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Original Research

Evaluation of efficacy of combinations of five endodontic sealers with five antibiotics against *Enterococcus Faecalis* – An in-vitro study

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Abstract:

Background: To evaluate and compare in vitro the antibacterial efficacy of five antibiotics when added individually to five endodontic sealers against *Enterococcus faecalis* (*EF*).

Materials & Methods: This controlled trial with systematic allocation method was carried out to detect the combined antibacterial activity of five endodontic sealers (Kerr sealer EWT, Endomethasone, AH26, AH Plus, Roekoseal) with five antibiotics regularly used (Amoxicillin, metronidazole, azithromyacin, gatifloxacin, doxycycline) on EF. For each sealer-antibiotic combinations, thirty BHI agar plates (15 aerobic and 15 anaerobic) were inoculated with EF, containing five sterile paper discs- three of various sealer- antibiotic combinations, one of sealer alone (positive control) & plain disc as negative control were incubated at 37°C for 48 hrs and the zone of inhibition was measured. Data analysis was done by ANOVA and Tukey's post- hoc test using SPSS(version 17).

Results: The findings of this study revealed that sealer-antibiotic combination containing amoxicillin had the significant difference (p<0.001) in the mean zone of inhibition compared to other combinations. Metronidazole showed the minimum zone of inhibition among used antibiotics. The sealers in the decreasing order according to their effectiveness on EF were Kerr sealer endomethasone, AH26, Rockseal, AH plus.

Conclusion: Addition of antibiotics to endodontic sealers enhances their antibacterial activity against Enterococcus faecalis.

Key Words: Antibiotics, endodontic sealer- antibiotic, endodontic sealer, enterococcous faecalis

Introduction

The main purpose of endodontic therapy is to clean and shape the root canal with the help of instruments and chemical irrigants, in order to eliminate or reduce the amount of microorganisms, when the pulp is necrotised.¹ The overall success rate of endodontics can be improved by sealers that exhibit both excellent sealing ability as well as antimicrobial properties. This enables the sealer to cope better with persisting residual infection and preventing the bacteria from reentering, from the oral cavity.² Endodontic sealers are used to prevent periapical exudates from diffusing into unfilled part of root canal & to prevent residual bacteria from reaching periapical tissues, via elimination of gaps between core filling material and canal walls.^{3,4} The zinc oxide eugenol based sealers exhibit their antimicrobial effects due to the eugenol content.⁵ Iodoform incorporated zinc oxide eugenol sealers exhibit enhanced antimicrobial effect due to the presence of iodoform a potent bactericidal agent and eugenol. The resin based sealers exhibit minimal antimicrobial effects mainly due to the absence of formaldehyde in most of the newer preparations.^{2,6}

It has been found in various studies that major factors associated with endodontic failure are the persistence of microbial infection in root canal system and/or periradicular area.^{7,8} Often microorganism may have survived biomechanical procedures⁹⁻¹¹ or invaded canal via coronal leakage of root filling.¹² Studies have revealed that root filled teeth with persisting periapical lesions usually harbour various bacterial species & Gram positive bacteria dominate the flora^{9,13}

Despite being an organism which accounts to a small proportion of the flora in untreated canals Enterococcus faecalis is a persistent organism plays a major role in etiology of persistent periradicular lesions after root canal treatment. It is also found in, 24-27% percentage of root canal failure cases surviving in the root canal as a single organism or as a major component of the oral flora.¹³⁻¹⁵ Enterococcus faecalis found usually in the necrotic pulp is a facultative gram positive cocci that occurs singly, in pairs or as short chains possessing the ability to grow in the presence or in the absence of oxygen found usually in necrotic pulp.¹⁶ It is difficult to eliminate Enterococcus faecalis through root canal medication once it has established itself in the dentinal tubules. Therefore, it might be advantageous if the sealer exerts some antimicrobial activity as the last element in the treatment regimen.^{15,17} Enterococcus faecalis is extremely resistant to current treatment modalities in endodontics. Following systemic administration of an antibiotic, concentration reaching root canal is negligible & unlikely to inhibit bacterial growth. The main advantage of local antibiotics compared to systemic use is that systemic consequences & complications are avoided & higher concentrations of the same reaches the target areas. ^{18, 19} Thus the purpose of this study was to evaluate in vitro antimicrobial effects of five antibiotics when added individually to five endodontic sealers against Enterococcus faecalis. The aim of the "to evaluate and present study is compare microbiologically the antibacterial activity of five endodontic sealers Kerr sealer, Endomethasone, AH26, AH plus, Roekoseal by addition of five antibiotics such as amoxicillin, metronidazole, azithromyacin, gatifloxacin, doxycycline against Enterococcus faecalis".

