Indian Journal of Dental Sciences.
October 2014 Supplementary Issue
Issue:4, Vol.:6

www.ijds.in

Original Article

Indian Journal
of Dental Sciences

E ISSN NO. 2231-2293 P ISSN NO. 0976-4003

Comparison Of Antimicrobial Efficacy Of Sodium Hypochlorite & Chlorhexidine At Different Concentrations - An Invitro Study

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Submission : 30th March 2013
Accepted : 29th May 2014

Quick Response Code

Abstract
The aim of this study was to evaluate the antimicrobial efficacy of sodium hypochlorite (1% and 3%) and chlorhexidine (1% and 2%) with or without the addition of organic load (sterile plasma 0.5%) against some bacterial samples Staphylococcus aureus(ATCC 25923), Enterococcus faecalis (ATCC 29212) using agar diffusion method. Muller Hinton agar plates were seeded with bacterial strains. Paper disks (7-mm diameter) saturated with 10µl of test solution for 1min were placed on the plates. In each Petri dish, one paper disk soaked with sterile saline solution was placed as negative control. Agar plates inoculated with bacterial strains were incubated for 24 hrs at 37S04;C. Diameters of the zones of bacterial inhibition were measured with a transparent ruler and recorded for each strain and solution. In half the specimens, 0.5% sterile plasma was added to simulate organic tissue present in the root canal. Bacterial growth was evaluated for each microorganism. Each test was repeated 5 times. Results revealed that all concentrations of CHX and NaOCl without organic load produced zones of antimicrobial activity against the tested strains. Whereas samples containing organic load exhibited inhibition zones although the diameters were smaller than in the absence of organic load.

Key Words
Antimicrobial agents, bacteria, endodontics, root canal irrigants.

Introduction

The harmful effect of microorganisms present in pulp and periapical pathologies has been studied since 1894, when Miller[1] proved the presence of bacteria in the interior of root canals and observed their importance in the etiology of such alterations. This was then confirmed by posterior studies [2],[3].

The use of chemical irrigants during chemo-mechanical canal preparation is important for disinfection and cleaning of the canal system[4]. As access to the root canal system is limited and the anatomy complex, microorganisms may remain in the dentinal tubules and in other irregular spaces. When these microorganisms find a supporting environment, they can proliferate and re-infect the root canal system. Studies have demonstrated that chemo-mechanical preparation combined with a non antiseptic irrigating solution was capable of reducing approximately 50% of bacteria in root canals[5]. While sodium hypochlorite (NaOCl) solution eliminated approximately 80% of bacteria.

An irrigant serves to flush out debris from within the instrumented root canals, dissolve organic tissue remnants, disinfect the root canal space and provide lubrication during instrumentation, without causing irritation to biological tissues[6],[7]. Sodium hypochlorite (NaOCl) has become the most popular agent for endodontic irrigation, even though its optimum working concentration has not been universally agreed[6]. Chlorhexidine gluconate (CHX), a less malodorous and toxic agent, has been suggested as an irrigant based on its antibacterial effects, substantivity and lower cytotoxicity than NaOCl, whilst demonstrating efficient clinical performance[8].

One of the most commonly used antimicrobial activity tests is the agar diffusion test, which involves the placement of paper disks, previously saturated with solutions, on Petri dishes containing agar with a selected microorganism. After a certain period of time, zones of inhibition of variable diameters form around the paper disks if the tested substance presents antimicrobial activity[9].

The purpose of this study was to evaluate, in vitro, the antimicrobial activity of NaOCl (1% and 3%) and CHX (1% and 2%) solutions against strains of bacteria found in infected root canals including facultative anaerobic microorganisms (E. Faecalis and S. aureus) using agar diffusion test and also to determine the influence of an organic load material. Sterile plasma (organic material) was added to simulate organic tissue present in the root canal system.

