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Comparison of the efficacy of calcium hydroxide in association with different proton pump inhibitors against Enterococcus faecalis- An in vitro study

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Abstract

Background & Aims: The viability of E. faecalis in infected root dentine has shown to be not completely affected by Calcium hydroxide. The aim of this study was to verify whether the association of proton pump inhibitors- omeprazole, lansoprazole and rabeprazole could increase the antimicrobial efficacy of calcium hydroxide against E. faecalis.

Methods: The Zone of Inhibition of Calcium hydroxide along with the proton pump inhibitors was measured against E. Faecalis (ATCC 29212) by well diffusion method. E. faecalis (ATCC 29212) was maintained on BHI broth and cultures of E. faecalis were grown overnight at 37°C in BHI broth for 24 h and inoculated on agar plate. The suspension of calcium hydroxide with water (for control) or with different concentrations of PPI's (25,20,15,10,5 ug/ml) was placed into the wells made in the agar plates. The plates were allowed to stand for 30 min and then incubated at 37 ± 1°C for 48 h. After 48 h the results were expressed as diameter of the clearing zone around the well measured in millimeters.

Results: At 15,20 and 25ug/ml concentration, there was significant difference between the effectiveness of calcium hydroxide with proton pump inhibitor and calcium hydroxide alone. Lansoprazole has shown significantly better results as compared to Omeprazole and Rabeprazole at 25,20,15 and 10ug/ml.

Conclusions: Under the conditions of this study, calcium hydroxide in association with Omeprazole, Lansoprazole and Rabeprazole have shown effective results in comparison with calcium hydroxide alone, and show a promising future in the elimination of Enterococcus Faecalis.

Keywords: Lansoprazole, E. Faecalis, Calcium hydroxide, Omeprazole, Rabeprazole.

Introduction: Enterococcus faecalis is a persistent organism that plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is able to survive in the root canal as a single organism and commonly found in a high percentage of root canal failures.[1]

This organism is highly associated with cases that are considered refractory to the endodontic treatment.[2]This is attributed to its ability to survive for many months with limited nutrients in an environment with minimum metabolic conditions.[3]

Ever since its introduction in 1920, calcium hydroxide has been considered the gold standard of intracanal medicaments, as it exhibits excellent bactericidal action, mediates degradation of bacterial lipopolysaccharides and controls inflammatory root resorption. [4,5]

Calcium hydroxide leads to release of hydroxyl ions in an aqueous environment. These hydroxyl ions are highly oxidant free radicals that react with several biomolecules, thus providing the antimicrobial activity of calcium hydroxide.[6]The viability of E. faecalis in infected root dentine has shown to be not affected by Calcium hydroxide.[7] The survival of E. faecalis in calcium hydroxide appears to be related to stress induced protein synthesis and a functioning proton pump, which is critical for survival of E. faecalis at high pH.[8]

The gastric H+/K+-ATPase are inhibited by the proton pump inhibitors (PPI's) via covalent binding to cysteine residues of the proton pump,[9] and this principle can probably be applied for inhibiting the proton pump activity in the cytoplasmic membrane of E. Faecalis, considering its mechanism of resistance to calcium hydroxide.

The aim of this study was to verify whether the association of proton pump inhibitors- omeprazole, lansoprazole, and rabeprazole could increase the antimicrobial efficacy of calcium hydroxide against E. faecalis.

Material and Methods

Preparation of the stock solutions

Each concentration from all the three proton pump inhibitors (PPI) was diluted serially up to 10^{-3} concentrations to get the concentrations in µg/ml. In this way, five concentrations of 25, 20, 15, 10 and 5 µg/ml were made for each PPI. E. Faecalis (ATCC 29212) was maintained on BHI broth and cultures of E. faecalis were grown overnight at 37°C in BHI broth for 24 h. The BHI broth was inoculated with E. faecalis from a freshly grown culture on an agar plate. 50 mg of calcium hydroxide (ProDent Ratnagiri, India) was weighed in tubes and 50 µl of each of the extract was mixed to it. The control received 50 µl of water.

