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Evaluation of antimicrobial efficacy of Omeprazole with Sodium hypochlorite against E. faecalis – An invitro study

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ABSTRACT

Introduction: The purpose of this in vitro study was to evaluate the antimicrobial efficacy of Omeprazole with Sodium hypochlorite against E. faecalis.

Methods: 30 extracted mandibular premolars were taken and their root canals were prepared till standardized No. 30 K file and the teeth were given to the microbiology laboratory for the formation of E. faecalis biofilms. The teeth were then randomly divided into 3 groups of 10 each. The teeth in the first group (control group) were irrigated with saline, teeth in the second group were irrigated with 3% Sodium Hypochlorite and the teeth in the third group were irrigated with combination of 8.5% Omeprazole and 3% Sodium Hypochlorite.

Results: Combination of 8.5% Omeprazole and 3% Sodium Hypochlorite was the most effective against E. faecalis followed by 3% Sodium Hypochlorite alone. The difference in the antimicrobial efficacy of the two groups was statistically significant.

Conclusion: Proton pump inhibitors increase the efficacy of Sodium Hypochlorite against E. faecalis.

Introduction

For many years, Sodium hypochlorite (NaOCl) have been used as a root canal irrigant due to its advantages like good antibacterial and tissue dissolving property.[1, 2]However, despite chemomechanical disinfection of the root canal, some microrganisms still persists and lead to root canal failures. Enterococcus faecalis is often found in a high percentage of root canal failures.[3,4]The frequency of *E. faecalis* association in primary endodontic infections ranges from 4 to 40%[5] and 24–77% [6]in persistent infections. Evans et al reported that E. faecalis is resistant to calcium hydroxide because of its proton pump.[7] E. faecalis has the ability to bind to dentin, invade dentinal tubules, survive starvation and also has the property of genetic polymorphisms.[8] E. faecalis has a proton pump in their plasma membrane which help in maintain the homeostasis of the cytoplasm and survive the high alkaline pH of the most commonly used root canal medicament, Calcium Hydroxide.[9]

Proton pump inhibitors are the drug of choice for gastric/duodenal ulcers and gastroesophageal reflux diseases as they cause an irreversible inhibition of the H+/K+ ATPase in the parietal cells of the stomach leading to reduced gastric acid secretion.[10] They also possess antioxidant properties by directly
acting on the neutrophils, monocytes, endothelial, and epithelial cells.[11]

Claudia Wagner et al in a recent study reported that Omeprazole with $Ca(OH)_2$ showed superior repair of rat periapical lesions and better efficacy against the endodontic microbiota when compared with the conventional Ca(OH)2 dressing.[12]Therefore, in the present study, 8.5% Omeprazole, the Proton Pump Inhibitor in combination with 3% Sodium Hypochlorite (NaOCL) was used to compare its antimicrobial efficacy against E. faecalis with 3% Sodium Hypochlorite alone.

Materials and methods

Thirty extracted human permanent single rooted lower premolars extracted for orthodontic/ periodontal purpose were taken. Teeth were cleaned using ultrasonic scalers to render them free from calculus following which they were stored in physiologic saline until use.

The teeth were decoronated at the cement-enamel junction using diamond disc to obtain a standardized root length of 14mm. Pulp were extripated and working length established 1mm short of the apex. Root canals of all the teeth were prepared with k-file upto the ISO size # 30. During preparation of the canals, saline was the only irrigant used. The roots were sterilized in an autoclave for 15 minutes at 121°C and 15 lb pressure. Efficacy of sterilization was tested by taking samples from the root canal with the use of paper points. These paper points were placed into a test tube of 1ml reduced transport fluid, vortexed for 10 seconds, then it was placed onto Brain Heart Infusion (BHI) agar incubated at 37°C for 48 hours and it was examined for the growth. No growth on the BHI indicated complete sterilization of the root samples.

Formation of E. faecalis biofilms

A suspension of E. faecalis was adjusted to 0.5 turbidity on Mcfarland scale (1.5×10^8bacteria/ ml). E. faecalis suspension of 10 microlitre were injected into the teeth using a micropipette, in a class 2 vertica laminar flow cabinet to prevent any airborne contamination. The inoculated specimens with E. faeclais were placed in vials filled with BHI agar, and then inoculum was added every day and inoculated airobically at 37° C for 3 days. After 3 days of inoculation, aliquots were taken from each root using a syringe and plated on BHI agar to verify the growth of E. faecalis. At the end of three days, teeth were removed from the vials and excess fluid was removed with paper points.

The teeth were randomly divided into 3 groups of 10 each, each depending on the experimental irrigant used.

Tests solution in the respective groups were kept in the root canal space for 30 seconds.

Group 1: 3ml of Saline (control group)
Group 2 : 3ml of 3% Sodium Hypochlorite
Group 3 : 3ml of 3% Sodium Hypochlorite + 8.5% Omeprazole (in equal amounts) (Omeprazole 8.5%, Dr Reddy`s labs private limited – Hyderabad)

Irrigation was accompanied using double side vented needle placed 1-2 mm short of the working length. A small amount of saline solution was introduced into the canal and an endodontic hand file as used in a filing motion to a level 1mm short of root apex. Sterile 30 gauge needle was used to collect 0.01 ml f sample from the canal. Using a bacterial loop, the bacterial suspension was placed on BHI agar. The plates were incubated at 37° C for 24 hours and then number of colony forming units (CFU's) were counted.

