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PANTOPRAZOLE – DOES IT ENHANCE THE ANTIBACTERIAL EFFICACY OF CALCIUM HYDROXIDE AGAINST *ENTEROCOCCUS FAECALIS?*

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ABSTRACT

This study is aimed to evaluate the *in vitro* efficacy of the association of pantoprazole (proton pump inhibitor) with calcium hydroxide (intracanal medicament) to eliminate the principal endodontic pathogen, *Enterococcus faecalis.* The antibacterial efficacy of three test solutions, 29% Calcium hydroxide (group I), - 29%$Ca(OH)_2$ with Pantoprazole 2mg/ml (group II) and 29%$Ca(OH)_2$ with Pantoprazole 4mg/ml (group III) was evaluated using the agar diffusion assay. 2%chlorhexidine (group IV) and sterile saline (group V) served as the positive and negative control respectively. The minimum inhibitory concentration (MIC) was determined by Broth microdilution assay. Agar diffusion assay showed zones of inhibition for groups I and IV. Chlorhexidine showed the maximum zone of inhibition compared to the other experimental groups. The MIC values - groups I, II and IV were 0.45 %, 0.45 % + 0.03 mg/ml, ≤ 0.01% respectively. The addition of the proton pump inhibitor- pantoprazole with $Ca(OH)_2$ does not enhance the antimicrobial efficacy of $Ca(OH)_2$.

KEY WORDS: Antimicrobial efficacy, Calcium hydroxide, *Enterococcus faecalis*, Pantoprazole.

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INTRODUCTION

Enterococcus faecalis has long been implicated in persistent root canal infection and the most common etiological agent in post treatment infections. *E. faecalis* establishes a persistent infection owing to its ability- to invade and adhere to the dentinal tubules with a depth of penetration ranging from 500-1000 µm, to elaborate an array of virulence factors (lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid), and to survive in harsh environmental conditions due to its potential to transform into the viable but non-cultivable (VBNC) state[1-3].Successful endodontic therapy relies on the absolute debridement and disinfection of the root canal system. Regardless of the complex root canal anatomy and the chemo-mechanical instrumentation, root canal irrigants and intracanal medicaments form a necessary adjunct in root canal treatment. The search for an effective intracanal medicament aims to achieve superior disinfection of the root canal system, long term clinical success of endodontic therapy and to increase the strength and stability of the radicular dentin collagen. Calcium hydroxide is being widely used as an intracanal medicament in root canal therapy as it exhibits excellent bactericidal action, mediates degradation of bacterial lipopolysaccharides, induces healing by hard tissue formation and controls inflammatory root resorption[4-7]. The antibacterial activity of $Ca(OH)_2$ against most endodontic microflora is attributed to its alkaline pH. On contact with aqueous fluids, calcium hydroxide dissociates into Ca^{2+} and OH^-. The OH^- ions promote the bacterial cell death by damaging the cytoplasmic membrane, denaturing the proteins and degrading the DNA. The three major reasons for bacterial survival and growth despite the use of calcium hydroxide dressing include, the capacity of some bacteria to survive in dentinal tubules and ramifications, neutral pH in the canal after a rapid use of all the $Ca(OH)_2$ and the microleakage of the temporary filling[8]. Nevertheless, *E. faecalis* is resistant to the antimicrobial activity of $Ca(OH)_2$ by virtue of its potential to withstand high alkalinity and by utilizing the existing functional proton pump in the cell membrane that maintains cell homeostasis[9].Proton pump inhibitors (PPIs) are routinely used in clinical practice as an adjunct to antimicrobials in the treatment of peptic ulcers of microbial origin. 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[10]. PPIs not only reduce acid secretion but also increase the sensitivity to antimicrobials, maintaining the alkaline pH. Previous studies conducted have shown that the association of omeprazole with calcium hydroxide displayed selective antimicrobial activity against endodontic microbes[11,12]. We hypothesized that the combination of a more potent PPI -pantoprazole with $Ca(OH)_2$ would exhibit a synergistic antibacterial effect against the noted endodontic pathogen, *E. faecalis.* Hence, this study aimed at evaluating the antimicrobial efficacy of calcium hydroxide with and without pantoprazole against *E. faecalis* in comparison with 2% chlorhexidine.

