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Assessment of Antimicrobial Efficacy of MTAD, Sodium Hypochlorite, EDTA and Chlorhexidine for Endodontic Applications: An *In vitro* Study

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Abstract: The key aim of endodontic treatment is to clean and debride the root canals and that is achieved biochemical and mechanical measures. Certain factors such as individual variations and complex anatomy and morphology of root canals have made debridement [both by mechanical and chemical means] a challenging task. The objective of this study was to evaluate the antimicrobial activity of recently introduced MTAD *in vitro* and comparing to commonly used irrigators such as NaOCl, chlorhexidine and EDTA under similar conditions and various pH levels. The results of this *in vitro* study were statistically analysed using Kruskal Wallis one-way ANOVA and Mann Whitney U test. It has been concluded that MTAD has the capability to eradicate E. faecalis.

Key words: MTAD · Root canal treatment · EDTA · Irrigators · Enterococcus faecalis

INTRODUCTION

The bacterial microorganisms are the major element that causes pulp pathologies requiring endodontic intervention. Such bacterial infection can spread in the surrounding areas affecting the dentin and periodical tissues [1, 2]. The major aim of endodontic therapy is to clean and debride the root canals and that is achieved biochemical and mechanical measures. Certain factors such as individual variations and complex anatomy and morphology of root canals have made debridement [both mechanical and chemical] a challenging task [3]. The beneficial role of chemical debridement using various kinds of root canal irrigators is well proven [4-8]. Various antiseptics and irrigating solutions are affective in removal of organic debris during mechanical preparation and destroying the microorganisms [9, 10].

Considering the bacterial flora, Enterococcus faecalis (E. faecalis) is the most numerous microorganism found in the diseased root canals [11]. A number of chemicals for example sodium hypochlorite, chlorhexidine, hydrogen peroxide, ethylenediamine tetraacetic acid (EDTA) have been used as intra canal irrigants for many years [6, 12-19]. In order to get the maximum benefits from any root canal irrigator, it must have the flushing action to remove debris as well as lubrication to facilitate mechanical instrumentation, dissolution of dentin and antimicrobial property [20]. Some other factor such as toxicity, smell or taste of the irrigator can be too problematic to use it in the oral cavity. All of the currently used root canal irrigator produce some kind of unwanted affects [3] or example, sodium hypochlorite (NaOCl), have toxicity issues, bad taste [21] and not very effective to remove the smear layer [22]. EDTA does not have disinfectant action [23] and cannot be used alongside NaOCl due to its capability to inactivate the chlorine released from NaOCL [24]. Similarly, chlorhexidine is well known for its antibacterial activity; however it remains ineffective in dissolution of the required tissue [25].

Due to such limitations, two or more root canal irrigators can be used in combination at the same time; such as MTAD. MTAD was introduced by Torabinejad [26] [US Patent 20030235804] for endodontic applications. MTAD is composed of doxycycline (an antibiotic), citric acid (dentin demineralizing) and tween 80 (for detergent action) in the aqueous solution [26]. Reflecting to its compositions, MTAD has the ability to remove the smear layer [27-29] and has been suggested to be more

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effective than sodium hypochlorite [5, 10, 11, 18, 30-33] and chlorhexidine [15] in disinfecting the root canals. The objective of this study was to evaluate the antimicrobial activity of MTAD *in vitro*. In addition, the antimicrobial effectiveness against E. faecalis was compared to commonly used irrigators NaOCl, chlorhexidine and EDTA under similar conditions and various pH levels.

MATERIALS AND METHODS

The comparative evaluation of various irrigant solutions including MTAD, NaOCl (5.25% and 1.0%), Chlorhexidine (2%), EDTA (17%) was performed for their antibacterial efficacy against E.faecalis. The normal saline (0.9 % w/v) was used as a control group.

Antimicrobial activity was assessed through bacterial suspensions of E.faecalis prepared using commercial McFarland's Standard 0.5 [turbidity=100-200 cfu/ml] [34]. Colonies were grown, suspended in the saline solution and the turbidity was adjusted to MacFarland's Standard 0.5 by colorimeter using 580nm filter. All bacterial solutions were utilized within half an hour of preparation to avoid any deterioration, color or turbidity variations. Effect of variation of pH on the antibacterial efficacy of the irrigants was tested by varying the pH of the TSA (Tryptic Soya Agar) media which was adjusted to 6.4, 6.8 and 7.4 using phosphate buffer and monitored by digital pH meter. This suspension was swabbed evenly on agar plate.

