Comparison of Antimicrobial Effect of Berberine as an Endodontic Irrigant with that of Other Common Root Canal Irrigants on Three Microorganisms Involved in Persistent Endodontic Infections

Zakiyeh Donyavi¹, Mohammad Reza Arabestani², Dara Dastan³, Mohammad Esmaeilzadeh⁴ & Nazanin Shahsavand⁵

¹ Assistant professor, Department of Endodontics, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
² Associate professor, Department of Microbiology, Hamadan University of Medical Sciences, Hamadan, Iran
³ Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
⁴ Assistant professor, Department of Pediatric Dentistry, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
⁵ Department of Endodontics, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.

Correspondence: Nazanin Shahsavand, Department of Endodontics, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran. Email: hamunindentist1@gmail.com

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Abstract

Background and Aim: The present study investigated the antimicrobial effect of Berberine as an endodontic irrigant on the microorganisms involved in persistent endodontic infections. In this experimental in vitro trial, organisms Enterococcus Faecalis, Staphylococcus Aureus, and Staphylococcus Epidermidis were assessed in a multi-species biofilm tooth model.

Methods: Seventy-five single-rooted anterior teeth were collected and standardized to a length of 10mm. The teeth were randomly assigned into 5 groups. The teeth were then autoclaved to confirm being sterile. Afterwards, a biofilm consisting of the three selected bacteria was inoculated into the teeth and they were incubated for 21 days.

Results: The comparison of the amount of reduction in viable bacterial counts after irrigation by different solutions among the groups was done by Kruskal-wallis test while the changes of viable bacterial counts before and after irrigation with each solution was done by Wilcoxon Signed Ranks test. No significant difference existed among the studied irrigation solutions regarding the mean reduction of total viable bacterial counts, neither did it exist regarding the viable staphylococcus aureus, staphylococcus epidermidis or Enterococcus faecalis counts after irrigation. MIC of berberine against staphylococcus aureus, staphylococcus epidermidis and Enterococcus faecalis species were 0.5µg/ml, 0.03µg/ml and 1µg/ml, respectively. These values were found to be 0.39µg/ml, 0.09µg/ml and 0.78µg/ml for NaOCl and 0.04µg/ml, 0.04µg/ml and 0.09µg/ml for chlorhexidine, respectively.

Conclusion: it is concluded that Berberine can be used as a natural alternative instead of conventional root canal irrigants. However, more studies are required in order to confirm that characteristics of this substance are appropriate from other aspects.

1. Introduction

The effect of bacteria and their products has been proven as the major cause of development of pulpal and periapical diseases in many studies (Siqueira Jr, Rôças, & Lopes, 2002). The main purpose of root canal treatment is to remove these microorganisms from root canal space and to achieve this, mechanical and chemical cleaning of the canal is essential (Tabrizizadeh, Zandi, Mehrjardi, & Mahmodizadeh, 2014). However, certain microorganisms, including facultative gram-positive bacteria and certain types of fungi, may survive even after precise chemomechanical preparation of the root canal system and cause persistent root canal infections (Nabavizadeh et al., 2014). Biofilm is a complex microbial community that is composed of a variety of bacteria that have different ecological needs and pathological potential. Biofilm not only effectively protects bacteria against host defense, but also resists
them against a number of disinfectants that are used as oral hygiene products or drugs in the treatment of infections (Pinheiro et al., 2004).

The successful treatment of these resistant infections depends on biofilm removal and the effective killing of bacteria in biofilms (Pinheiro et al., 2004).

Enterococcus faecalis is a resistant microorganism that plays an essential role in persistent radical pericardial lesions after root canal treatment. It has also been found in 24% to 77% of endodontically treated teeth with apical periodontitis, as a single organism or as part of bacterial flora (Nosrat et al., 2009).