MATERIALS AND METHODS

Standardization of inoculum

E. faecalis ATCC 29212 (American Type Culture Collection) was revived from the lyophilized stock in Muller Hinton Broth (MHB, HiMedia Laboratories Pvt Ltd, India) and subcultured on MacConkey agar (HiMedia Laboratories Pvt Ltd, India). Culture purity was assessed based on the microscopic morphology and colony morphology. Fresh broth culture was prepared in MHB incubated at 37˚C for 4hours. The cell density was adjusted to1.5×10^8 *cfu*/ml using 0.5 standard of McFarland scale.

Preparation of the stock solutions

Group I - 29%$Ca(OH)_2$ (w/v) (ProDent, Rathanagiri, India)
GroupII - 29%$Ca(OH)_2$ (ProDent, Rathanagiri, India) + Pantoprazole(2mg/ml) (ALKEM laboratories, India.)
Group III - 29%$Ca(OH)_2$ (ProDent, Rathanagiri, India) + Pantoprazole(4mg/ml) (ALKEM laboratories, India.)
Group IV- 2% Chlorhexidine (Asep RC, India) (positive control)
Group V - Sterile saline (Nirlife, Nirma Ltd, India) (negative control)

Agar diffusion assay

Agar diffusion assay was performed by Kirby-Bauer method[13]. *E. faecalis* ATCC 29212 was seeded on the surface of Muller Hinton Agar (MHA) plates to form a lawn culture. Different concentrations of the test solutions were added to the respective wells punched out on the agar medium and the plates were incubated at 37˚C for 24 hours.

Minimum Inhibitory Concentration (MIC)

Broth Microdilution assay was performed to determine the MIC of the test solutions (groups I - IV) as per CLSI guidelines, 2013[13]. The analysis was performed by using doubling serial dilutions of the test solutions from wells 1 to 11 of each row of sterile microtitre plates (Zellkulter, Germany). The last well of each row served as the culture control. The assay was performed in triplicate. The lowest concentration of the test solution that almost completely (99%) inhibited the growth of *E. faecalis* was recorded as the MIC. Minimum bactericidal concentration (MBC) was determined by spot inoculating onto Muller Hilton agar (MHA) plates. Plates were incubated at 37˚C overnight and were looked for the absence of growth.

Statistical analysis

Statistical analysis was done using SPSS software, version 10.0. The data were analyzed by Kruskal Wallis Test and Chi-square test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Agar diffusion assay

Table 1 shows the mean and standard deviations of the zones of inhibition (in mm) for the test solutions against *E. faecalis*. Chlorhexidine 2% (group IV) exhibited pronounced antibacterial activity against *E. faecalis*. $Ca(OH)_2$ (group I) expressed antimicrobial activity nevertheless, the activity was lesser compared to the positive control (group IV) (p=0.000). $Ca(OH)_2$ with pantoprazole (2mg/ml) & $Ca(OH)_2$ with pantoprazole (4mg/ml) showed no zone of inhibition similar to that of the negative control (group V- saline). Multiple comparisons done by Post Hoc tests revealed a statistically significant difference between $Ca(OH)_2$ and $Ca(OH)_2$ with pantoprazole (4 mg/ ml and 2mg/ml) (p= 0.000, 0.000).

Table 1
Antibacterial efficacy of the test solutions

Groups	Test solution	Mean diameter (in mm) ± SD of the zone of inhibition		
		40 µl	50µl	60µl
I	29%$Ca(OH)_2$	13.33±0.577	14±1.000	14.67±0.577
II	29%$Ca(OH)_2$+ Pantoprazole(2mg/ml)	6±0.000	6±0.000	6±0.000
III	29%$Ca(OH)_2$+ Pantoprazole(4mg/ml)	6±0.000	6±0.000	6±0.000
IV	2%Chlorhexidine	23.67±0.577	24.67±0.577	29.00±1.00
V	Sterile saline	6±0.000	6±0.000	6±0.000

Minimum Inhibitory Concentration

The MIC of CHX against planktonic cells of *E. faecalis* ATCC 29212 was found to be ≤0.01%. No statistically significant difference was observed between $Ca(OH)_2$ and $Ca(OH)_2$ + pantoprazole (0.45% *Vs* 0.45%+0.03mg/ml, p= 0.564).

