Original Article

Antimicrobial Efficacy of Endomethasone and Sealapex Sealers against Specific Endodontic Pathogens

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Abstract:

Background: Antimicrobial efficacy is one of the desired properties of root canal sealer to eliminate the residual microorganisms from complex root canal system unaffected by chemomechanical preparation and intracanal medication. The aim of the study is to compare the antimicrobial effects of two different sealers, i.e. zinc oxide based Endomethasone and calcium hydroxide based Sealapex against *E. faecalis, S. aureus and C. albicans* which are considered most resistant microorganisms in the oral cavity.

Methods: Agar diffusion method was used in this study. The sealers were prepared according to manufacturer's instruction and placed in prepared wells 36 agar plates inoculated with *E. faecalis, S. aureus and C. albicans*. All plates were incubated for 5 days at 37^{0}C. The zone of inhibition of each sealer was measured after 24 hours, 48 hours and 5 days interval in order to evaluate the antimicrobial efficacy in different time duration. Statistical analysis was performed by ANOVA, independent sample 't' test and paired 't' test as applicable. P value <0.05 was considered as statistically significant.

Results: Endomethasone produced largest zone of inhibition at all-time interval than that of Sealapex against all tested microorganisms. However, the zone of inhibition of Endomethasone reduced significantly after 48 hours. The antimicrobial efficacy of Sealapex gradually increased at all times interval.

Conclusion: The antimicrobial efficacy of Endomethasone against *E. faecalis, S. aureus and C. albicans* is higher than that of Sealapex.

Key words: Antibacterial activity, root canal sealer, agar diffusion test, Endomethasone, Sealapex.

Introduction:

Elimination of microorganisms from the root canal system and prevention of subsequent reinfection is the main objective of endodontic treatment.[1] The means for achieving this goal are powerful chemo-mechanical debridement, an intracanal dressing, adequate three-dimensional obturation, and coronal restoration.[2] However, many residual bacteria and fungi have been found in the dentinal tubules, crevices, canals, fins, and the apical ramifications of the root canal[3] even after these procedures. As a consequence, root canal treatment leads to failure. Enterococcus faecalis *(E. faecalis), Staphylococcus aureus (S. aureus) and Candida albicans (C. albicans)* are considered to have the highest resistance in the oral cavity, with the potential to cause failure of root canal treatment.[4,5]

The use of an endodontic sealer with antibacterial properties may help to eliminate residual microorganisms unaffected by chemo-mechanical preparation of root canal system[6] and thus, increases

the chances of a successful endodontic treatment outcome and may be advantageous especially in clinical situations of persistent or recurrent infection. The most commonly used root canal sealers in endodontic treatment are mainly of three types depending on their composition. These are zinc oxide eugenol based, calcium hydroxide based and epoxy resin based root canal sealers.[7] Although many studies have been performed to investigate the antimicrobial efficacy of calcium hydroxide based and zinc oxide based sealer, most of the studies reported evaluated initial microbial inhibition only, but it seems equally important to determine the effect over a longer time interval.

Therefore, the aim of the present study was to evaluate the antimicrobial efficacy Sealapex and Endomethasone at different time intervals in the presence of facultative microorganisms such as *E. faecalis, S. aureus and C. albicans.*

Materials and Methods:

The studied root canal sealers were: calcium hydroxide based sealer- Sealapex (Kerr, Romula, MI, USA) and zinc oxide based sealer-Endomethasone (Septodont, France). The microbial strains used in this study were: *E. faecalis (ATCC 29212), S. aureus (ATCC-25923) and C. albicans* (confirmed by germ tube test). All the microbial strains were collected from the Department of Microbiology and Immunology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Antimicrobial efficacy of Sealapex and Endomethasone was observed against E. faecalis, S. aureus and C. albicans by agar diffusion method. The methodology utilized by Miglani R, et al. (2007) was followed. The sampling units were selected purposively or as convenient. A total number of 36 (12 for each microorganism) petridishes of Mueller Hinton Agar were taken. From 3 to 5 colonies of each microbial strain were picked from subculture plate using sterile swab stick and suspended in 5 ml of normal saline in screw capped test tube to adjust the turbidity to 0.5 McFarland standards. Each microorganism was scattered on the dried surface of Mueller Hinton Agar media in three different planes with a swab. Now two perforations were made in the media with a cork borer of 4 mm in diameter. The sealers were manipulated according to manufacturer's instructions. The prepared holes were then filled with 0.11 gm of sealer. The plates were maintained at room temperature for 1 hour to allow prediffusion of the material and then incubated at 37^{0}C for 5 days. Plates were observed after 24 hours, 48 hours and 5 day intervals at which time the diameter of the zone of inhibition was measured with an endodontic millimeter ruler with accuracy of 0.5 mm. Tests were replicated 12 times.

Data were processed and analyzed using SPSS (statistical package for social science) version 18. The analysis of variance (ANOVA), and independent sample 't' test and paired 't' test were performed to know the effects of each variable and to reveal the statistical significance. P-value <0.05 was considered as significant.

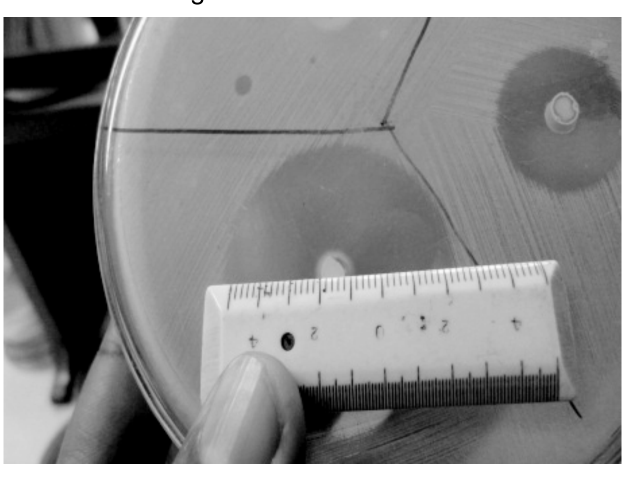

Fig.1: Inhibition zones formed by sealers.

Results:

Table-I: The mean diameter of zones of inhibition produced by Endomethasone and Sealapex against *E. faecalis*, *S. aureus* and *C. albicans*. (n=36)

Micro-organism	Time interval	Zone of Inhibition Endomethasone (mm)	Zone of Inhibition Sealapex (mm)
E.faecalis	24 hours 48 hours 5 days	28.5±1.38 28.91±1.57 27.45±2.08	12.45±1.82 14.62±1.66 15.45±1.35
S.aureus	24 hours 48 hours 5 days	36.33±3.79 34.91±3.39 33.91±3.44	12.12±1.62 12.83±0.91 13.75±1.21
C.albicans	24 hours 48 hours 5 days	27.45±1.61 28.33±1.46 27.62±1.46	11.75±0.99 13.16±1.37 13.50±1.36

Note: Data are expressed as mean ±SD.

The mean diameter of zones of inhibition produced by Endomethasone and Sealapex are presented in table-I.

It was seen that the maximum zone of inhibition produced by Endomethasone was against S. aureus at 24 hours intervals (36.33 ± 3.79 mm), while minimum antimicrobial efficacy was seen for

Sealapex against *C. albicans* (11.75±0.988) at 24 hours interval.

It was revealed that Endomethasone produced largest zone of inhibition against *E. faecalis, S.aureus and C. albicans* which was significantly higher in comparison to that of Sealapex at all-time intervals (p<0.001) (table-II).