Statistical Analysis

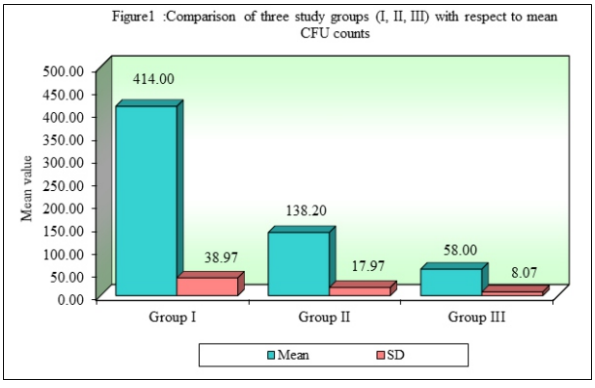
The values collected were analyzed using Kolmogorov-Sirnov Test, one way ANOVA and Newman-Keuls multiple posthoc procedures.

Results

Groups	Group I	Group II	Group III
Mean	414.00	138.20	58.00
SD	38.97	17.97	8.07
Group I	-		
Group II	P=0.0001*	-	
Group III	P=0.0001*	P=0.0001*	-

*p<0.01

Table 1: Pair wise comparison of three study groups (I, II, III) with respect to CFU counts by Newman-Keuls multiple posthoc procedures



The irrigant in Group 3 (8.5% Omeprazole +3% Sodium Hypochlorite) was the most effective against E. faecalis as the number of colony forming units were the least in Group3 following irrigation. Group 2, 3% Sodium Hypochlorite alone also reduced the CFU as compared to the control group, however, the antibacterial efficacy of 3% Sodium Hypochlorite was further enhanced when it was combined with 8.5% Omeprazole (Group 3). The difference between each group was statistically significant.

Discussion

In the present study, it was decided to use 8.5% Omeprazole, the Proton Pump Inhibitor in combination with 3% Sodium Hypochlorite (NaOCL) and to compare its antimicrobial efficacy against E. faecalis with 3% Sodium Hypochlorite alone. The result of the present study showed that the combination of 3% Sodium Hypochlorite and 8.5% Omeprazole showed the maximum antibacterial efficacy against E. faecalis (Mean 414.00 and SD 38.97) followed by 3% Sodium Hypochlorite alone (Mean 138.20, SD 17.97). Therefore, in the present study, Omprazole increased the antibacterial efficacy of Sodium Hypochlorite against E.faecalis.

Sodium Hypochlorite has been most commonly used root canal irrigant used in Endodontics and several studies have shown that it is lethal to E. faecalis, yet it has been recovered very frequently from the failed root canal cases.[13-15]

E. faecalis is frequently associated with persistent endodontic infections as it possesses extraordinary ability to withstand adverse conditions and survive within the dentinal tubules as it secretes serine protease, gelatinase, and collagen binding protein which all help in dentin binding.[16] It can also withstand prolonged periods of starvation and can utilize serum as a nutritional source which also helps it in dentin binding. Another reason attributed to its survival in root canal treated teeth is its ability to resist intracanal dressing of calcium hydroxide for over10 days.[16]

Proton pump inhibitors have been commonly used along with antibiotics for the treatment of peptic ulcers of microbial origin (Helicobacter pylori). [17]Omeprazole was the first PPI (Proton pump inhibitor)to be developed; it is a highly lipophilic weak base, which can easily cross the cell membrane.[18]PPIs do not exhibit antimicrobial activity when used alone, but is reported to have a direct effect on the proton pump of certain bacterial species.[17] PPIs not only reduce acid secretion but also increase the sensitivity to antimicrobials, maintaining the alkaline pH.[19,20]

According to Booth[21], to survive in an acidic or alkaline condition, most bacterial cells maintain a pH homeostasis by maintaining the internal pH within a narrow range, this helps the bacterial cell to maintain the normal function of its enzymes and proteins. The pH homeostasis consists of two basic mechanisms, passive mechanism and an active mechanism. As a passive mechanism, the bacterial cells maintain a low cell membrane permeability to ions and a buffering ability of the cytoplasm. Active mechanism consists of controlled transport of cations (potassium, sodium and protons) across the cell membrane.[22-24] In acidic environments, a cation antiport system raises the internal pH by expelling protons across the cell wall. In an alkaline medium, cations/protons are pumped into the cell to lower the internal pH.[25,26] Kinoshita et al[27] determined the role of the proton pump in maintaining survival of E. faecalis in a high pH environment, in this study CCCP was used to shut down the pump, and there was a 20- fold reduction in cell survival after 30 min exposure to high pH compared to cells that were not exposed to CCCP. These results show that a functioning proton pump, which drives protons into the cell to acidify the cytoplasm, is critical for the survival of E. faecalis in a highly alkaline environment. However, when the environmental alkalinity reaches pH 11.5 or above, this life-saving mechanism of the bacterial cells is overwhelmed.

Results of the present study are in accordance with the study done by Claudia Wagner et al[28]. The improved results of Group III(8.5% Omeprazole and 3% Sodium Hypochlorite) may be due to presence of Omeprazole, the Proton Pump Inhibitor which is known to block proton movement across cell membrane1 and hence homeostasis of bacteria is not maintained and further due to the presence of NaOCL (pH 11.0) which has antibacterial and oxidative properties destroys the cytoplasam and inhibits dehydrogenases in microorganisms[24], might have played a key role in further reduction of the bacterial count.

Conclusion

Within the limitations of the study, it was concluded that Omeprazole, the proton pump inhibitor increased the antibacterial efficacy of Sodium Hypochlorite against E. faecalis.

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