Evaluation of the Antibacterial efficacy of Omeprazole with Sodium Hypochlorite as an Endodontic Irrigating Solution- An Invivo Study

Padma Gandi¹, Sravanthi Rajah Vasireddi², Sindhura Reddy Gurram³, Kishore Darasani⁴

¹Professor & Head, Department of Conservative Dentistry & Endodontics, SVS Institute of Dental Sciences, Andhra Pradesh, India; ²Reader, Department of Conservative Dentistry & Endodontics, SVS Institute of Dental Sciences, Andhra Pradesh, India; ³Senior Lecturer, Department of Conservative Dentistry & Endodontics, SVS Institute of Dental Sciences, Andhra Pradesh, India; ⁴Post Graduate Student, Department of Conservative Dentistry & Endodontics, SVS Institute of Dental Sciences, Andhra Pradesh, India.

ABSTRACT

Background: Proton pump inhibitors are used in endodontic disinfection of root canals for elimination of enterococcus faecalis. This invivo study on Wistner Rats is carried out to determine antimicrobial efficacy of proton pump inhibitor in combination with sodium hypochlorite & Mixture of Isomer of tetracycline, acid and detergent (MTAD) against E. Faecalis

Materials & Methods: Periapical lesions were induced on the 30 Incisor teeth of 30 male Wistner rats (10 per group). After 28 days, root canals of each tooth were instrumented to 35# k file, during the process of instrumentation canals were irrigated with their respective irrigation solutions. Group-1: 2% CHX + 5.2% NaOCL, Group-2: MTAD(Dentsply Tulsa Dental, Tulsa, OK) + 5.2% NaOCL, Group-3: 8.5% Omeprazole (Dr Reddy’s labs private limited – Hyderabad) + 5.2% NaOCL. Microbiological samples were collected by using #35 sterilized paper points after 28 days of inducing periapical lesions, Sample (S1) was collected before instrumentation and Sample (S2) was collected after instrumentation and Irrigation data were subjected to analysis of variance, followed by Newman Keuls Post Hoc test.

Results: Microbiological Analysis revealed significant decrease of colony forming units from S1 to S2 Samples in all the 3 groups.

Conclusion: – Our data showed that association of Omeprazole with NaOCL showed a superior antibacterial efficacy against Enterococcus faecalis in comparison with other irrigants.

Key words: Sodium Hypochlorite, Chlorhexidine, Gluconate, MTAD, Omeprazole.


Source of Support: Nil
Conflict of Interest: None Declared
Received: 15th December 2012
Reviewed: 8th January 2013
Accepted: 22nd February 2013

Address for Correspondence: Dr. Padma Gandi. Department of Conservative Dentistry & Endodontics, SVS Institute of Dental Sciences, Andhra Pradesh, India. e-mail: padmagandi@gmail.com

Introduction

It is well established that pulp and periapical disease as well as failed root canal therapy (RCT) are due to the presence of microbes in the root canal system¹. Reduction and elimination of successful endodontic therapy. Although instrumentation of the root canal is the primary method of canal debridement, irrigation is a critical adjunct. Nevertheless, because of the
Evaluation of the Antibacterial efficacy…. Gandi P et al

Figure 1: Study Animals

Figure 2: Intra Peritoneal Injection of Xylazine (10mg/kg) combined with Ketamine (100mg/kg)

anatomical complexities of many root canals, organic residues and bacteria located in the dentinal tubules cannot be sufficiently cleaned, even after meticulous mechanical procedures. Therefore, various substances have been used during and immediately after root canal preparation to remove debris and necrotic pulp tissue and to eliminate microorganisms from the root canal². Irrigation serves as a physical flush to remove debris as well as serves as a antimicrobial agent, tissue solvent and lubricant. Root canal irrigation plays a key role in the success of endodontic treatment, because it helps in the progressive removal of the smear layer and neutralizes the root canal microbial flora³. There is no single irrigating solution that alone sufficiently covers all of the functions required from an irrigant. Optimal irrigation is based on the combined use of 2 or several irrigating solutions, in a specific sequence, to predictably obtain the goals of safe and effective irrigation⁴.

Several antimicrobial rinses are used in Endodontics including Sodium Hypochlorite, Chlorhexidine and Biopure MTAD. Various concentrations of sodium Hypochlorite (NaOCl) have been used as root canal irrigants for many decades. The main advantages of NaOCl are its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms⁵,⁶. Chlorhexidine is a cationic bisbiguanide, it is bacteriostatic at lower concentrations and bactericidal at higher concentrations (2% Chlorhexidine) and shows the property of substantivity⁷. Despite its usefulness as a root canal irrigant, it cannot be advocated as the main irrigant in standard endodontic procedures as it lacks the property to dissolve necrotic tissue remnants and fails to remove smear layer, so it can be advocated only as a final irrigant. Biopure MTAD (Dentsply, Tulsa OK) is a mixture of tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween 80). When used as a root canal irrigant, MTAD has been reported to safely remove the smear layer⁸ and effectively eliminate Enterococcus faecalis⁹. Studies have found E. faecalis to be a commonly recovered microbe in failing root canals¹⁰-¹². Enterococcus faecalis is often found in a high percentage of root canal failures. It plays a major role in the etiology of persistent periapical lesions after root canal treatment. Gomes et al¹³ showed that E. faecalis is frequently found in primary necrotic areas. Evans et al¹⁴ showed that it is resistant to calcium hydroxide because of its proton pump. E. faecalis can also survive by genetic polymorphisms and its ability to bind to dentin, invade dentinal tubules, and survive starvation¹⁵.

