JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 411-416

Fighting the Survivor Endodontopathogen..... *Enterococcus faecalis*

Kavita Dube[1], Jagadish[2] and Vinitha[3]

[1]Department of Endodontics, Hitkarini Dental College & Hospital, Jabalpur, India.
[2]Department of Endodontics, [3]Department of Microbiology,
R.V. Dental College & Gospital, Bangalore, India.

(Received: 20 May 2011; accepted: 05 July 2011)

Root canal (endodontic) treatment is a common dental procedure performed to salvage infected teeth. However, endodontic failures are not uncommon. This study evaluated the efficacy of three intracanal medicaments against Enterococcus faecalis, the most commonly isolated organism from endodontic failure cases. The root canals of human teeth were intentionally infected with isolates of *E. faecalis*, following which the root canals were medicated with: Group A: Calcium hydroxide mixed with saline, Group B: Calcium hydroxide mixed with 2% Chlorhexidine and Group C: Calcium hydroxide mixed with Iodoform (Vitapex), the specimens were incubated at 37^0 C for 7 days. After this, the medicaments were removed from the canal by irrigation with sterile distilled water; dentinal shavings were collected from the canals which were weighed and cultured on Trypticase soy agar. The number of colony forming units per milligram (mg) was calculated and the data subjected to statistical analysis. The Calcium hydroxide – Chlorhexidine group reduced the bacterial count significantly better followed by the Calcium hydroxide – Iodoform group which showed significantly superior results in comparison to Calcium hydroxide – saline group.($p<0.001$).It is concluded that the addition of Chlorhexidine to Calcium hydroxide could present an effective strategy for the treatment of teeth with post- endodontic failures.

Key words: *E. faecalis*, Calcium hydroxide, Chlorhexidine, Iodoform, Endodontic, Retreatment.

Endodontics is a specialized clinical discipline concerned with the prevention, control and treatment of the root canal infection. Since 1890, when Miller first observed microorganisms associated with pulp tissue, microorganisms have been implicated in infections of endodontic origin.

* To whom all correspondence should be addressed.
Mob.: +91-9589015200; Tel.: 0761-4035746
E-mail: kvanjnedubey@gmail.com

Although the majority of the bacteria found in the root canal system may be eliminated by the biomechanical cleaning and shaping of the root canal space, few microorganisms might still survive these challenges due to the anatomical complexities of many root canals, such as dentinal tubules, ramifications, deltas and fins which cannot be sufficiently cleaned, even after meticulous mechanical procedures[1]

The major cause of root canal treatment failure is the persistence of microorganisms in the canal. *Enterococcus faecalis* is a recalcitrant candidate among the causative agents of failed

endodontic treatment[2]. *E. faecalis* is rarely present in primary apical periodontitis, but it is the dominant microorganism in root filled teeth presenting with post treatment apical periodontitis[3].

Calcium hydroxide is the most commonly used intracanal medicament. *E. faecalis* is claimed to be resistant to Calcium hydroxide. This has been attributed to a cell wall associated functioning proton pump which serves to acidify its cytoplasm at high ph, an adaptive response at alkaline Ph and a stress induced protein synthesis[4]. Considering the high prevalence of *Enterococcus faecalis* in cases with failed endodontic treatment, this study was undertaken to establish medicaments with pronounced anti bacterial activity against this organism.

MATERIAL AND METHODS

Forty extracted human maxillary anterior teeth were collected, stored, disinfected and handled as per the recommendation and the guidelines laid down by Occupational Safety and Health Administration (OSHA) and Center for Disease Control (CDC).The apical 5 mm and two thirds of the crown were cut off with a rotating diamond disk in a straight hand piece at slow speed. A # 10 round bur was used to enlarge the root canal of the middle segment to standardize the inner diameter of the canal. The smear layer was removed by irrigating the canals with 10% citric acid. The segments were sterilized by autoclaving at 121°C for 20 minutes. This was repeated three times. The segments were placed in trypticase soy broth containing a culture of *E. faecalis* ATCC29212 (1.5×10^8 Colony forming units / ml) which correspond to 0.5 Mc Farland units and incubated at 37°C for 5 days. The broth was replaced with fresh broth containing cultures of *E. faecalis* (1.5×10^8 Colony forming units / ml) on the third day. A sample was taken on the first, third and fifth day and cultivated on blood agar plates to confirm the purity of E .faecalis in the inoculum. (Fig. 1) After 5 days, the specimens were taken out from the broth and the canals were blotted dry with sterile paper points. The specimens were randomly divided into three experimental groups and one control group. (n = 10 in each group.)