DISCUSSION

Our data showed that the following experimental groups (I- calcium hydroxide; IV- chlorhexidine) exhibited antimicrobial action against *E. faecalis*. Previous studies have documented the excellent antimicrobial efficacy of 2%chlorhexidine when used as an intracanal medicament against *E. faecalis.* Also, chlorhexidine is reported to be bacteriostatic at lower concentrations and bactericidal at higher concentrations and shows the property of substantivity[14-16]. In our study, chlorhexidine exhibited pronounced antibacterial activity against *E. faecalis* with a very low MIC of ≤ 0.01%.The antibacterial activity of calcium hydroxide is related to the release and diffusion of OH^- ions and the velocity of its release depends on the vehicle with which it is manipulated. Previous studies have reported that, viscous vehicle retards the release of OH^- ions while, an aqueous vehicle accelerates the same[17,18]. Sterile distilled water was used as vehicle in this study for preparation of the stock solution of $Ca(OH)_2$. The antimicrobial activity of $Ca(OH)_2$ is attributed to release of OH^- ions and hence the increase in pH that induces a series of events *viz.,* alteration of bacterial cell wall; damage to the bacterial cell membrane, DNA; denaturation of proteins, endotoxin & lipopolysaccharide[5,19-21]. However, *E. faecalis* neutralizes the alkaline pH of $Ca(OH)_2$ by its proton pump inhibitory action. The reduced antibacterial efficacy of $Ca(OH)_2$ against *E.faecalis* in this study may be due to the difference in the methodology when compared to other experimental studies[22-24]. A previous *in vivo* study has revealed that, the combination of a PPI, omeprazole with $Ca(OH)_2$ when used as an intracanal medicament exhibited increased antimicrobial efficacy against *E. faecalis* and superior healing of periapical lesions with increase in reparative bone areas in male Wister rats in comparison to the conventional agent $Ca(OH)_2$[11]. Another study has shown that 8.5% Omeprazole + 5.2% NaOCl when used as the final irrigant exhibited a superior bactericidal activity against *E. faecalis* and healing of the periradicular lesions with decrease in the colony forming units (*cfu*) in comparison with other irrigants viz., Chlorhexidine & MTAD[12]. Nevertheless, omeprazole has been reported to cause significant interactions with drugs metabolized by the cytochrome P450 system in the liver (ie, CYP2C19, 3A4, 2D6, 2C9). Differences do exist among the PPIs in their pharmacokinetics, pharmacodynamics and potential for drug interactions[25]. Panataprazole is reported to be a valuable alternative for other PPIs. Pantoprazole in combination with two antimicrobials, metronidazole and clarithromycin/amoxicillin is found to be effective in the treatment of *Helicobacter pylori* infection and is well tolerated with minimal potential for drug interactions[10]. We hypothesized that combination of pantoprazole with $Ca(OH)_2$ would enhance the latter's antibacterial efficacy by inhibiting the bacterial proton pump and maintaining an alkaline pH. Conversely, we did not observe any increment in zone of inhibition for $Ca(OH)_2$ with pantoprazole in both the groups II (2mg/ml) & III (4mg/ml) compared to group I ($Ca(OH)_2$). Also, no significant reduction in the MIC was observed when $Ca(OH)_2$ was combined with pantoprazole. Of note, pantoprazole did not enhance the antibacterial activity of $Ca(OH)_2$, instead reduced the same (p=0.000). This could be attributed to the negative $Ca(OH)_2$ - pantoprazole drug interaction. However, further studies need to be carried out to evaluate the chemical interaction between pantoprazole & $Ca(OH)_2$ and its antibacterial efficacy against *E. faecalis in vivo*.

CONCLUSION

Within the limitations of the study, we conclude that pantoprazole did not enhance the *in vitro* antibacterial activity of $Ca(OH)_2$ against *E. faecalis.*