In a recent study by Claudia Wagner et al¹⁶ showed that association of Omeprazole with Ca(OH)2 favoured a superior repair of rat periapical lesions and seemed to display different
selective activity over endodontic microbiota in comparison with conventional Ca(OH)2 dressing. So we decided to use Omeprazole the Proton Pump Inhibitor in combination with 5.2% Sodium Hypochlorite (NaOCL) and to compare its antimicrobial efficacy with other combination of irrigants such as MTAD with 5.2% NAOCL and 2% Chlorhexidine Gluconate with 5.2% NAOCL.

Rationale for Checking E. faecalis

Studies indicate that the prevalence of E. faecalis is low in primary endodontic infections and high in persistent infections. E. faecalis is also more commonly associated with asymptomatic cases than with symptomatic ones. Although E. faecalis possesses several virulence factors, its ability to cause periradicular disease seems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth. Our challenge as endodontic specialists is to implement methods to effectively eliminate this microorganism during and after root canal treatment. There are currently 23 Enterococci species and these are divided into five groups based on their interaction with mannitol, sorbose, and arginine. E. faecalis belongs to the same group as E. faecium, E. casseliflavus, E. mundtii, and E. gallinarum. These five species form acid in mannitol broth and hydrolyze arginine, however they fail to form acid in sorbose broth. After establishing that the gram-positive coccus is a member of one of the five groups in the Enterococcus genus, several conventional tests are used to identify the specific species. E. faecalis can normally be identified by further testing with arabinose, tellurite, and pyruvate. E. faecalis is arabinose negative and except for some atypical variants, is the only member of the group to utilize pyruvate and to tolerate tellurite.

Materials and Methods

30 Incisor teeth of 30 male Wistner rats (180-200g body weight, 10 animals per group) were used (figure 1). Experimental protocols were approved by local Animal Ethic committee and animals were maintained following guide to management and use of experimental animals. On day 1 each Rat was anesthesized by an intra peritoneal injection of xylazine (10mg/kg) combined with ketamine (100mg/kg) (figure 2). Pulp exposure was performed on incisor tooth by using ½ no. round bur to depth of bur diameter (figure 3) followed by instrumentation of canal with #10 no. k file (Dentsply mailfield, SA) endodontic instrument (figure 4), under irrigation with saline solution. Exposed pulps were left open to oral environment to allow formation of periapical lesions for 28 days.

Irrigating Solutions Used:

5.2% Sodium Hypochlorite (NAOCL, Vishal dentocare, pvt limited, India), 2% Chlorhexidine Gluconate CHX, Dentachlor, Ammdent, India), MTAD(Dentsply Tulsa Dental, Tulsa,OK) and 8.5% OMEPRAZOLE (Dr Reddy’s labs private limited,Hyderabad) all irrigation solutions were irrigated using 30 gauze side vented needle & Syringe( Nirlife, Nirma limited, India)
**Figure 4:** Initial instrumentation with 10# k file

**Figure 5:** Irrigation with combined Omeprazole and 5.2 % NaOCL

**Group-1:** 2% CHX + 5.2% NAOCL (Irrigated separately for 30 sec each)

**Group-2:** MTAD(Dentsply Tulsa Dental, Tulsa, OK) + 5.2% NAOCL (Irrigated separately for 30 sec each)

**Group-3:** 8.5% OMEPRAZOLE (omeprazole 8.5%, Dr Reddy’s labs private limited – Hyderabad) + 5.2% NAOCL (Both are combined and irrigated for 30 sec)

**Procedure for Root Canal Preparation:**
On day 28 Rats were divided into 3 Groups reanesthetized and root canals were instrumented to 35# k file Dentsply maillefer, SA) during the process of instrumentation canals were irrigated with their respective irrigation solutions (figure 5).

**Microbiological Analysis:**
Microbiological samples were collected by using #35 sterilized paper points 28 days after inducing periapical lesions,

S1: Sample (S1) was collected before Instrumentation and Irrigation

S2: Sample (S2) was collected after Instrumentation and Irrigation

In all cases absorbent paper points were aseptically transferred to 1.5ml microcentrifuge tubes (aliquots) containing brain heart infusion broth. Then solution is agitated by vortexing to suspend attached bacteria into solution. To estimate the number of colony forming units per ml, serial decimal dilutions (upto 10^-3) were prepared. Aliquots of 100µl of each dilution and original suspension were spread onto surface of blood agar and incubated aerobically at 370 c for 24hrs.