Group I - $Ca(OH)_2$ + distilled water (control)

GroupII - $Ca(OH)_2$ + Omeprazole (Dr Reddy's Lab Ltd , India)

Group III - $Ca(OH)_2$ + Lansoprazole (Cipla Ltd, India)

Group IV - $Ca(OH)_2$ + Rabeprazole (Lupin Ltd, India)

Well diffusion method was used to derive the results. Wells of 7 mm diameter and 4 mm depth were punched in the agar plates. The suspension of calcium hydroxide with different concentrations of PPI's (25,20,15,10,5 ug/ml) was placed into this well. The Calcium hydroxide suspension in distilled water served as control. The plates were allowed to stand for 30 minutes and then incubated at 37 ± 1°C for 48 h. After 48 h the results were expressed as diameter of the clearing zone around the well measured in millimeters using a zone measurement scale (HI Media, India).

Results: At 5ug/ml, there was no significant difference between the effectiveness of calcium hydroxide with proton pump inhibitors and calcium hydroxide alone. However, at 15, 20 and 25ug/ml, there was significant difference between groups.

Table1.

Concentration	Omeprazol (Mean±SD)	Lansoprazole (Mean±SD)	Rabeprazol (Mean±SD)	F value	P value
25 ug/ml	16±0.71	14±0.71	12.8±1.3	14.519	0.001**
20 ug/ml	13.2±1.30	11±1.0	11.2±1.1	5.692	0.018*
15 ug/ml	12±0.71	10±0.71	9.4±0.54	21.385	0.001**
10 ug/ml	10.4±1.14	8.6±0.54	8±0.71	11.143	0.002*
5 ug/ml	7.6±0.54	7.8±0.44	7.4±0.54	.750	0.493

One way ANOVA,*p≤0.05 (significant), **p≤0.001 (highly significant),p>0.05 (insignificant)

Table 2.

Concentration	Combinations	Mean difference	p-value
25 ug/ml	Omeprazole vs Lansoprazole	2.0	0.015*
	Omeprazole vs Rabeprazole	3.2	0.001**
	Lansoprazole vs Rabeprazole	1.2	0.155
20 ug/ml	Omeprazole vs Lansoprazole	2.2	0.025*
	Omeprazole vs Rabeprazole	2.0	0.041*
	Lansoprazole vs Rabeprazole	0.2	0.959
15 ug/ml	Omeprazole vs Lansoprazole	2.0	0.001**
	Omeprazole vs Rabeprazole	2.6	0.001**
	Lansoprazole vs Rabeprazole	0.6	0.352
10 ug/ml	Omeprazole vs Lansoprazole	1.8	0.013*
	Omeprazole vs Rabeprazole	2.4	0.002*
	Lansoprazole vs Rabeprazole	0.6	0.513
5 ug/ml	Omeprazole vs Lansoprazole	0.2	0.816
	Omeprazole vs Rabeprazole	0.2	0.816
	Lansoprazole vs Rabeprazole	0.4	0.462

Post hoc test (Tukey).*p≤0.05 (significant),**p≤0.001 (highly significant),p>0.05 (insignificant)

Discussion

The pH of calcium hydroxide has been determined as 12.5, and most bacterial species found in the root canal environment are eliminated when in direct contact with calcium hydroxide for a short period of time.[6]

Enterococcus faecalis has been isolated in 38% of teeth that had recoverable microorganisms. The recovery of E. faecalis in the root canals of failed cases implies an ability of E. faecalis to survive, suggesting that it is an important factor in endodontic failures.[10]

According to Booth, the pH homeostasis is based on two principal components: a passive function consisting of a low cell membrane permeability to protons and other ions and a buffering ability of the cytoplasm which it derives from its protein content and from the synthesis of glutamate as a counter ion for potassium accumulation; and an active mechanism that functions mainly through controlled transport of cations (potassium, sodium and protons) across the cell membrane.[11,12]

Out of the many factors proposed for the resistance of *E. faecalis* with calcium hydroxide, the most important is the presence of a proton pump, which helps to maintain cytoplasmic pH[8] by pumping protons into the cell to lower the pH. However, at a pH of 11.5 or greater, *E. faecalis* is unable to survive.