Removal of this bacterium by root canal therapy is very difficult because the bacterium penetrates into the dentinal tubules and stabilizes. Its ability to form biofilms and tolerate adverse environmental conditions explains its relatively high prevalence. Therefore, using a root canal irrigant that is effective on these bacteria can help to prevent refractory endodontic infections (Imanshahidi & Hosseinzadeh, 2008; Sundqvist, Figdor, Persson, & Sjögren, 1998). Staphylococci are also commonly found in persistent endodontic infections (Endo & Dias Filho, 2015; Wei, Xu, & Wu, 2011).

Root canal mechanical preparation is the initial procedure for cleansing the canal and irrigants are an essential complement to the treatment (Shuping, Ørstavik, Sigurdsson, & Trope, 2000). Selection of a proper irrigating solution is important because of their different functions in removing debris, smear layer and bacteria from the root canal system. Different formulations of detergents have different functions on the pulp, necrotic tissues and microorganisms (Baumgartner, Hutter, & Siqueira, 2006). Among these materials, sodium hypochlorite has been widely used as a successful irrigating solution after its introduction by Walker. Almost all studies have indicated its beneficial antimicrobial effects. Although this solution is used as a cleansing agent for the treatment of necrotic teeth due to its antimicrobial activity and its unique properties in tissue solubility, but it is toxic to vital tissues and its extrusion from apex causes pain and emphysema. On the other hand, it is cssn induced allergic reactions and its taste is unfavorable for patients and its vapor is an eye stimulus (Baumgartner, Hutter, & Siqueira, 2006; Leonardo et al., 2002; Ceri et al., 1999). Therefore, the study is still underway to find a solution with desirable antimicrobial properties and less side effects.

Chlorhexidine gluconate (CHX) is another detergent that has a wide antimicrobial spectrum as well as good substantivity by binding to hydroxyapatite as a final cleanser (Rôças, Siqueira Jr, & Santos, 2004).

Due to the continuous increase in antibiotic-resistant strains due to the inappropriate use of antibiotics the effects of synthetic detergents, researchers have recently started research into plant alternatives. Herbal detergents are harmless and non-toxic and have shown potent antibacterial effects in vitro (Leonardo et al., 2002). The antibacterial effects of green tea and Triphala, Monrinda Citrifolia, Zataria Multiflora Boiss, Satureja Jamzad Khuzistanica, Carvacol and Arctium Lappa have been studied as a detergent or intracanal drug in infectious root canals (Baumgartner, Hutter, & Siqueira, 2006; Leonardo et al., 2002; Ceri et al., 1999; Rôças, Siqueira Jr, & Santos, 2004).

Berberine, a quaternary ammonium salt of the isoquinolones group is found in plants such as Berberis Vulgaris, Goldenseal and Coptis Chinesis (Clauditz, Resch, Wieland, Peschel, & Götz, 2006). Berberis Vugaris (Barberry) is well known in Iran, and various parts of this plant such as root, leaf and fruit juices are used as traditional drugs (Siqueira Jr & Lima, 2002).

The mechanical treatment of the root canal is the initial method of cleansing the canal and washing solutions are an essential supplement to the treatment. Endodontic antimicrobial agents should be compatible with periapical tissues, in addition to being effective on pathogens. However, there are some disadvantages of some common irrigants, including their cytotoxicity, and adverse tissue effects in case of unintentional outflow from the canal. Therefore, there has recently been a growing trend towards the use of natural products in endodontic treatments (Nabavizadeh et al., 2014). Berberine has been studied in several studies because of its antimicrobial properties and biocompatibility, in order to determine its antibacterial and antifungal effects and its ability to be used as a root canal cleanser.

Considering the fact that in many studies, the most prevalent bacteria in persistent endodontic infections is Enterococcus faecalis and to a lesser extent are Staphylococcus aureus and Staphylococcus epidermidis, the aim of this study was to investigate the effect of Berberine as a natural root canal irrigants on these microorganisms.

