Research Article

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Does Proton Pump Inhibitor Omeprazole! Enhance the Antibacterial Efficacy of Calcium Hydroxide-Based Root Canal Sealer Against *Enterococcus Faecalis*?

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ABSTRACT

Background: The proton pump present in the cell membrane of *Enterococcus Faecalis*, enables it to survive in adverse conditions. Thus, the use of proton pump inhibitor (PPI) along with a calcium hydroxide Ca $(OH)_2$-based endodontic sealer (Apexit plus) might help eradicate *E. faecalis* after obturation of the root canals. The aim of the present *in vitro* study was to evaluate the antimicrobial efficacy of PPI omeprazole in conjunction with the Apexit plus sealer against *E. faecalis*.

Materials and Methods: The antibacterial efficacy of 29 mg Apexit plus (group I), 2mg Omeprazole (group II), 4mg Omeprazole (group III), 29mg Apexit plus with 2mg Omeprazole (group IV), and 29mg Apexit plus with 4mg Omeprazole (group V) was evaluated against E. faecalis using the direct contact test. Sterile saline was used as a mixing medium and also served as the negative control (group VI). The number of colonies forming units were counted on agar plates, the results were statistically analysed.

Results: The results of this study showed that the antibacterial efficacy was significantly improved following the addition of omeprazole to Apexit plus ($P \leq 0.05$).

Conclusion: The combination of omeprazole and Apexit plus appears to give promising results in controlling post endodontic infections.

Keywords: Antimicrobial efficacy, Apexit plus sealer, *Enterococcus faecalis*, Omeprazole, proton pump inhibitor.

INTRODUCTION

The persistence of microorganisms in the root canal is believed to be one of the main causes for endodontic treatment failure (1-3). Enterococcus faecalis is one of the most common among these microorganisms, as it endures starvation in cleaned and obturated canals in spite of the various intracanal disinfecting measures used during treatment (4). Because of the high resistance of *E. faecalis* to several medicaments including calcium hydroxide (5), it is difficult to eradicate them from the root canal by conventional means (6).

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E. faecalis has a functioning proton pump within its cell membrane, which acidifies the cytoplasm when exposed to high pH levels, so that enzymes and proteins can continue to perform their normal functions, thereby maintaining the pH homeostasis (7, 8). Thus, the ability of *E. faecalis* to survive in adverse conditions, can be attributed to this proton pump.

Omeprazole is a proton pump inhibitor (PPI), used for the treatment of gastrointestinal disorders. Several in vitro studies have demonstrated that omeprazole by exerts some antimicrobial activity against Helicobacter pylori (9). It aids in the reduction of gastric acid production by the parietal cells of the gastric membrane through via specific inhibition of the gastric H+/K+ ATPase system (proton pump) (10,11). Wagner, et al. (12)(2011) used an intracanal medicament consisting of omeprazole and calcium hydroxide which showed better repair of periapical lesions in rats, when compared with the conventional calcium hydroxide dressing: moreover, this combination displayed different selective activities over the endodontic microbiota.

Apexit plus is a calcium hydroxide based sealer. The two major advantages of calcium hydroxide based sealers are that they stimulate the periapical tissues to promote healing and they also exert an antimicrobial effect. However, study by Zhang et al (13) demonstrated poor antimicrobial activity of Apexit against *E. faecalis*. Based on these reports, the present study hypothesizes that the combination of omeprazole and Apexit plus may exhibit a synergistic antibacterial effect against *E. faecalis*. Hence, this study aimed at evaluating the antimicrobial efficacy of Apexit plus with and without omeprazole against *E. faecalis* using a direct contact test.

MATERIALS AND METHODS

This study was performed at the Department of Microbiology, SVS institute of medical sciences Telangana, India. The microorganism selected for this in vitro study was *E. faecalis* (ATCC 29212). A culture of the experimental strain was allowed to grow in brain heart infusion (BHI) broth for 24 hours at 37°C. The density was adjusted to 0.5 McFarland turbidity standard scale (1.5 × 10 8 CFU/ml).

Omeprazole (KRS Pharmaceutical Pvt Ltd, India) and Apexit Plus sealer (Ivoclar Vivadent) were used in this study. The two agents were prepared and divided into six groups after weighting and mixed with 1 ml of sterile saline in their respected eppendorf tubes. Apexit plus sealer was mixed as per manufacturer's instructions.

Group preparation

Group I - 29mg Apexit plus
Group II - 2mg omeprazole
Group III - 4mg omeprazole
Group IV – 29mg Apexit plus with 2mg Omeprazole
Group V - 29mg Apexit plus with 4mg Omeprazole
Group VI – Sterile Saline (control group)

Subsequently, 1ml of bacterial suspension was carefully added to each of the six eppendorf tubes, which were then incubated at 37°C for 24 hours. After incubation, each group was streaked on to separate blood agar plates and incubated at 37^0C for 24hours. The colony forming units (CFU) were enumerated using a digital colony counter. This procedure was repeated 12 times.

Statistical Analysis

Statistical analysis was carried out using SPSS software, version 10.0. Comparison between the groups was done by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. The value of significance was set as $P \leq 0.05$.

RESULTS

Figure 1 shows the mean values of CFU's for the test groups against *E. faecalis*. One way ANOVA showed significant differences ($p \leq 0.05$) among the six groups (Table 1). The combination of Apexit plus with omeprazole (4mg/ml) in Group V was found to be more effective against *E. faecalis* followed by that of Apexit plus with Omeprazole (2mg/ml) in Group IV. The effectiveness of Apexit plus alone, against *E. faecalis* was less when compared with the combination groups but higher than that of omeprazole alone. The negative control saline demonstrated the least effectiveness among the six groups in the study. Multiple comparisons performed by the Bonferroni's Multiple Comparison Test revealed a statistically significant difference between Apexit plus and Apexit plus with omeprazole (4 mg/ ml and 2mg/ml) ($p \leq 0.05$).

