

Minimal Inhibitory Concentration and Minimal Bactericidal Concentration of Various Intracanal Medicaments against *Enterococcus faecalis*: Microbial Analysis

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ABSTRACT

Aim: The aim of the study was to compare the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of three intracanal medicaments against *Enterococcus faecalis* (*E. faecalis*).

Materials and methods: Three intracanal medicament groups were used in the current study. Each group had five samples. Group I—calcium hydroxide paste, group II—triple antibiotic paste (TAP) (metronidazole, ciprofloxacin, minocycline), group III—double antibiotic paste (DAP) (metronidazole, ciprofloxacin). The nutrient agar was then stored at room temperature for 2 days before use to verify that it had remained sterile. Brain heart infusion broth was also prepared and stored in 5 mL vials for 2 days. The serial dilution was done with concentrations ranging from for each intracanal medicament in the other test tubes. The lowest dilution inhibiting the growth was taken as MIC. A loopful of the broth dilutions was taken and streaked on nutrient agar plates. The growth of bacteria was checked after incubation for 24 hours at 37°C. The lowest dilution which showed no growth of bacteria was taken as the minimum bactericidal concentration.

Results: Growth of *E. faecalis* was found to be inhibited even with the lowest dilution of the TAP itself which was 1.8 ± 0.7 µgm per mL while it was a little higher for calcium hydroxide paste with a dilution of 4.39 ± 1.6 µgm per mL and of 5.01 ± 1.69 µgm per mL for DAP ($p < 0.05$). While the bactericidal effect was seen at 2.19 ± 0.8 µgm per mL for triple antibiotic paste, 4.39 ± 1.69 µgm per mL for CaOH, and 5.63 ± 1.38 µgm per mL for DAP ($p < 0.05$).

Conclusion: It had been found that MIC and MBC were least for TAP compared to other intracanal medicaments. The addition of minocycline in a TAP increased the antimicrobial property.

Clinical significance: For successful root canal treatment choosing effective intracanal medicament is important to debride the microorganism completely even at lower concentration. The present study provides a minimum concentration of some intracanal medicament used in root canal treatment.

Keywords: Calcium hydroxide, *Enterococcus faecalis*, Minimal bactericidal concentration, Minocycline, Minimal inhibitory concentration.

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INTRODUCTION

For a successful endodontic treatment, complete debridement of microorganisms from the root canal is important.¹ In case of complex root canal configuration, successful debridement through mechanical instrumentation alone cannot remove all the bacterial load. The use of chemical intracanal irrigators and medicaments is requisited to remove the infected tissues and eradicate microorganisms from the root canal system.² In endodontics intracanal medicaments have been used for microorganism elimination, rendering canal contents, reducing posttreatment pain, and providing an anesthesia effect. Bacteria that are present in root canals form biofilms, which are more resistant to chemomechanical debridement procedures. In the case of anatomical complexities of the root canal system like lateral and accessory canals, isthmi, and apical deltas complete elimination of microorganisms following biomechanics preparation of the root canal system is not possible. Complete elimination of microorganisms is necessary for apical repair of mature and immature teeth.²

A wide number of microorganisms are present in root canal systems. Among the microorganisms that infect the canal, *Enterococcus faecalis* (*E. faecalis*) is the most common cause of periapical infection and re-infection of root canal-treated teeth.³ In persistent periradicular infections, *E. faecalis* had been isolated in about 24–77% of individuals which resulted in root canal failure

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because *E. faecalis* survives even in very high alkaline atmosphere and deep tubular invasion. It multiplies by adhering to biofilm and colonizes.^{4,5} *E. faecalis* also exhibits resistance to varied intracanal medicaments and has the capability to survive in the absence of other microbes.⁶ Thus for successful root canal treatment complete debridement of *E. faecalis* is more important.

The most commonly used intracanal medicament is calcium hydroxide. The high alkalinity of calcium hydroxide is responsible for antibacterial activity.⁷ Release of hydroxyl ions results in antibacterial activity. The antibacterial activity of calcium hydroxide

is due to lysis of the bacterial cell wall, protein denaturation, damage to bacterial cytoplasm, and damage to the deoxyribonucleic acid. Since there is an increased risk of systemic side effects and inability of systemic antibiotics within the infected root canal and the peri apical tissues, local application of antibiotics may be a more effective mode. Triple antibiotic paste (TAP) (composed of ciprofloxacin, metronidazole, and minocycline) or double antibiotic paste (DAP) (composed of ciprofloxacin and metronidazole) are most commonly used as an intracanal medicament as a substitute for calcium hydroxide.^{8,9}

Recently, during root canal treatment for disinfection of the canal, TAP which contains ciprofloxacin, minocycline, and metronidazole, has been commonly used.¹⁰⁻¹² When the TAP is used as an intracanal medicament in extracted teeth that were damaged and hopeless due to their extensive caries, it eliminates the microorganisms from the root canal system and dentinal tubules.^{13,14} Due to the staining property of minocycline, a DAP has been made which includes a mixture of ciprofloxacin and metronidazole which also acts as a good intracanal medicament.