Material And Method

Sodium hypochlorite with initial concentration of 4% was diluted in sterile water to make final concentration of 1% and 3% and Chlorhexidine gluconate with initial concentration of 20% was diluted in sterile water to make final concentration of 1% and 2% respectively. Sterile plasma (0.5%) was added as organic load material.

Plates containing Muller–Hinton agar were seeded with bacterial strains Staphylococcus aureus (ATCC 25923), Enterococcus faecalis (ATCC 29212). Paper disks (7-mm diameter) saturated with 10 µL of test solution for 1 min were placed on the plates. In each Petri dish, one paper disk soaked with sterile saline solution was placed as negative control.

Agar plates inoculated with bacterial strains were incubated for 24 hrs at 37S04;C.Diameters of the zones of bacterial inhibition were measured with a transparent ruler and recorded for each strain and solution.Test was repeated 5 times. Data were statistically analyzed using SPSS software; student's t-test was used to compare the diameters of the zones of bacterial inhibition to determine the influence of sterile plasma on the effectiveness of the solutions ($P < 0.01$).Sterile plasma interfered with the antimicrobial activity of 1% CHX and 1% and 3% NaOCl against E. faecalis;and 1% and 3% NaOCl against S. aureus.

To compare the antimicrobial activity of tested solutions the ANOVA and Bonferroni's tests ($P < 0.05$) were performed. In the presence of sterile plasma, 2% CHX had the most reliable antimicrobial result for all strains tested. However, in the absence of sterile plasma, 2% CHX was the most effective antimicrobial against E. faecalis and S aureus. The level of significance was kept at 0.05%.

Results

The outcome of the present study the mean diameters of the zones of bacterial inhibition for each irrigant solution against the bacterial strains, with or without the organic load, are shown in **Table 1.1**. All concentrations of CHX and NaOCl without organic load produced zones of antimicrobial activity against the tested strains. However, when higher concentrations of the solution were used, larger inhibition halos were formed.

Whereas samples containing organic load exhibited inhibition zones although the diameters were smaller than in the absence of organic load. Organic load interfered with the antimicrobial activity of 1%, 2% CHX and 3% NaOCl against the tested strains resulting in smaller inhibition halos. In the presence of organic load, 2% CHX had the most reliable antimicrobial result for all strains tested as shown in the **bar graph Fig 1.2**.

Table: 1.1: Arithmetical means of the diameters (mm) of the bacterial inhibition zones for Sodium Hypochlorite and Chlorhexidine solutions on microorganisms in the agar diffusion test.

Solution	E.Faecalis Without Organic Load	With Organic Load	S.Aureus Without Organic Load	With Organic Load
1% Sodium Hypochlorite	8.0mm ± 0.74	8mm ± 0	10mm ± 0.84	8mm ± 0
3% Sodium Hypo- Chlorite	10mm ± 0.89	8.5mm ± 0	11mm ± 0.71	9mm ± 0
1% Chlorhexidine	12mm ± 0.70	11.5mm ± 1.58	12.5mm ± 0.61	12.5mm ± 0.42
2% Chlorhexidine	13.5mm ± 0.57	13mm ± 0.70	14.5mm ± 1.0	13mm ± 0.84

Discussion

Complete debridement and disinfection of the pulpal space are considered to be essential for predictable long-term success in endodontic treatment. Residual pulpal tissue, bacteria, and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation[10]. Therefore, several irrigant solutions have been recommended for use in combination with canal preparation. However, the efficacy of these procedures also depends upon the vulnerability of the involved species. Anaerobic bacteria, especially black-pigmented Gram-negatives, have been linked to the signs and symptoms of endodontic disease[11] but facultative bacteria, such as Enterococcus faecalis, have also been isolated from pathologically involved root canals, being considered one of the most resistant species in the oral cavity and a possible cause of failure of root canal treatment[12].

