffusion test. Gentamicin was used as the positive control because it showed the greatest inhibition zone compared to the other tested antibiotics in the pilot study. We used test materials in 400 µg/ml aqueous preparations. There is a controversy regarding the most suitable concentration of aqueous Ca(OH)₂ preparations.

Table 1. Mean Zone of Microbial Growth Inhibition Provided by Test Drugs Against *Enterococcus Faecalis*

Groups (n=10)	Mean (mm)	SD*
Diclofenac	9.10 ^a	2.02
Ibuprofen	12.90 ^b	2.64
Ca(OH)₂	1.00 ^c	0.00
Amoxicillin	20.67 ^d	0.82
Positive Control (Gentamicin)	15.70 ^e	1.49
Negative Control	0.00 ^{cf}	0.00

Groups identified by different superscript letters are significantly different (p<0.05), * SD= Standard Deviation

Behnen et al. tested three different concentrations of $\text{Ca}(\text{OH})_2$ regarding antibacterial activity against *E. faecalis* and showed that 10% and 40% aqueous $\text{Ca}(\text{OH})_2$ were significantly more effective than the 50% solution [24]. They proposed that the ionic dissociation rate in low viscous preparations is higher than thick preparations. Safavi and Nakayama confirmed it and theorized that in saturated solutions of slightly soluble materials like $\text{Ca}(\text{OH})_2$, the ionic concentration will remain constant as long as some undissolved material is present. More viscous preparations of $\text{Ca}(\text{OH})_2$ seem to have the same ionic concentration as thin preparations [25, 26]. Blanscet et al. [26] studied the effect of various concentrations of aqueous $\text{Ca}(\text{OH})_2$ on its antibacterial activity against *E. faecalis*. They showed that the antibacterial activity of $\text{Ca}(\text{OH})_2$ in 400 $\mu\text{g}/\text{ml}$ and 600 $\mu\text{g}/\text{ml}$ concentrations was not significantly different. We used *E. faecalis* as the test bacterium because it has been shown to be associated with resistant endodontic infections [27, 28]. Results of the agar diffusion test showed that antibiotics (amoxicillin, gentamicin) had the greatest antibacterial activity followed by NSAIDs (ibuprofen, diclofenac). $\text{Ca}(\text{OH})_2$ failed to show antibacterial activity against *E. faecalis*. In the second stage, we used tube dilution assay to measure MIC of the test materials. This test is a somewhat quantitative method and a complement for agar diffusion assay. The result of tube dilution test was in harmony with agar diffusion assay. Tested antibiotics were effective in low concentrations (25 $\mu\text{g}/\text{ml}$) followed by tested NSAIDs (50 $\mu\text{g}/\text{ml}$). Dutta et al. showed that the MIC of diclofenac sodium against 45 strains of mycobacterium is 10-25 $\mu\text{g}/\text{ml}$, which is much higher (5-6 times) than the MIC of the conventional anti-mycobacterial drugs [19, 21, 22]. In our study, $\text{Ca}(\text{OH})_2$ was unable to prevent the bacterial growth at any concentrations. This is in agreement with other studies which showed weak antibacterial effect of $\text{Ca}(\text{OH})_2$ against *E. faecalis* [29, 30].

One interesting finding of this study was the profound antibacterial activity of ibuprofen against *E. faecalis*. He et al. demonstrated the antibacterial activity of ibuprofen against six common periodontal pathogens [11]. The exact mechanism of this antibacterial activity of diclofenac and ibuprofen is unclear. However, studies have proposed inhibition of bacterial DNA synthesis [9] or impairment of membrane activity [19, 21] as possible underlying mechanisms. Some studies have shown that incorporation of anti-inflammatory agents such as corticosteroids or diclofenac in the composition of intracanal dressings reduces the inter-appointment pain [17, 31, 32]. Therefore, ibuprofen or diclofenac with both anti-inflammatory and antibacterial activity may theoretically propose as a substitute for $\text{Ca}(\text{OH})_2$ as the main component of the intracanal medication. The results of this study should be interpreted with caution. Agar diffusion test is a simple and well-standardized method of antibacterial testing [33]. It is the least costly of all susceptibility methods [33]. The media, equipment and supplies required for the test are readily accessible to most clinical laboratories. This method is especially well suited for determining the antimicrobial ability of water-soluble materials. A number of materials may be tested quickly using this method and a variety of products can be evaluated including liquids, solid materials and coated antimicrobial surfaces. However, this method has some disadvantages. Inhibition zones do not always have clear or regular boundaries and are influenced by the diffusion rate of materials through the agar, which in turn is affected by concentration, molecular weight and solubility of antimicrobials [34-36]. Furthermore, it is necessary to mention that determining MIC in vitro by the tube dilution method may not reflect the in vivo conditions where bacteria grow as biofilm on complex root canal surfaces. Further studies are underway to determine the antibacterial activity of NSAIDs against bacterial biofilms on root canal dentin.

CONCLUSION

Within the limitations of this in vitro study, it is concluded that:

1. Ibuprofen and diclofenac have significantly more pronounced antibacterial activity against *E. faecalis* in comparison with Ca(OH)₂.
2. The antibacterial activity of ibuprofen and diclofenac against *E. faecalis* is less than antibiotics (amoxicillin and gentamycin).

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