Original Article

Evaluation of Antimicrobial Efficacy of Calcium Hydroxide along with Proton Pump Inhibitor against Enterococcus Faecalis

Namitha Divakar¹, Sunil Paramel Mohan², Manoj Kumar Pulyodan¹, Arun Tom², Deepthi Karukayil², Maya Somasundaram²

¹Department of Conservative Dentistry and Endodontics, Sree Anjaneya Institute of Dental Sciences, Calicut, Kerala, India, ²Department of Oral Pathology and Microbiology, Sree Anjaneya Institute of Dental Sciences, Calicut, Kerala, India

Aim: The aim of this study was to evaluate in vitro the efficacy of pantoprazole, a proton pump inhibitor, alone, and in combination with calcium hydroxide against Enterococcus faecalis, a star survivor in endodontic pathology. Materials and Methods: Teeth were inoculated with E. faecalis strains MTCC 439 and were divided into groups. Group 1 used saline as the negative control, Group 2 used calcium hydroxide, Group 3 used pantoprazole alone, and Group 4 used calcium hydroxide along with pantoprazole. They were incubated and dentine harvesting was performed. Colonies were counted using a digital counting machine. Data were statistically analyzed. Results: Group 4 was found to be the most effective against the pathogen. Discussion: This study indicates that the association of calcium hydroxide with the proton pump inhibitor pantoprazole can be successfully used as an intracanal medicament against E. faecalis.

KEYWORDS: Calcium hydroxide, Enterococcus faecalis, proton pump inhibitors

Received : 03-02-2020.
Revised : 05-02-2020.
Accepted : 02-03-2020.
Published : 28-08-2020.

INTRODUCTION

Enterococcus faecalis is a gram-positive facultative anaerobe. It is part of polymicrobial flora of untreated or retreated root canal infections. Seen as the primary cause in persistent infections and failed root canal treatments, it survives harsh environments such as extreme pH and temperature of 60°C. It resists bile salts, detergents, heavy metals, ethanol, azide, and desiccation, and endures long periods of nutritional starvation. The prevalence of the star survivor in primary endodontic infection is 40% and in persistent endodontic infection 24%–77%. It invades and colonizes inside dentinal tubules. It alters the host response and suppresses the action of lymphocytes. It possesses lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid, and uses serum as the nutritional source. It resists intracanal medicaments, that is, calcium hydroxide (CH) by maintaining pH hemostasis. It can colonize root canal and survive without the support of other bacteria and competes with other cells. It forms a biofilm that renders it more resistant to phagocytosis, antibodies, and antimicrobial agents. There are bacteria and fungi that use a proton pump in their plasma membrane used primarily for energy metabolism and constant cytoplasmic pH maintenance. This property allows E. faecalis to maintain the homeostasis of the cytoplasm and to survive the high alkalinity of approximately 11.5 of CH. Moreover, the buffering effect of dentin neutralizes the high pH of CH.

Cleaning and shaping procedures and irrigation are not able to adequately eliminate endodontic microbiota because of less contact time. Intracanal medicaments have been used extensively in root canal disinfection due to the virtue of longer contact time with the endodontic pathogens. Ever since its introduction into endodontics by Hermann in 1920, CH has been considered the gold standard of intracanal

Address for correspondence: Dr. Namitha Divakar, Department of Conservative Dentistry and Endodontics, Sree Anjaneya Institute of Dental Sciences, Nandanam, Kunnunmel, Paroppady Kannadikkal Road, Vengeri (PO), Calicut 673010, Kerala, India. E-mail: namitha.divakar@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms. For reprints contact: reprints@medknow.com

How to cite this article: Divakar N, Mohan SP, Pulyodan MK, Tom A, Karukayil D, Somasundaram M. Evaluation of antimicrobial efficacy of calcium hydroxide along with proton pump inhibitor against Enterococcus faecalis. J Pharm Bioall Sci 2020;12:S352-4.
medicaments. However, *E. faecalis* resists CH dressing even up to 10 days due to an inherent proton pump.[2]

**Aim**

The aim of this study was to assess the efficacy of pantoprazole alone and in combination with CH against *E. faecalis*, *in vitro*.