Table-II: Mean difference and P-value between the zones of inhibition produced by Endomethasone and Sealapexa gainst *E. faecalis*, *S. aureus* and *C. albicans.* (n= 36)

Micro-organisms	After 24 hrs		After 48 hrs		After 5 days	
	Mean difference	P value	Mean difference	P value	Mean difference	P value
E. faecalis	16.04116	.000***	14.29167	.000***	12.0000	.000***
S. aureus	24.2083	.000***	22.08333	.000***	20.16667	.000***
C. albicans	15.70833	.000***	15.16667	.000***	14.12500	.000***

For statistical analysis one way ANOVA was performed. Independent sample t' test was done for comparison between the groups*** = Significant at p < 0.001, ** = Significant at p < 0.01, * = Significant at p < 0.05, n = total number of subject.

Table-III: Mean difference and P value of zone of inhibition produced by Endomethasone and Sealapex in between different time intervals.

Time interval	Endomethasone		Sealapex	
	Mean difference	P value	Mean difference	P value
24 hours vs. 48 hours	.04167	.842ns	-1.43056	.000***
48 hours vs. 5 days	1.4584	.000***	-.69444	.000***

Data were analyzed by paired 't' test. *** = Significant at p < 0.001, ns = not significant.

Changes in the average antimicrobial efficacy of the sealers against all tested microorganisms at different duration are presented in table-III. The change in the mean antimicrobial efficacy (substantiated by the formation of zone of inhibition) of Endomethasone was not significant between 24 hours and 48 hours but decreased after 48 hours which was very highly significant (p<0.001). The mean antimicrobial efficacy of Sealapex increased at all-time intervals which was very highly significant (p< 0.001).

Discussion:

Several methods such as Agar Diffusion Test and Direct Contact Test have been proposed for the analysis of antimicrobial activity of root canal sealers. ADT is said an effective for measuring the zone of inhibition.[8] The advantage of this method is to creation of direct comparisons of root canal sealers against test microorganisms and the visual indication of which sealer has the potential to eliminate microorganisms in the local microenvironment of the root canal system.[3]

The results of the present study revealed that both sealers, showed antimicrobial activity substantiated by the formation of growth inhibition zones against all strains of microorganisms. However, among the sealers, the antimicrobial efficacy of Endomethasone was higher in comparison to that of Sealapex. It produced the largest inhibitory zones against all microorganisms at all-time intervals, which was highly significant (P<0.001). The results found in the present study corresponded to the results of Gomes BP et al. (2004), Saha et al. (2010)[9], Al Khatib et al. (1990).[10] The findings of the current study did not correspond to the results of Shantiaee et. al (2010).[11] Difference in the microorganism strains used, testing method and type of zinc oxide eugenol based sealer may be the main reasons of these controversies. Shantiaee used pure zinc oxide eugenol sealer against *Streptococcus mutants and Prevotella melaninogenicus*. However, in the present study Endomethasone showed maximum antimicrobial activity at 24 hours intervals which also corresponded to the study of Saha et al. (2010).[9] There were no significant changes in the antimicrobial efficacy of Endomethasone between 24 hours and 48 hours but the efficacy reduced after 48 hours which was highly significant (p<0.001).

Previous study has shown that the antimicrobial activity of Endomethasone was due to their chemical components. Kaplan AE, et al. (1999) stated that the most effective antimicrobial sealers contain eugenol and formaldehyde.[12] A gradual and continuous release of formaldehyde from the paraformaldehyde in the sealer after setting, accounts for its antimicrobial activity.[13] Furthermore, eugenol is also a potent bactericidal agent.[14]

Regarding the antibacterial activity of Sealapex, previous study showed that it was based on its ionic dissociation into calcium (Ca^{2+}) and hydroxyl (OH^-) ions causing an increase in pH (12.5).[15] A pH >9 results loss of biological activity of the cytoplasmic membrane of microbes[16], but the initial antimicrobial efficacy of the sealer was weak because of their low solubility and diffusibility in agar. The results of the

present study revealed that the antimicrobial efficacy of Sealapex increased at all-time intervals and reached to optimal level at 5 days which was highly significant (p< 0.001). Heling I and Chandler NP (1996) also showed that Sealapex's antimicrobial effect increases with time.[17] Disintegration of sealer and an increase in the available amount of hydroxyl ion over time may be the possible explanation. Secretion of calcium ion also reacts with carbon dioxide, reducing the source of respiration for anaerobic bacteria.[18]