Sodium hypochlorite solution is, to date, the most commonly employed root canal irrigant. However, no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25%. NaOCl provides good tissue solvent action[13],[14], has a broad spectrum of antimicrobial activity [4],[5],[15], acts as a lubricant for instrumentation and can flush loose debris from root canals[10]. The major disadvantages of NaOCl are its cytotoxic effect if injected into the periapical tissues[15], its foul smell and taste, its ability to bleach clothes, and it's potential for causing corrosion[16]. It is also known to produce allergic reactions[17].

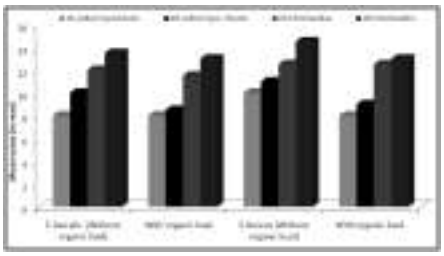

Fig: 1.2: Bar graph representing the arithmetical means of the diameters (mm) of the bacterial inhibition zones for Sodium Hypochlorite and Chlorhexidine solutions at different concentrations on E. faecalis and Staph. aureus.

Chlorhexidine gluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At low chlorhexidine concentrations, small molecular weight substances will leak out, specifically potassium and phosphorous, resulting in a bacteriostatic effect. At higher concentrations, chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking[18].

Present study evaluated the antimicrobial activity of sodium hypochlorite and chlorhexidine gluconate irrigant solutions against different bacterial strains using agar diffusion test. Sterile plasma was added as an organic load to simulate the organic content within the root canal and to verify the effectiveness of the antimicrobial activity[19]. Organic materials have been shown to interfere with the antimicrobial activity of canal irrigating solutions[20], and no previous study has analyzed the effectiveness of irrigating solutions in the presence of an organic load.

In the present study, we used strains that best represented endodontic infections and that were good models to be tested for antimicrobial sensitivity. Two facultative anaerobic bacteria strains were tested (E. faecalis and S. aureus) because these are present in all phases of the development of an infection in root canals[21],[22]. Although sodium hypochlorite is the most clinically used irrigating solution, there is no agreement about its optimum concentration[5].

Various studies tested many irrigating solutions in both in vitro and in vivo, and concluded that an ideal solution is that which combines both maximum antimicrobial effects with minimum toxicity[15].

Chlorhexidine solution presents a wide spectrum antimicrobial action, substantivity and is relatively nontoxic. However, it does not dissolve organic material.

The present study showed all concentrations of CHX and NaOCl without organic load produced zones of antimicrobial activity against the tested strains. However, when higher concentrations of the solution were used, larger inhibition halos were formed. Whereas samples containing organic load exhibited inhibition zones although the diameters were smaller than in the

absence of organic load. Similar results have been reported in previous investigations[4],[23],[24]. Using the same test, Yesilsoy et al. demonstrated the antimicrobial activity of 0.12% CHX against a number of bacterial strains, including P. gingivalis. Siqueira Jr et al. reported that 0.2% and 2% CHX was antimicrobial against P. gingivalis and P. endodontalis.

Organic load interfered with the antimicrobial activity of 1%, 2% CHX and 3% NaOCl against the tested strains resulting in smaller inhibition halos. In the presence of organic load, 2% CHX had the most reliable antimicrobial result for all strains tested. A factor that might explain this finding is the possible formation of a high molecular weight substance, as a result of a reaction between the NaOCl and organic load that could hamper the diffusion of the irrigating solution through the media resulting in low or absence of inhibition zones[9]. By contrast CHX has good diffusibility through the agar gel[4],[8],[24].

Conclusion

Within the limitations of this in vitro study, it may be concluded that Chlorhexidine should be used in conjunction with sodium hypochlorite as it does not have tissue dissolving capabilities. However Chlorhexidine should be the last irrigant to be used as it has better antimicrobial efficacy as well as substantivity

Also presence of organic load does not have a major impact on its antimicrobial efficacy as compared to sodium hypochlorite.

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Source of Support : Nill, Conflict of Interest : None declared