Group A

Calcium hydroxide with saline

Pure Calcium hydroxide powder (Deepthi chemicals) was mixed with sterile saline on a sterile glass slab to form a paste. The paste was loaded in a sterile syringe and the canals were injected with the medicament.

Group B

Calcium hydroxide with iodoform (vitapex-(Neo Dental Chemical Products Company limited. Japan)

The canals of the samples in this group were injected with the medicament (Vitapex).

Group C

Calcium hydroxide with 2% chlorhexidine gluconate solution

Pure Calcium hydroxide powder (Deepthi chemicals) was mixed with 2% Chlorhexidine Gluconate (Loba chemicals) solution on a sterile glass slab to form a paste. The paste was loaded in a sterile syringe and the canals were injected with the medicament.

Group D

Control group

The canals of these specimens were not medicated.

After injecting the medicaments under aseptic conditions, the specimens were transferred separately into sterile glass beakers containing moist sterile gauze (to prevent drying of the medicament).The specimens were incubated at 37° C for 7 days. After seven days, under aseptic conditions each specimen was individually removed using sterile forceps, the root canals were irrigated with sterile saline to remove completely the medicament. The canals were dried with sterile absorbent points. Each canal was prepared manually with a new sterile # 40 Hedstrom file. The dentin removed from the canal was collected on pre weighed sterile aluminum foil. The weight of dentinal shavings was measured in each case and then the shavings transferred to 1 ml of trypticase soy broth in a test tube. The contents were shaken up in the test tube and using a sterile metal loop the sample was streaked on trypticase soy agar plates and incubated at 37°C for 24 hours. The same procedure was repeated for every sample. After 24 hours; the colony forming units were counted. Using the recorded weight of dentin shavings, the number of colony forming units per mg was calculated and the data subjected to statistical analysis.

RESULTS

The Kruskal-Wallis test (Table 1 & 2) and Mann-Whitney test (Table 3) were used for statistical analysis. Though all the experimental groups reduced the bacterial counts, the Calcium hydroxide – Chlorhexidine group (Fig. 2) showed significantly better results followed by the Calcium hydroxide – Iodoform group (Fig. 3) which showed significantly superior results in comparison to Calcium hydroxide – saline group. (Fig. 4)

From the above table it is seen that there is a statistically significant difference in the CFU/mg (x100) of the 4 groups (P<0.001)

It is observed that there is a statistically significant difference between all the groups (P<0.001).

Table 1. Kruskal wallis test(ranks)

Medicaments	N	Mean rank
Control	10	35.5
Calcium hydroxide + saline	10	5.5
Calcium hydroxide +Iodoform	10	15.5
Calcium hydroxide +chlorhexidine	10	25.5
Total	40	

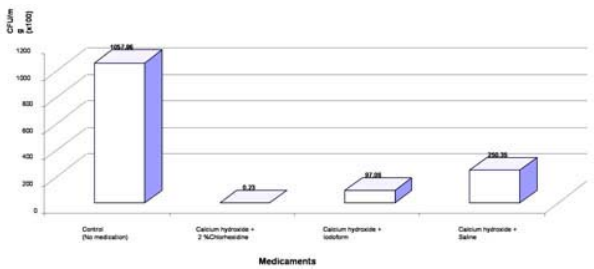
Table 2. Kruskal wallis test (test statistics a,b)

Test statistics	CFU/mg ×100
Chi - square	36.879
df	3
Asymp.sig.	.0000

a-Kruskal Wallis test
b-Grouping variable-medicaments

Table 3. Multiple comparisons using mann-whitney test:

Medicament 1	Medicament 2	P-value
Control	Ca(OH)2 + CHX	<0.001
Control	Vitapex	<0.001
Control	Ca(OH)2 + Saline	<0.001
Ca(OH)2 + CHX	Vitapex	<0.001
Ca(OH)2 + CHX	Ca(OH)2 + Saline	<0.001
Vitapex	Ca(OH)2 + Saline	<0.001



Graph 1. Mean CFU/mg (x100) in different medicaments

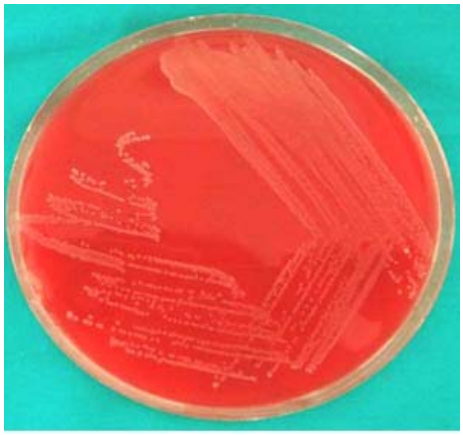


Fig. 1. Culture on blood agar showing purity of the inoculum

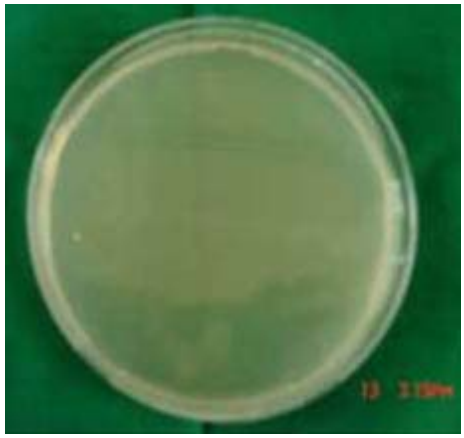


Fig. 2. Culture on trypticase soy agar (Calcium hydroxide - Chlorhexidine group)

Fig. 3. Culture on trypticase soy agar (Calcium hydroxide - Iodoform group)

Fig. 4. Culture on trypticase soy agar (Calcium hydroxide - saline group)

Fig. 5. Culture on trypticase soy agar (Control group)

DISCUSSION

The species *Enterococcus faecalis* has been recovered in a high proportion of Endodontic failures – approximately one third of the canals of root filled teeth with periapical lesions. Enterococci are part of the normal flora in the oral cavity and gastrointestinal tract. In Endodontics, the prevalence of isolating *E. faecalis* in the root canal increases significantly if the canal has been left unsealed between appointments and, in particular, when appointments are many[3]. *Enterococcus faecalis* was chosen for inoculation in the study because it is considerably resistant to intracanal medication with Calcium hydroxide and frequently demonstrated in post endodontic failure cases.

The experimental model used in this study was adapted from that established by Orstavik and Haapasalo[5] for the study of infection and disinfection of tubules. The model was modified by adapting it to extracted human teeth rather than bovine incisors. After intentionally infecting with the organism *E. faecalis*, the specimens were treated with the medicaments for one week. The ability of E faecalis to colonize the root dentin was measured to assess the antimicrobial activity imparted by the medicaments.

Pastes containing Iodoform (Tri iodo methane) have been exhaustively indicated as antiseptics due to Iodine release in nascent state when in contact with secretions or Endodontic infections. The antimicrobial action of Iodoform occurs from releasing Iodine which gives it high reactivity[6]. The antimicrobial action of Iodine is rapid, even at low concentrations, but the exact mode of action is not fully known. It is thought that iodine attacks key groups such as proteins, nucleotides, and fatty acids, resulting in cell death[7]. Vitapex which is a combination of Calcium hydroxide and Iodoform in silicone oil was used in this study.

Chlorhexidine gluconate is a broad spectrum antibacterial agent. It is a cationic bisguanide. Being positively charged, it adsorbs onto microbial cell surfaces and reacts with negatively charged groups causing a reduction of the surface charge. The molecules of Chlorhexidine gluconate can adsorb on to the dentin and prevent microbial colonization on the dentin surface for sometime (substantive antimicrobial activity)[8].It was decided to combine Calcium Hydroxide with 2% Chlorhexidine gluconate solution and use it as an intracanal medicament.