Materials and Methods

Enterococcus faecalis (ATCC 29212) was taken in single use disposable vials from the American Type Culture Collection (Manassas, VA). The bacterium was grown and maintained on Brain Heart Infusion (BHI) agar or broth (Difco, Sparks, MD, USA) from which new stock culture plates (Figure 1a) were prepared periodically & stored in the refrigerator.

A culture of EF was grown overnight at 37°C in BHI broth. Bacterial growth was checked by changes in turbidity at 24 hours (Figure 1b). Brain heart infusion agar plates were inoculated with EF by spreading the culture over the surface of the plate to develop a lawn of EF and the excess was discarded. Eugenol containing sealers - Kerr sealer (Sybron Endo), Endomethasone (Septodont) and resinbased sealers - AH Plus (Dentsply), AH 26 (Dentsply), RoekoSeal (Roeko, Langenau) were chosen, as they are widely used in dentistry and various studies have been undertaken to find out their effectiveness against EF.^{1-5, 20-28} Five antibiotics - Amoxicillin (Ranbaxy, India), Azithromyacin (Protec, Cipla, India), Metronidazole (J.B Chemicals, India), Gatifloxacin (Dr. Reddy labs, India) Doxycycline(Pristine Health Care, India), were chosen on the basis of their effectiveness against EF as supported by various articles in dental literature.^{4,10,18,27,29} Sealer samples were prepared by adding 10% of antibiotic to powder/ paste of the sealers weight and were mixed according to the manufacturer's instructions. Sterile paper discs were saturated with the sealer- antibiotic combinations by exposing the paper discs to the sealer mixture until uniform coverage were visually achieved. The saturated paper discs were assigned to the BHI agar plates consisting of five paper discs: three experimental groups, only sealer serving as the positive control and a plain disc serving as a negative



Figure 1: a) Enterococcus faecalis stock culture b) Change in turbidity in BHI broth and c) Zone of inhibition after 48 hr.

control. Antibiotic sensitivity for E. faecalis was performed on BHI agar by disc diffusion method by Kirby – Bauer method. Thirty agar plates were made for each sealerantibiotic combination, 15 were incubated aerobically and 15 anaerobically at 37[°]C for 48 hrs and diameter of zone of inhibition (Figure 1c) was measured in mm. Mean zone of inhibition of all the sealer-antibiotic combinations were measured. Descriptive statistics were analyzed using SPSS (version 17). The observations were tabulated and analyzed using ANOVA and multiple comparisons were made by Tukey post hoc tests.

Results

antibiotics - Amoxicillin Five (Ranbaxy, India). Azithromyacin (Protec, Cipla, India), Metronidazole (J.B Chemicals, India), Gatifloxacin (Dr. Reddy labs, India) Doxycycline(Pristine Health Care, India), were chosen on the basis of their effectiveness against EF as supported by various articles in dental literature.^{4,10,18,27,29} Sealer samples were prepared by adding 10% of antibiotic to powder/ paste of the sealers weight and were mixed according to the manufacturer's instructions. Sterile paper discs were saturated with the sealer- antibiotic combinations by exposing the paper discs to the sealer mixture until uniform coverage were visually achieved. The saturated paper discs were assigned to the BHI agar plates consisting of five paper discs: three experimental groups, only sealer serving as the positive control and a plain disc serving as a negative control. Antibiotic sensitivity for E. faecalis was performed on BHI agar by disc diffusion method by Kirby – Bauer method. Thirty agar plates were made for each sealerantibiotic combination, 15 were incubated aerobically and 15 anaerobically at 37[°]C for 48 hrs and diameter of zone of inhibition (Figure 1c) was measured in mm. Mean zone of inhibition of all the sealer-antibiotic combinations were measured. Descriptive statistics were analyzed using SPSS (version 17). The observations were tabulated and analyzed using ANOVA and multiple comparisons were made by Tukey post hoc tests.

Statistical analysis using ANOVA revealed that the mean zone of inhibition of all the sealers in positive control group was significantly higher in condition than in aerobic condition (p<0.001). However, endodontic sealers showed significant increase (p<0.001) in their antibacterial activity against EF by the addition of antibiotics in both anaerobic & aerobic conditions. Multiple comparisons using Tukey HSD revealed statistically that Kerr sealer with amoxicillin showed the maximum mean zones of inhibition which was found to be significantly higher (p<0.001) as compared to all the other sealers. Endomethasone and AH 26 had significantly higher (p<0.001) mean zones of inhibition as compared to AH Plus and Roekoseal. All the sealer-amoxicillin combination showed maximum zone of inhibition where as all the sealer-metronidazole combination showed the minimum zone of inhibition, which was significant statistically (p<0.001)



Figure 2: Mean values of different Zone of inhibition in anaerobic conditions.