In the regulation of cytoplasmic pH in an alkaline environment, extensive work with Enterococcus hirae (formerly Streptococcus faecalis) has confirmed a fundamental role for a potassium/proton antiport system .[13,14]

In a study by Kinoshita et al, the role of the proton pump in maintaining survival of E. faecalis in a high pH environment was determined. CCCP (carbonyl cyanide-m-chlorophenylhydrazone) 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. They concluded that a high concentration of nutrients was required for bacterial growth in the absence of proton motive force.[15]

Ganesh et al observed that at 12 hours there was statistically significant growth inhibition by calcium hydroxide and lansoprazole 6.25 µg/ml. At 24 hours the maximum Inhibition of E. faecalis was demonstrated by both calcium hydroxide and chlorhexidine 2%, and calcium hydroxide and lansoprazole (6.25 µg/ml). Pantoprazole did not enhance the bactericidal effect of calcium hydroxide on E. faecalis compared to lansoprazole, which was statistically significant.[16]

Similarly, Suresh and Abraham observed no effect of pantoprazole associated with calcium hydroxide on E. faecalis[5]. Evans et al used a PPI carbonyl cyanide-m-chlorophenylhydrazone and calcium hydroxide and found

20-fold more reduction in microbial load compared to plain calcium hydroxide.[7]

Wagner et al was the first to eradicate E. faecalis using calcium hydroxide supplemented with omeprazole and compared it with conventional calcium hydroxide intracanal dressing using a rat model of periapical lesion. Calcium hydroxide supplemented with omeprazole showed superior healing rates as compared to conventional calcium hydroxide dressing. [17] The association of omeprazole with calcium hydroxide has shown a superior repair of rat periapical lesions in comparison with the conventional calcium hydroxide dressing and also displayed a different selective activity over the endodontic microbiota.[17] Literature search showed no study comparing the effect of rabeprazole, lansoprazole and omeprazole when combined with Calcium hydroxide. In this study, Lansoprazole has shown significantly better results as compared to Omeprazole and Rabeprazole at 25,20,15 and 10ug/ml. At 5ug/ml, (Table 1) there was no statistical significance between the zone of inhibition of calcium hydroxide alone and calcium hydroxide along with the PPIs. However, as the concentration of the drugs increased from 5ug/ml to 15ug/ml, 20ug/ml and 25ug/ml, the zone of inhibition of the groups II, III and IV became significantly greater in comparison to the group I (calcium hydroxide alone), with the highest zone of inhibition of lansoprazole at 25ug/ml. (Fig. 2-5)

Conclusions

Under the conditions of this study, calcium hydroxide in association with Omeprazole, Lansoprazole and Rabeprazole have shown effective results in comparison with calcium hydroxide alone, and show a promising future in the elimination of Enterococcus Faecalis.

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Figure1: Graphical representation comparing the mean zone of inhibition of all the groups

Figure 2: Zone of inhibition with calcium hydroxide mixed with different concentrations of Lansoprazole.

Figure 3: Zone of inhibition with calcium hydroxide mixed with different concentrations of Rabeprazole.

Figure 4: Zone of inhibition with calcium hydroxide mixed with different concentrations of Omeprazole.

Figure 5: Zone of inhibition with calcium hydroxide mixed with distilled water.