All the medications demonstrated resistance to microbial colonization significantly greater than that of positive controls. Best results were obtained with the use of 2% CHX in conjunction with Calcium hydroxide. This could be attributed to the substantive antimicrobial activity of Chlorhexidine. This is in concurrence with the results of previous studies by Siren et al.[9]Vitapex performed significantly better than the Calcium hydroxide – Saline group as well as the positive control. The use of an oily vehicle promotes low solubility and diffusion of paste within the tissues, thus it remains in the root canal for longer time. This is similar to the results of an in vitro study done by Cwikla et al.[8]

Supplementing the antibacterial efficacy of Calcium hydroxide with Iodoform or 2% Chlorhexidine gluconate improved the efficacy of the intracanal medicament against *E. faecalis*. Chlorhexidine gluconate can be used as a routine root canal irrigant and in combination with Calcium hydroxide as an intracanal medicament. For increased efficacy the concentration of Chlorhexidine can be increased and commercial preparations incorporating Calcium hydroxide and Chlorhexidine gluconate as a paste would be beneficial for the clinician and help in eradication of bacteria resistant to Calcium hydroxide. Its use in retreatment cases could be particularly beneficial.

In this study, the medicaments were tested in vitro with *Enterococcus faecalis* in monoculture. However Endodontic diseases are primarily caused by mixed infections. The medicament that is effective against a single microbe may not necessarily be effective against the complex microbial flora in vivo. Thus the effectiveness of the medicaments needs to be tested against various other Endodontopathogens.

CONCLUSIONS

The efficacy of Calcium hydroxide is improved by addition of specific antibacterial agents .The Calcium hydroxide – Chlorhexidine group reduced the bacterial count significantly

better followed by the Calcium hydroxide – Iodoform group which showed significantly superior results in comparison to Calcium hydroxide – saline group.($p<0.001$).However, in this study, the medicaments were tested in vitro with *Enterococcus faecalis* in monoculture. The effectiveness of the medicaments needs to be tested against various other Endodontopathogens also.

Clinical applicablity

Chlorhexidine gluconate can be used as a routine root canal irrigant and in combination with Calcium hydroxide as an intracanal medicament. For increased efficacy ,the concentration of Chlorhexidine can be increased and commercial preparations incorporating Calcium hydroxide and Chlorhexidine gluconate as a paste would be beneficial for the clinician and help in eradication of bacteria resistant to Calcium hydroxide.

REFERENCES

1. Shafer E, Bossman K. Antibacterial efficacy of Chlorhexidine and two Calcium hydroxide formulations against *Enterococcus faecalis*. *Int Endod J* 2005; **31**(1): 53-56.
2. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment & the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Path* 1998; **85**(1): 86-93.
3. Portenier I, Tuomos M.T, Waltimo, Haapasalo M. *Enterococcus faecalis* – the root canal survivor and 'star' in post treatment disease. *Endodontic Topics* 2003; **6**: 135-159 .
4. Evans, Davies J.K, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide .*Int Endod J* 2002; **35**: 221-228.
5. Haapasalo M, Orstavik D. *In vitro* infection and disinfection or dentinal tubules. *J Dent Res* 1987; **66**: 1375-9.
6. Estrela A, Estrela CRA, Hollanda ACB, Decurcio DA, Pécora JD. Influence of iodoform on antimicrobial potential of calcium hydroxide antimicrobial potential of calcium hydroxide. *J Appl Oral Sci.* 2006; **14**(1): 33-7.
7. Atthanasiadis B, Abott PV, Walsh LJ. The use of calcium hydroxide,antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J* 2007; **52**Suppl 1: 64-82.
8. Stephen J. Cwikla, Myriam Bélanger, Steeve Giguère, Ann Progulske-Fox, Frank J. Vertucci. Dentin tubule disinfection using three calcium hydroxide formulations. *J Endod* 2004; **31**(1): 50-52.
9. Siren EK, Haapasalo MPP, Waltimo TMT, Orstavik D. In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on *Enterococcus faecalis*. *Eur J Oral Sci* 2004; **112**: 326-331.