According to the study results, it seems that Endomethasone is more appropriate to eliminate facultative microorganisms than Sealapex and may help to eliminate these residual microorganisms unaffected by chemomechanical preparation of root canal system, thus, improving the success rate of endodontic treatment. Furthermore, it may be more advantageous than Sealapex, especially in clinical situations of root canal treatment failure such as persistent or recurrent infection.

Conclusions:

Based on the present study, it can be concluded that zinc oxide eugenol based sealer, Endomethasone is more effective in the formation of inhibition zone towards all the microorganisms at all times intervals when comparing to Sealapex. However, as the culture medium can influence the solubility, ion release and alkalinity of sealers which are essential conditions for antimicrobial effect[19] to be more conclusive, further studies by other suggested methods such as direct contact test or collecting the flora from root canal should be performed to evaluate antimicrobial activity of the sealers.

References:

1. Dalton BC, Orstavik D, Phillips C, et al. Bacterial reduction with nickel–titanium rotary instrumentation. J Endod 1998;24:763-67.
2. Molander A, Reit C, Dahlen G, et al. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7.
3. Gomes BPFA, Pedroso JA, Jacinto RC, et al. In vitro evaluation of the antimicrobial activity of five root canal Sealers. Braz Dent J 2004;15(1):30-35.
4. Peciuliene V, Reynaud AH, Balciuniene I, et al. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001;34:429-34.
5. Gomes BPFA, Ferraz CCR, Vianna ME, et al. In vitro antimicrobial activity of calcium hydroxide pastes and their vehicles against selected microorganisms. Braz Dent J 2002;13:155-61.
6. Kayaoglu G, Erten H, Alacam T, et al. Short-term antibacterial activity of root canal sealers towards Enterococcus faecalis. Int Endod J 2005;38:483-88.
7. Sundqvist G, Figdor D, Persson S, et al. Microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
8. Leonardo MR, Bezerra da Silva LA, Tanomaru Filho M, et al. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod 2000;26(7):391-94.
9. Saha S, Sonali S, Samadi F, et al. Antimicrobial effect of six endodontic sealers: an in vitro evaluation. Endod DentTraumatol 2010;15:42-45.
10. Al-Khatib ZZ, Baum RH, Morse DR, et al. The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Oral Pathol Endod 1990;70:784-90.
11. Shantiaee Y, Dianat O, Janani A, et al. In vitro evaluation of the antibacterial activity of three root canal sealers. Iranian Endod J 2010;5(1):1-5.
12. Kaplan AE, Picca M, Gonzalez MI, et al. Antimicrobial effect of six endodontic sealers: an in vitro evaluation. Endod Dent Traumatol 1999;15(1):42-45.
13. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. J Endod 1983;9:372-74.
14. Hume WR. The pharmacologic and toxicological properties of zinc oxide-eugenol. JADA 1986;113:789-91.
15. Sjogren U, Figdor D, Spangberg L, et al. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24(3):119-25.
16. Estrela C, Sydney GB, Bammann LL, et al. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Brazil Dent J 1995;6:85-90.
17. Heling I, Chandler NP. The antimicrobial effect within dentinal tubules of four root canal sealers. J Endod 1996;22(5):257-59.
18. Kontakiotis G, Nakou M, Georgopoulou M. Invitro studies of the indirect action of calcium hydroxide on the anaerobic flora of root canal. Int Endod J 1995;28:285-89.
19. Siqueira JF, Goncalves RB. Antibacterial activities of root canal sealers against selected anaerobic bacteria. J Endod 1996;22:78-80.