Since proper irrigation of the root canal is necessary for successful endodontic treatment using medicaments with high antimicrobial properties is very important. Only limited studies have compared MIC and MBC values of various intracanal medicaments against *E. faecalis*. Thus aim of the present study was to compare the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of three intracanal medicaments against *E. faecalis*. A hypothesis was formulated that MIC and MBC would be the same for all the intracanal medicaments against *E. faecalis*.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India. The present study was a single-blinded study conducted at the Department of Microbiology, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India, from April to October 2022. There were three intracanal medicament groups used in the current study. Each group had five samples each. Group I—calcium hydroxide paste (Prime Dental RC Cal), group II— TAP [metronidazole (Flagyl), ciprofloxacin (cipross), minocycline (minolox)], group III—DAP [metronidazole (Flagyl), ciprofloxacin (cipross)].

Nutrient agar was used to prepare five agar plates. Agar was mixed and adequate agar was poured to cover the surface of a 125 mm diameter Petri dish. The nutrient agar dishes were then stored at room temperature for 2 days before use to verify that they had remained sterile. BHI broth was also prepared and stored in 5 mL vials for 2 days.¹⁵

Placement of intracanal medicament was done by one operator. Initially, test tubes with 0.5 mL of BHI broth were taken and 0.5 mL of intracanal medicaments (calcium hydroxide, triple antibiotic paste, double antibiotic paste) were added for all five samples in each group. Then the serial dilution was done with concentrations

ranging from (25 µg, 12.5 mg, 6.25 µg, 3.15 µg, 1.56 µg, 0.78 µg, and 0.39 µg) for each intracanal medicament in the other test tubes. *E. faecalis* (ATCC 11700) bacterial strain was grown in BHI broth for 24 hours at 37°C which was procured from the Department of Microbiology, SRM University. 200 µL of the broth culture of this bacterial suspension which was adjusted to 0.5 McFarland was added to each test tube and this mixture was incubated at 37°C for 24 hours. After 24 hours the tubes were visually checked for turbidity (bacterial growth). The MIC was evaluated by checking turbidity and the minimal bactericidal concentration (MBC) was evaluated using the zone of inhibition method. The turbidity and zone of inhibition were evaluated by a separate operator who was unaware of the three intracanal medicament groups. The minimal dilution inhibiting the growth was taken as MIC.

The minimal dilution which showed no growth of bacteria was taken as MBC. The MIC and the MBC were calculated by the macro-dilution method. The MIC and MBC values were tabulated and statistical analysis was done.

Statistical Analysis

Statistical analysis was done by IBM Statistical Package for the Social Sciences version (SPSS) 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The comparison of MIC and MBC within the group was done with a one-way analysis of variance and multiple groups pairwise comparison was done using the Bonferroni *post hoc* test. The values were considered statistically significant when the *p*-value was <0.05 with a confidence interval (CI) of 95%.

RESULTS

The mean values for minimum inhibitory concentration for calcium hydroxide, triple antibiotic paste, and DAP were 4.39 µg, 1.87 µg, and 5.01 µg, respectively while the standard deviation (SD) was 1.69, 0.71, and 1.69, respectively, which were statistically significant (Table 1). Multiple pairwise comparisons show that the mean difference between CaOH with TAP and DAP were 2.51 and -0.62, respectively, while the mean difference between TAP with CaOH and DAP was -2.51 and -3.13 which were statistically significant. The mean difference between DAP with CaOH and TAP were 6.2 and 3.13 respectively (Table 2).

Table 2: Multiple pairwise comparisons of MIC

(I) Group	(J) Group	Mean difference (I-J)	<i>p</i> -value
CaOH	TAP	2.51200	0.049
	DAP	-0.62000	1.000
TAP	CaOH	-2.51200	0.049
	DAP	-3.13200*	0.015
DAP	CaOH	-0.62000	1.000
	TAP	3.13200*	0.015

Table 1: Mean and SD of MIC of various intracanal medicaments

Group	Mean (µg)	Standard deviation	Standard error	95% CI for mean		F	<i>p</i> -value
				Lower bound	Upper bound		
CaOH	4.3900	1.69794	0.75934	2.2817	6.4983	6.57	0.012
Triple AP	1.8780	0.71107	0.31800	0.9951	2.7609		
Double AP	5.0100	1.69794	0.75934	2.9017	7.1183		

Table 3: Mean and SD of minimal bactericidal concentration (MBC) of various intracanal medicaments

Group	Mean (μg)	Standard deviation	Standard error	95% CI for mean		F	p-value
				Lower bound	Upper bound		
CaOH	4.3900	1.69794	0.75934	2.2817	6.4983	8.15	0.006
TAP	2.1960	0.87088	0.38947	1.1147	3.2773		
DAP	5.6300	1.38636	0.62000	3.9086	7.3514		

Table 4: Multiple pairwise comparisons of MBC

(I) group	(J) group	Mean difference (I-J)	p-value
CaOH	TAP	2.19400	0.077
	DAP	-1.24000	0.527
TAP	CaOH	-2.19400	0.077
	DAP	-3.43400*	0.005
TAP	CaOH	1.24000	0.527
	DAP	3.43400*	0.005

The mean values for minimum bactericidal concentration for calcium hydroxide, triple antibiotic paste, and DAP were 4.39 μg , 2.19 μg , and 5.63 μg , respectively while the SD was 1.69, 0.87, and 1.38, respectively which were statistically significant (Table 3). Multiple pairwise comparisons show that the mean difference between CaOH with TAP and DAP were 2.19 and -1.24 respectively, while the mean difference between TAP with CaOH and DAP was -2.19 and -3.43 which were statistically significant. The mean difference between DAP with CaOH and TAP was 1.24 and 3.43, respectively (Table 4).