2. Materials and Method

In this experimental in vitro trial, organisms Enterococcus Faecalis, Staphylococcus Aureus, and Staphylococcus Epidermidis were assessed in a multi-species biofilm tooth model. Seventy-five single-rooted anterior teeth were collected. The canals were prepared by crown-down technique by ProTaper rotary files using normal saline as an irrigant during cleaning and shaping procedures. Smear layer removal was done by 1 minute application of 17% EDTA (10 mL) and 5.25% NaOCl (5 mL). The roots were cut to standardized 10 mm sections and the root apices
were sealed using cyanoacrylate glue in order to prevent apical leakage and the outer surfaces of the teeth were covered with a layer of nail laquer. The teeth were randomly assigned into 5 groups: Berberine (2mg/mL), 5.25% NaOCl, Chlorhexidine gluconate 2%, mixture of Berberine 1mg/ml + Chlorhexidine gluconate 2% and Normal saline (control group). The teeth were then autoclaved to confirm being sterile. Afterwards, a biofilm consisting of the three selected bacteria (Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis) was inoculated into the teeth and they were incubated for 21 days. After incubation, the root canals were irrigated 3 times with 10ml of normal saline and dried with sterile #40 paper points. In order to take initial samples, dentin chips were collected by using #40 hedstrom files and added to microtubes containing 1ml normal saline and 1% tween 80 and shook for 30 seconds so that the microorganisms were separated. They were then cultured on Blood Agar medium and viable microorganisms were counted (S1). After that, the canals were flooded with irrigants for 5 minutes. They were then dried with #40 paper point and each irrigant was neutralized by 5ml of the appropriate solution so as to prevent the carry-over effect. The neutralizer was dried with sterile paper point after 1 minute and sampling was performed as done before and the microorganisms were cultured on Blood Agar medium. The blood Agar media were incubated at 35°C for 48 hours and the viable bacteria were counted (S2). Ultimately, the S1 and S2 counts were compared. The comparison of the amount of reduction in viable bacterial counts after irrigation by different solutions among the groups was done by Kruskal-wallis test while the changes of viable bacterial counts before and after irrigation with each solution was done by Wilcoxon Signed Ranks test.

3. Results

No significant difference existed among the studied irrigation solutions regarding the mean reduction of total viable bacterial counts, neither did it exist regarding the reduction of viable staphylococcus aureus, staphylococcus epidermidis or Enterococcus faecalis counts after irrigation.

However, the effect size for BBR, NaOCl, CHX, BBR+CHX and saline was respectively:

0.43, 0.62, 0.62, 0.42 and 0.3 against Enterococcus faecalis
0.51, 0.59, 0.62, 0.55 and 0.52 against Staphylococcus aureus
0.54, 0.62, 0.6, 0.61 and 0.4 against Staphylococcus epidermidis
and 0.55, 0.62, 0.62, 0.55 and 0.48 against the total biofilm bacteria.

MIC values against staphylococcus aureus, staphylococcus epidermidis and Enterococcus faecalis species were:

0.5µg/ml, 0.03µg/ml and 1µg/ml for BBR
0.39µg/ml, 0.09µg/ml and 0.78µg/ml for NaOCl
And 0.04µg/ml, 0.04µg/ml and 0.09µg/ml for chlorhexidine, respectively.

Table 1. MBC, MIC and inhibition zone diameter in the use of different irrigants against bacterial species