REFERENCES

1. Love RM, *Enterococcus faecalis*- a mechanism for its role in endodontic failure. Int Endod J, 34(5): 399-405, (2001).
2. Stuart CH, Schwartz SA, BeesonTJ and Owatz CB. *Enterococcus faecalis* – its role in root canal treatment failure and current concepts in retreatment. J Endod, 32(2): 93-98, (2006).
3. Saleh IM, Ruyter IE, Haapasalo M and Orstavik D, Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers *in vitro.* Int Endod J, 37(3):193-198, (2004).
4. Byström A, Claesson R and Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol, 1(5):170-175, (1985).
5. Safavi KE and Nichols FC. Effect of calcium hydroxide on bacterial lipopolysaccharide. J Endod, 19(2): 76-78, (1993).
6. Soares I, Goldberg F, Massone EJ and Soares IM. Periapical tissue response to two calcium hydroxide-containing endodontic sealers. J Endod, 16(4):166-169, (1990).
7. Sahamanta CG, Gabriel AS, Isela FCR, Laura VFG and Hugo MZ. Antimicrobial activity with mixture of calcium hydroxide and propolis, Int J Pharm Bio Sci, 2(4): 203 – 210, (2011).
8. Tang G, Samaranayake LP and Yip HK. Molecular evaluation of residual endodontic microorganisms after instrumentation, irrigation and medication with either calcium hydroxide or Septomixine. Oral Dis, 10(6): 389-397, (2004).
9. Evans M, Davies JK, Sundqvist G and Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J, 35(3):221–228, (2002).
10. Cheer SM, Prakash A, Faulds D and Lamb HM. Pantoprazole: an update of its pharmacological properties and therapeutic use in the management of acid-related disorders, Drugs, 63(1):101-133, (2003).
11. Wagner C, Barth VC Jr, de Oliveira SD and Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an *in vivo* study. J Endod, 37 (9) :1253-1257 (2011).
12. Gandi P, Vasireddi SR, Gurram SR and Darasani K, Evaluation of antibacterial efficacy of omeprazole with sodium hypochlorite as an Endodontic irrigating solution- an *In vivo* study. J Int Oral Health, 5(2):14-20, (2005).
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute, 2013.
14. Kawashima N, Wadachi R, Sudha H, Yeng T and Parashos P. Root canal medicaments. Int Endod J, 59(1): 5-11, (2009).
15. Semenoff TADV, Semenoff-Segundo A, Borges AH, Pedro *FML,* Caporossi LS and Rosa-Júnior A. Antimicrobial activity of 2% chlorhexidine gluconate, 1% sodium hypochlorite and paramonochlorophenol combined with furacin against *S.aureus*, *C.albicans*, *E. faecalis* and *P. aeruginosa.* Rev Odonto Cienc, 25(2): 174-177, (2010).
16. Dametto FR, Ferraz CCR, Gomes BP, Zaia AA, Teixeira FB and de Souza Filho FJ. *In vitro* assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis.* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 99(6):768-772, (2005).
17. Athanassiadis B, Abbott PV and Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Dent J, 52(1 Suppl): S64-82, (2007).
18. Gomes BP, Ferraz CC, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB and Souza-Filho FJ. *In vitro* antimicrobial activity of calcium hydroxide pastes and their vehicles against selected

microorganisms. Braz Dent J, 13(3):155-161,(2002).

19. Sjogren U, Figdor D, Spangberg L and Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intra-canal dressing. Int Endod J, 24(3):119–125, (1991).
20. Silva L, Neson-Filho P, Leonardo MR, Rossi MA and Pansani CA, Effect of calcium hydroxide on bacterial endotoxin *in vivo*. J Endod, 28(2): 94–99, (2002).
21. Mohammadi Z, Shalavi S and Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: a review, Chonnam Med J, 48(3): 133–140, (2012).
22. Madhubala MM, Srinivasan N and Ahamed S. Comparative evaluation of Propolis and Triantibiotic mixture as an intracanal medicament against *Enterococcus faecalis,* J Endod, 37(9): 1287-1289, (2011).
23. Kandaswamy D, Venkateshbabu N, Gogulnath D and Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, *Morinda citrifolia* juice, 2% povidone iodine and calcium hydroxide. Int Endod J, 43(5): 419-423, (2010).
24. Mozayeni MA, Haeri A, Dianat O and Jafari AR. Antimicrobial effects of four intracanal medicaments on *Enterococcus faecalis*: an *In vitro* study. Iran Endod J, 9(3): 195-198, (2014).
25. Thomson AB. Are the orally administered proton pump inhibitors equivalent? A comparison of lansoprazole, omeprazole, pantoprazole and rabeprazole. Curr Gastroenterol Rep, 2(6):482-493, (2000).