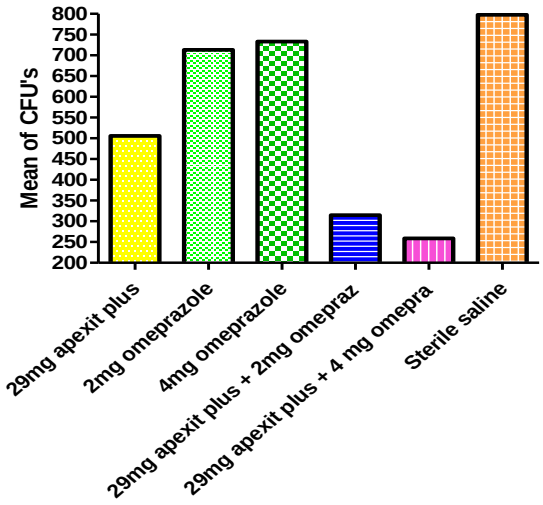

Fig 1. CFU values obtained for the different groups used in this study.

Table 1: Mean values obtained from different groups against *E. faecalis.*

Parameters	N	Minimum	Maximum	Mean	SD	P-Value
29mg apexit plus	12	470	540	505.5	23.2	0.000
2mg omeprazole	12	680	760	713.3	26.8	
4mg omeprazole	12	700	770	733.3	23.3	
29mg apexit plus + 2mg omepraz	12	280	352	314.6	21.8	
29mg apexit plus + 4 mg omepra	12	229	288	258.9	18.0	
Sterile saline	12	730	850	797.9	32.6	

DISCUSSION

Bacteria that adhere superficially to the root canal walls are more likely to be eradicated by Chemo-mechanical debridement. However, those which infect the dentinal tubules and are located in the undebrided areas of the root canal may cause recurrent infection (14). Successful root canal therapy is not just the removal of the microbial entity, but also involves the prevention of future re-infections using biocompatible sealing agents (15).

In the present study we investigated the antimicrobial efficacy of Apexit plus sealer with or without Omeprazole against *E. faecalis*. *E. faecalis* was used in this study as it is known to be the most common etiological agent in post treatment infections. *E. faecalis* establishes a persistent infection because of its ability to invade and adhere to the dentinal tubules with a depth of penetration of 500-1000 µm, and to survive harsh environmental conditions (16). In addition, it is resistant to the high alkalinity and antimicrobial activity of $Ca(OH)_2$ to the presence of the functional proton pump in the cell membrane, which maintains cell homeostasis (17).

Two concentrations of Omeprazole (2mg & 4mg) were used in this study: sterile saline was used as negative control. Results showed that group V (29mg Apexit+4mg Omeprazole) gave better results compared to group IV (29mg Apexit + 2mg omeprazole). This was followed by group III (4mg omeprazole), group II (2mg omeprazole), group I (29mg Apexit) and lastly Group VI (saline).

Apexit plus was used in this study as it is a calcium hydroxide based endodontic sealer, with poor antibacterial activity against *E. faecalis* (13). Apexit plus releases hydroxide ions OH^- and alters the integrity of the cytoplasmic membrane of the bacterial cells, thereby increasing the pH levels (above 12.5) and creating unfavorable conditions for microbial growth (18). However, *E. faecalis* is resistant to unfavorable alkaline conditions owing to the presence of a proton pump inhibitor. This study hypothesized that the combination of omeprazole and Apexit plus would enhance the antimicrobial efficacy of the endodontic sealer by inhibiting the *E. Faecalis* proton pump and maintaining an alkaline pH. An increase in the concentration of omeprazole was found to increase the effectiveness of Apexit plus against *E. faecalis*. An in vivo study, using the combination of omeprazole with $Ca(OH)_2$ as an intracanal medicament demonstrated increased antimicrobial efficacy against E. faecalis and superior healing of periapical lesions with increase in reparative bone formation in male Wister rats when compared with the conventional $Ca(OH)_2$ agent (25).

According to Evans et al (19) bacterial cells in an alkaline medium maintain pH homeostatsis by pumping protons into the cell to lower the internal pH. Omeprazole easily crosses the cell membrane as it is a weak base that has acceptable stability under

alkaline conditions, and rapidly degrade in acidic medium to shut down the pump (20). Concentration of 2 and 4 mg were used in the present study, in accordance with a previous report by Malathi et al (21). Interestingly, the 4mg omeprazole appeared to be more effective against E. faecalis than the 2mg concentration.

It has been reported that, a viscous vehicle retards the release of OH^- ions, whereas an aqueous vehicle accelerate its release (22, 23). Sterile saline was used as vehicle in the present study for preparation of the stock solution. It was also used as a negative control, demonstrating the least effectiveness against *E. faecalis.*

CONCLUSION

Within the limitations of the study, we conclude that omeprazole did enhance the in vitro antibacterial activity of Apexit plus sealer against *E. faecalis*. The findings of the present study show that the combination Apexit plus sealer and omeprazole may have promising effects in endodontic clinical settings. However, further studies are required to determine the minimal inhibitory concentration, the chemical interaction between the two agents, and its antibacterial efficacy against *E. faecalis* in vivo.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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