<table>
<thead>
<tr>
<th>Halo of lack of growth</th>
<th>MIC</th>
<th>MBC</th>
<th>group</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm11</td>
<td>µg/ml5/0</td>
<td>µg/ml1</td>
<td>Berberine</td>
<td>S. aureus</td>
</tr>
<tr>
<td>mm30</td>
<td>µg/ml04/0</td>
<td>µg/ml09/0</td>
<td>CHX 2.0%</td>
<td>S. aureus</td>
</tr>
<tr>
<td>mm40</td>
<td>µg/ml39/0</td>
<td>µg/ml78/0</td>
<td>NaOCl 5.25%</td>
<td>S. aureus</td>
</tr>
<tr>
<td>mm28</td>
<td>µg/ml04/0</td>
<td>µg/ml09/0</td>
<td>CHX+Berberine</td>
<td>S. aureus</td>
</tr>
<tr>
<td>mm19</td>
<td>µg/ml03/0</td>
<td>µg/ml06/0</td>
<td>Vancomycin</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>mm38</td>
<td>µg/ml04/0</td>
<td>µg/ml09/0</td>
<td>Berberine</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>mm41</td>
<td>µg/ml09/0</td>
<td>µg/ml19/0</td>
<td>NaOCl 5.25%</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>mm38</td>
<td>µg/ml04/0</td>
<td>µg/ml09/0</td>
<td>CHX+Berberine</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>mm20</td>
<td>µg/ml1</td>
<td>µg/ml2</td>
<td>Vancomycin</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>mm21</td>
<td>µg/ml09/0</td>
<td>µg/ml19/0</td>
<td>Berberine</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>mm29</td>
<td>µg/ml78/0</td>
<td>µg/ml56/1</td>
<td>NaOCl 5.25%</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>mm20</td>
<td>µg/ml09/0</td>
<td>µg/ml19/0</td>
<td>CHX+Berberine</td>
<td>E. faecalis</td>
</tr>
</tbody>
</table>

4. Discussion

According to the results of the study conducted by Xie et al, Berberine alone and in combination with chlorhexidine reduced the number of live bacteria in the form of a laboratory protocol based on a study on a multibacterial biofilm (including Fusobacterium nucleatum, Enterococcus faecalis and Prevotella intermedia) (Xie, Johnson, Wenckus,
According to the results of the current study, sodium hypochlorite has the ability to destroy *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* as other rinse solutions. Species of *Staphylococcus epidermidis* and *Enterococcus faecalis* decreased significantly after irrigation compared to the counts before irrigation. Neither did it exist regarding the reduction in viable *Staphylococcus aureus*, *Staphylococcus epidermidis* or *Enterococcus faecalis* counts after irrigation.

No significant difference existed among the studied irrigants regarding the mean reduction of total viable bacterial counts and the number of viable bacterial counts of *Staphylococcus aureus*, *Staphylococcus epidermidis* or *Enterococcus faecalis* species decreased significantly after irrigation compared to the counts before irrigation.

**5. Conclusion**

No significant difference existed among the studied irrigants regarding the mean reduction of total viable bacterial counts, neither did it exist regarding the reduction in viable *Staphylococcus aureus*, *Staphylococcus epidermidis* or *Enterococcus faecalis* species after irrigation.

However, in all solutions (except in the normal saline group for *Enterococcus faecalis* species), the number of total viable bacterial counts and the number of viable bacterial counts of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* species decreased significantly after irrigation compared to the counts before irrigation.

**MIC of Berberine against staphylococcus aureus, staphylococcus epidermidis and Enterococcus faecalis**

 MIC of Berberine against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* species were 0.5 μg/ml, 0.03 μg/ml and 1 μg/ml, respectively. These values were found to be 0.39 μg/ml, 0.09 μg/ml and 0.78 μg/ml for NaOCl and 0.04 μg/ml, 0.04 μg/ml and 0.09 μg/ml for chlorhexidine, respectively.

Altogether, since the effective concentrations of berberine are achievable in vitro, and because of the similar antibacterial efficacy of berberine compared to that of other conventional irrigants, and considering it’s natural and herbal essence and the fact that this substance is biocompatible and not cytotoxic (in contrary to some conventional irrigants which can cause adverse effects if unintentionally extruded beyond the canal space), it is concluded that Berberine can be used as a natural alternative instead of conventional root canal irrigants. However, more studies are required in order to confirm that characteristics of this substance are appropriate from other aspects.
References


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