**Statistical Analysis:**
Result were analysed as mean ± standard error of mean of 10 animals in each experimental group. Data were subjected to analysis of variance, followed by Newman Keuls Post Hoc test.

**Results**
Results of this investigation showed that Group 3 had the maximum zones of inhibition against the E.faecalis. Comparatively Group 2 showed maximum zones of inhibition than Group 1(Table 1).

**Microbiological Analysis** After placement of respective intra canal medicaments in all the 3 groups in

Group 1 - There was a mean reduction of 51% in colony forming units of E.faecalis

Group 2 - There was a mean reduction of 79% in colony forming units of E.faecalis.

Group 3 - There was a mean reduction of 82% in colony forming units of E.faecalis.
Discussion
In present study we decided to use Omeprazole the Proton Pump Inhibitor in combination with 5.2% Sodium Hypochlorite (NaOCL) and to compare its antimicrobial efficacy with other combination of irrigants such as MTAD with 5.2% NAOCL and 2% Chlorhexidine Gluconate with 5.2% NAOCL in rat model of periapical lesions. Usefulness of rat models to emulate human endodontic lesions has been well established. Several in vitro studies have shown Sodium Hypochlorite solution lethal to E. faecalis, yet when E. faecalis-infected dentine is exposed to sodium hypochlorite solution, some cells of E. faecalis may survive\textsuperscript{17-19}. The recovery of E. faecalis in the root canals of failed cases after endodontic retreatment \textsuperscript{20} also implies an ability of E. faecalis to survive.

The results of this invivo study showed that the combination of 8.5% Omeprazole and 5.2% NaOCL (Group-III) is more effective against E.faecalis than combinations of MTAD and 5.2% NAOCL (Group-II) and 2% Chlorhexidine (CHX) and 5.2% NaOCL (Group-I). In this study CHX Group did not perform any better than MTAD and Omeprazole Groups but it is important to note that this study did not address the property of substantivity of the medicament.

Shabahang and Torabinejad\textsuperscript{21} showed the efficacy of BioPure MTAD against E.faecalis. Within the parameters of this study, Group-II that is combination of MTAD and 5.2% NaOCL had an observable effectiveness against E.faecalis similar to Group-III when compared to Group-I. Its effectiveness is attributed to its anticollagenase activity, low pH, and ability to be released gradually over time\textsuperscript{22}. The effects of MTAD are enhanced when sodium hypochlorite is used as an irrigant during instrumentation. Use of NaOCl as an initial rinse followed by the use of MTAD as a final rinse result in a 30% reduction in the antimicrobial substantivity of MTAD\textsuperscript{23}. So in the present study MTAD is used as an initial rinse followed by the use of 5.2% NaOCl as final rinse. According to Booth\textsuperscript{2}, in acid or alkaline environments, most bacterial cells maintain pH homeostasis in which the internal pH is kept within a narrow range so that enzymes and proteins maintain normal function. The pH homeostasis is based on two principal components: a passive function consisting of a low cell membrane permeability to ions and a buffering ability of the cytoplasm; and an active mechanism that functions mainly through controlled transport of cations (potassium, sodium and protons) across the cell membrane\textsuperscript{24-26}. In acidic environments, a cation antiport system is thought to raise the internal pH by expelling protons across the cell wall. In an alkaline medium, cations/protons are pumped into the cell to lower the internal pH. Extensive work with Enterococcus hirae (formerly Streptococcus faecalis) has confirmed a fundamental role for a potassium/proton antiport system in maintaining cytoplasmic pH in an alkaline environment\textsuperscript{27-28}. Kinoshita et al\textsuperscript{29} determined the role of the proton pump in maintaining survival of E. faecalis in a high pH environment, in this study CCCP 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. These results show that a functioning proton pump, which drives protons into the cell to acidify the cytoplasm, is critical for the survival of E. faecalis in a highly alkaline environment. Presumably, when the environmental alkalinity reaches pH 11.5 or above, this life-saving mechanism is overwhelmed.

Results of this study support the findings of Claudia Wagner et al\textsuperscript{16}. The improved results of Group III may be due to presence of Omeprazole the Proton Pump Inhibitor which is known to block proton movement across cell membrane\textsuperscript{15}.
and hence homeostasis of bacteria is not maintained and further due to the presence of NaOCL (pH 11.0) which has antibacterial and oxidative properties destroys the cytoplasm and inhibits dehydrogenases in microorganisms, might have played a key role in further reduction of the bacterial count.

**Conclusion**

Our data showed that association of Omeprazole with NaOCL showed a superior antibacterial efficacy against E. faecalis in comparison with irrigants. In the changing face of dental care, continued research on E. faecalis and its elimination from the dental apparatus may well define the future of the endodontic specialty.

**References:**