Discussion

Bacteria or their by-products are considered to be the etiologic agents of pulpal necrosis and periapical lesions.²⁸ Multiple factors contribute to the endodontic failures which include intraradicular infection, extraradicular infection, foreign body reaction, and cysts.^{30,31} However, it is believed that most treatment failure occurs due to the survival of microorganisms in the apical portion of the root filled tooth.^{7,13,28} Enterococcus faecalis was chosen as test organism because it is associated with persistent apical inflammation in clinical situations and also it is difficult to eliminate this organism from the root canal system.^{11,13,17,29} Furthermore, EF is able to survive the antimicrobial effect of calcium hydroxide^{17,19} and its survival in calcium hydroxide appears to be the result of a functioning proton pump that drives protons into the cell to acidify the cytoplasm.^{16,28} Its survival in sodium hypochlorite has been attributed to the possibility that the solution is buffered by dentin or variation in some EF strain that may allow them to survive low concentration of sodium hypochlorite.^{17,30}

In this study, sterile paper discs were used by Kirby – Bauer Method, as it is a test in which antibiotics are used by disc diffusion, to test whether particular bacteria are susceptible to specific antibiotics. In this method the antibiotics & antibacterial agents from the sealers comes out through the disc and acts on the culture causing formation of clear zones known as 'Zone of Inhibition'.^{31,32} Chronic periradicular lesions associated with pulp necrosis do not have adequate blood supply or endodontically treated teeth don't have pulp at all. So, the concentration of antibiotics reaching root canal system in systemic administration is negligible and not beneficial.¹³

Holescher et al ⁴ found that the sealer-antibiotic groups exhibited antimicrobial activity peaking around 10% concentration of antibiotic. Increase in antibiotics concentration, upto 50% did not further increase the zone of inhibition. Hence, in the present study 10% antibiotic concentration was utilized. It has been demonstrated that EF was resistant to benzylpenicillin, ampicillin, clindamycin, metronidazole and tetracycline but sensitive to erythromycin and vancomycin. In this study zinc oxide eugenol based sealer was chosen, as it is widely used in dentistry and various studies have been undertaken to know their effectiveness against EF.^{1-5,18-25} Zinc oxide eugenol based sealers (Kerr sealer, Endomethasone) have good antimicrobial activity due to eugenol which is a potent antimicrobial agent. ^{5,21,33}

Resin based sealers (AH26, AH Plus, Roekoseal) has very little antimicrobial property as compared to zinc oxide eugenol containing sealers except AH26. AH26 shows antimicrobial activity due to the release of formaldehyde.^{6,23} This release of formaldehyde is in freshly mixed AH26, which lasts for 48 hrs. Once set, the formaldehyde concentration in $\sim \times 200$ that of the fresh mix and subsequently decreases over the next 7 days.³⁴ AH plus showed the least zones of inhibition, this may be due to the diffusibility of the sealer in media, the interaction of the sealer with media components and the microenvironmental conditions. In study by Razmi et al ²⁶ and Holescher et al⁴ mean diameter of the zone of inhibition of amoxicillin in sealer- antibiotic combination was significantly larger than any other sealer antibiotic combinations and in the current study also sealeramoxicillin showed the greatest zone of inhibition which was significantly higher (p<0.001) as compared to all other sealer- antibiotic combinations. Furthermore, it has been reported that average zone of inhibition of amoxicillin to be 31.6mm.⁴ In the current study the average zone of inhibition of Kerr sealer - amoxicillin was 32.62 mm for anaerobic and 31.52 mm for aerobic. The present study found that certain antibiotics (Amoxicillin, Metronidazole, Azithromycin, Gatifloxacin) when added to Kerr sealer, Endomethasone, AH26, AH Plus, Roekoseal enhanced the antimicrobial activity of the sealer against EF.

Conclusion

All five antibiotic agents when added into endodontic sealers showed significant increase in their antibacterial properties in anaerobic & aerobic conditions. All the sealer-amoxicillin combination showed the maximum zone of inhibition where as all the sealer-metronidazole combination showed minimum zone of inhibition, which was statistically significant. Thus, we can conclude that the antibacterial agents and type of conditions (aerobic/anaerobic) have significant effect on the diameter of zones of inhibition.

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