**Materials and Methods**

Forty single-rooted human mandibular incisors stored in 0.1% thymol solution were selected for the study. Decoronation of the teeth below the cementoenamel junction at 15 mm from apex was performed using a rotary diamond point. Cementum was removed and root canals were accessed with a size 10K file and enlarged up to a 30K file. The specimens were immersed in an ultrasonic bath of 17% ethylene diamine tetra acetic acid for 5 min, then in 3% NaOCl for 5 min to remove all debris. Autoclaving of the specimens were performed for two cycles, one at 121°C and the other with specimens immersed in 1 mL of tryptone soya (TS) broth in microcentrifuge tubes. Ten teeth were randomly selected to form the negative control. The test organism used in this study was *E. Faecalis* MTCC 493. *Enterococcus faecalis* was grown on TS agar for 24 h. The culture was immersed in 5 mL of TS broth and incubated at 37°C for 4 h. The remaining 30 teeth were placed in microcentrifuge tubes containing 1 mL of the TS broth. 50 μL of the test inoculums containing the microorganism were transferred into each of the microcentrifuge tubes. After 24 h, the specimens were transferred into fresh broth containing *E. faecalis* in a laminar flow chamber. Subculturing of 5 μL of the broth was carried out for purity checking from the incubated dentine specimens in TS broth on TS agar plates. Contamination of the specimens was carried out for 21 days. The specimens were irrigated with 10 mL of sterile saline at the end of 21 days to remove the incubation broth.[3] They were assigned into the remaining groups.

Group I: Saline—negative control

Group II: CH

Group III: Pantoprazole (0.4 U/μg)

Group IV: Pantoprazole with CH (1:1).

The medicaments were placed inside the canals and sealed at both ends with paraffin wax and incubated at 37°C aerobically for 7 days. Dentine harvesting was performed at two depths, the first at 200 and the last at 400 μm, with Gates-Glidden drill no 4. The dentine shavings thus collected were transferred into 1 mL of sterile TS broth and in an aerobic environment incubated, for 24 h at 37°C. After 24 h, the contents were serially diluted, 100 μL of the broth in 100 μL of sterile saline five times. 50 μL of the dilution were plated on TS agar plates and reincubated for 24 h. Colony counting was performed using a digital (LAPIZ Colony Counter Medica Instrument MFG. CO, Mumbai, India) unit and readings tabulated.

**Results**

Statistical analysis was performed using Kruskal–Wallis test and *post hoc* test. A statistically significant difference was observed between the groups. The intracanal medicament in Group 4 (pantoprazole with CH) was found to be the most effective against *E. faecalis* as the number of colony-forming units (CFU) was the least in Group 4. In Group 3, pantoprazole alone also reduced the CFU as compared to the control group; however, the antibacterial efficacy of CH was further enhanced when it was combined with pantoprazole. The difference between each group was statistically significant (*P* < 0.05).

**Discussion**

Pantoprazole is a new generation proton pump inhibitor (PPI) used extensively in medicine for gastric disorders. Shin and Sachs[4] state that pantoprazole 40 mg was found to be more effective than omeprazole 20 mg and, in addition, shows a more rapid onset of action. The addition of pantoprazole to CH showed synergism. This synergistic effect may be due to the irreversible inhibition of proton pump in the cell membrane of the *E. Faecalis*. Evans et al.[1] showed that survival of *E. faecalis* in CH is not related to stress-induced protein synthesis but due to a proton pump that pumps protons into the cell for cytoplasm acidification. The present data are in accordance with their study, which proves that the combination of a PPI, pantoprazole with CH when used in root canal disinfection, showed increased antimicrobial efficacy against *E. faecalis*. Other properties include anti-inflammatory and reparative effects, which enhance periapical healing.[5]

Our study has shown that pantoprazole alone was not able to eradicate *E. faecalis* significantly, but when combined with CH, the bacterial count dropped down notably. The direct effect of pantoprazole on the proton pump present inside the cell of bacteria is primarily seen as the reason behind, which increases the internal pH, thus enhancing the effectiveness of CH.[6]

Haapasalo et al.'s[7] *in vitro* model for disinfection of dentinal tubules was modified in this study to assess the efficacy of endodontic intracanal medicaments. Cementum was removed from the specimens so that *E. faecalis* could infect the dentine without hindrance.
Dentinal shavings at 200 and 400 µm were tested, because CH is known to penetrate only up to 200–300 µm. CH pastes release OH\(^{-}\) ions, in a time period of 5 days as shown by Beltes et al.\(^{[8]}\) and so a 7-day period was selected for maximum release.