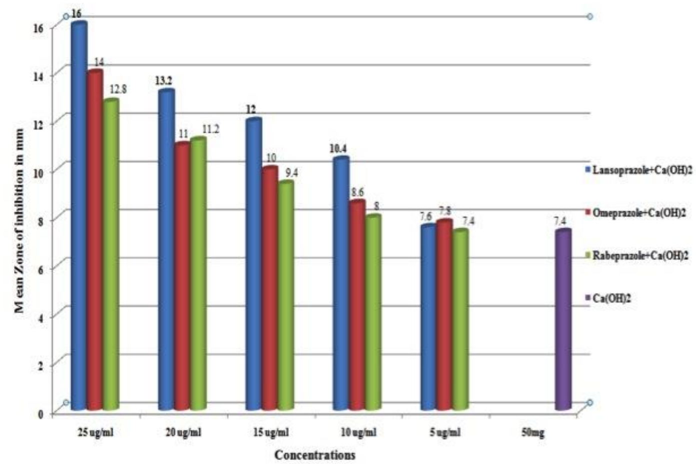

Figure 1

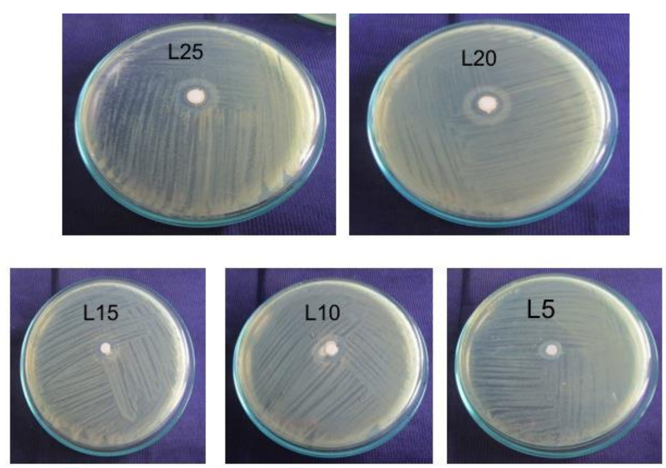

Figure 2

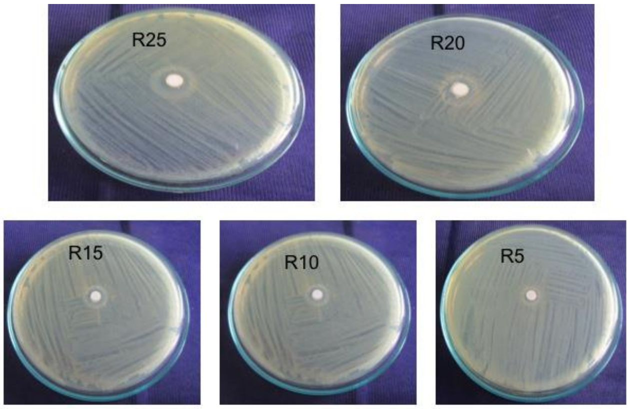

Figure 3

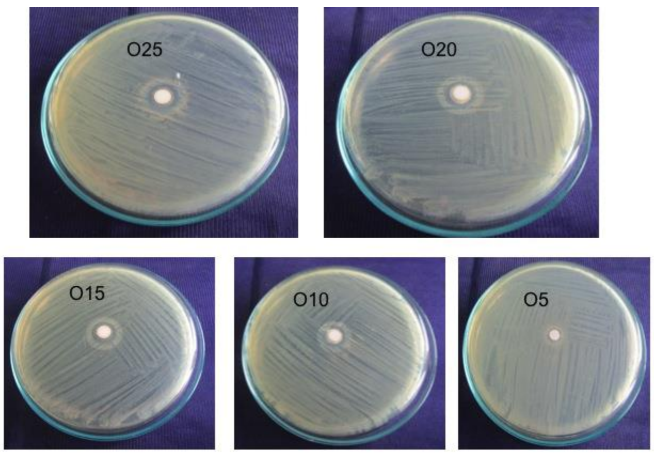

Figure 4

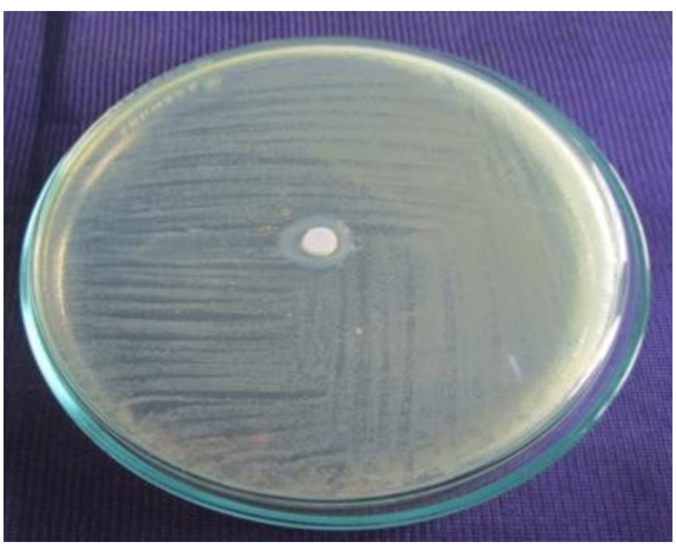

Figure 5