Compared to the other two intracanal medicaments TAP showed the lowest MIC (1.87 $\mu\text{g} \pm 0.71$) and MBC (2.19 $\mu\text{g} \pm 0.87$) values against *E. faecalis*. Calcium hydroxide showed lesser MIC (4.39 $\mu\text{g} \pm 1.69$) and MBC (4.39 $\mu\text{g} \pm 1.69$) values compared to double antibiotic paste.

DISCUSSION

Intracanal medicament has been used as an interim appointment dressing for many years. The commonly used commercial synthetic medicaments are calcium hydroxide, antibiotics, etc. However, the majority had been reported for its toxic effect, development of resistant strains, and depletion of the immune response.²

The most common microorganism that is isolated from teeth with failed endodontic treatment is *E. faecalis*. Thus *E. faecalis* was studied because it is a most commonly occurring, opportunistic facultative anaerobic organism that's nonfastidious, easy to culture and it had been used successfully in previous studies. It is often resistant to antibacterial agents.^{16,17} Thus, it has been used as the target microorganism in the current study. Several studies have demonstrated the resistance of the bacteria to calcium hydroxide which is the most common intracanal medicament used in endodontic treatment.¹⁸ This has drawn attention to the use of other medicaments like double antibiotic and triple antibiotic paste. A study shows that silver nanoparticles have potential bactericidal effects against *E. faecalis* at a concentration of 5 mg/mL. Silver nanoparticles contain antibacterial properties which can be added to the intracanal medicaments, sealers, and irrigants.

In a retrospective study, Bose et al. showed that calcium hydroxide and TAP which possess very good antimicrobial properties can aid further development of the pulp dentin complex when used as an intracanal medicament in grossly decayed teeth.¹⁹

Sato et al. advocated that when the TAP is used it can even disinfect the lateral and apical canals efficiently.¹⁴ In another study conducted on animals advocated the effectiveness of TAP in the disinfection of immature teeth with apical periodontitis.^{20,21} Pinheiro et al. advocated that the 21 microbial isolates from intracanal of restored teeth with periapical periodontitis were susceptible to doxycycline and tetracycline, which was similar to the present study that suggested minocycline was the most effective medicament against *E. faecalis*.²²

Minimal inhibitory concentration (MIC) is defined as the minimal concentration of an antimicrobial that will inhibit the growth of a microorganism after incubating overnight and is important in microbial laboratories to confirm the resistance of microorganisms when treated with antimicrobial agents and also to evaluate the activity of a new antimicrobial agent.

The present study showed that the minimum inhibitory concentration and the minimum bactericidal concentration of TAP were less compared to the DAP and calcium hydroxide paste. The TAP has a greater antibacterial effect compared to the other two intracanal medicaments. The minocycline combination was very effective against *E. faecalis*. Although metronidazole and ciprofloxacin showed antibacterial activity their ability to kill *E. faecalis* was less compared to minocycline and the TAP, according to both the agar diffusion and MIC tests. Thus minocycline was the most potent component of the TAP against *E. faecalis*.²¹

The antimicrobial activity of the TAP and its three components against *E. faecalis* were compared to calcium hydroxide and double antibiotic paste. The results of this present study have indicated that calcium hydroxide had some antibacterial efficacy against *E. faecalis*, but only at concentrations of 3.25 $\mu\text{g}/\text{mL}$. The zones of inhibition formed by calcium hydroxide were much smaller than the zones formed by the triple antibiotic group, which concluded that calcium hydroxide is not as effective as an intracanal TAP. It is also proved that calcium hydroxide also has some antibacterial effects compared to double antibiotic paste.²³⁻²⁵

Thus TAP has been proven to be a better intracanal medicament than calcium hydroxide and shows more antibacterial effects in combination with components such as chlorhexidine or any other medical extracts. The limitation of the current study was, only the dilution rate at which the inhibition was noted were recorded for each intracanal medicaments and zone of inhibition was not measured.

Further research should be undertaken to compare with other intracanal medicament also. A substitute for minocycline also should be found to reduce staining properties and the antibacterial effects of TAP in combination with any other herbal extracts can be determined in further studies.

CONCLUSION

It has been concluded that MIC and MBC were the least for TAP compared to other intracanal medicaments. Calcium hydroxide was found to be more effective than double antibiotic paste. The TAP

shows excellent antimicrobial properties against *E. faecalis* and can be considered a more useful root canal medicament compared to calcium hydroxide paste and double antibiotic paste. Even though TAP has a very good antibacterial activity, staining of teeth due to minocycline still been a problem for dental surgeons.

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