The buffering action of dentin always proved the key factor in decreased efficacy of CH all throughout the endodontic literature\(^{[9]}\). In our study also CH failed to disinfect tubules when compared to other medicaments. The proficiency of \textit{E. faecalis} to infiltrate dentinal tubules even up to a depth of 1000 µm is accredited to efaA and ace, bacterial adhesion factors\(^{[3]}\).

Although omeprazole was the first PPI to be developed, it has definite disadvantages over pantoprazole. It is a weak base, lipophilic, and easily crosses the cell membrane.\(^{[10]}\) Wagner et al.\(^{[11]}\) used a rat model of periapical lesion and compared it to conventional CH intracanal dressing. They obtained superior healing rates with CH supplemented with omeprazole than conventional CH dressing.\(^{[10]}\) Suresh and Abraham,\(^{[12]}\) using \textit{in vitro} agar diffusion assay showed that addition of pantoprazole to CH did not augment the antimicrobial efficiency of CH in comparison with chlorhexidine. The negative results may be attributed due to the difference in the tested drugs and methodology used.

This study indicates that the association of CH with the PPI pantoprazole improved the effects of the CH. Research needs to be directed to authenticate that the same results will also be reproduced inside polymicrobial root canal system, where direct contact of the medicament with the microflora is not always possible chiefly due to the difference in consistency and the type of base in which it is mixed which allows their easy insertion and complete placement in the root canal system.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of \textit{Enterococcus faecalis} to calcium hydroxide. Int Endod J 2002;35:221-8.
2. Portenier I, Waltimo T, Ørstavik D, Haapasalo M. The susceptibility of starved, stationary phase, and growing cells of \textit{Enterococcus faecalis} to endodontic medicaments. J Endod 2005;31:380-6.
3. Vasudeva A, Sinha DI, Tyagi SP, Singh NN, Garg P, Upadhyay D. Disinfection of dentinal tubules with 2\% chlorhexidine gel, calcium hydroxide and herbal intracanal medicaments against \textit{Enterococcus faecalis}: an \textit{in vitro} study. Singapore Dent J 2017;38:39-44.
4. Shin JM, Sachs G. Pharmacology of proton pump inhibitors. Curr Gastroenterol Rep 2008;10:528-34.
5. Dammann HG, Burkhardt F. Pantoprazole versus omeprazole: influence on meal-stimulated gastric acid secretion. Eur J Gastroenterol Hepatol 1999;11:1277-82.
6. Mehta S, Verma P, Tikkur AP, Chandra A, Bains R, Banerjee G. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. Eur J Dent 2017;11:053-7.
7. Haapasalo HK, Sirén EK, Waltimo TM, Ørstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine: an \textit{in vitro} study. Int Endod J 2000;33:126-31.
8. Beltes PG, Pissiotis E, Koulaouzidou E, Kortsaris AH. \textit{In vitro} release of hydroxyl ions from six types of calcium hydroxide nonsetting pastes. J Endod 1997;23:413-5.
9. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-9.
10. Kedika RR, Souza RF, Spechler SJ. Potential anti-inflammatory effects of proton pump inhibitors: a review and discussion of the clinical implications. Dig Dis Sci 2009;54:2312-7.
11. Wagner C, Barth VC Jr, de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an \textit{in vivo} study. J Endod 2011;37:1253-7.
12. Suresh M, Abraham TA. Pantoprazole: Does it enhance the antibacterial efficacy of calcium hydroxide against Enterococcus faecalis. Int J Pharm Bio Sci 2015;6:734-9.