The study deals with disc diffusion method to determine the antibacterial efficacy of the irrigants by measuring the zones of inhibition. Ten 6mm sterile filter paper disks were used for each irrigator under investigation. Each paper disc was loaded with an aliquot of 20μl of test irrigant and dried in hot air oven at 37°C for 30 min.

These test irrigants loaded disks were placed in their respective quadrants on the TSA plate and then incubated at 37°C for 24 hours. The antibacterial efficacy was calculated based on the areas of inhibition [in millimeters] appearing due to diffusion in the surrounding medium. These zones of inhibition were observed to assess the efficiency of the irrigants i.e. higher the antibacterial efficacy greater will be the zones of inhibition and vice versa. All data was evaluated and variables were compared by Kruskal Wallis One-way ANOVA followed by Mann Whitney U test; [$p < 0.05$]. The statistical analysis was performed using a software package (SPSS; Version19).

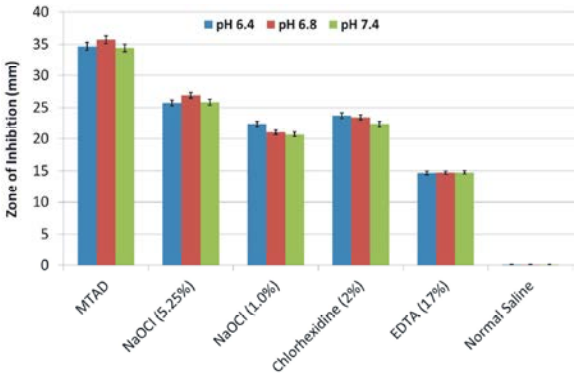

Fig. 1: Zone of inhibition exhibited by different root canal irrigators at various levels of pH

RESULTS

The results for antimicrobial efficacy of various irrigators at different pH levels have been shown in figure 1. No zone of inhibition was observed adjacent to the filter paper disk loaded with sterile saline regardless of pH.

Statistical analysis using Kruskal Wallis One way ANOVA and Mann Whitney U tests suggested a significant difference between MTAD, NaOCl (5.25% and 1.0%), Chlorhexidine (2%), EDTA (17%); [$p<0.05$] in terms of bacterial inhibition at all pH levels.

For pH 6.4, the mean value in MTAD (34.6 ± 0.5) was considerably greater than the mean values of NaOCl (26.4 ± 0.5 mm for 5.25 % and 21.0 ± 1.0 mm for 1.0%), Chlorhexidine (23.4 ± 0.5 mm), EDTA (14.6 ± 0.5 mm) and normal saline (0.0 ± 0.0) ($p<0.05$). Hence, MTAD resulted in the largest inhibition zone, followed by NaOCl (5.25 %), Chlorhexidine, NaOCl (1%) and EDTA. Normal saline (control group) did not show any zone of inhibition for all pH levels. Similar results were observed at pH level of 6.8 and 7.4. By changing pH level [6.4-7.4], no significant difference was observed in zones of inhibition for any irrigator. No particular trend could not be established between for a pH range of [6.4-7.4] irrigators ability to produce inhibition zones.

DISCUSSION

The type and incidence of microorganism found in infected root canal have been studied by various researchers [9, 14]. Eradication of microorganisms is the most important aspect of endodontic treatment and it has been accepted that thorough biomechanical preparation followed by the use of an effective irrigant will reduce the bacterial population in the infected root canals [7, 9].

The microorganisms enduring in the apical parts of the obturated root canals of are one of major threat to the failure of endodontic therapy. The different zones of inhibition produced by various root canal irrigators were statistically analyzed. The absence of zone of inhibition observed in this study with saline solution may be due to its lack of antibacterial property while *Dametto et al* [35] in their in-vivo study have reported 95% reduction in the debris and bacterial content may be due to the flushing action of saline solution.

Regarding NaOCl irrigators, it was evident that the higher concentration i.e. 5.25% showed better antibacterial effect and this has also been supported by the earlier studies [9, 11, 13]. EDTA (17%) was found to have greater zones of inhibition that was considerably greater than saline ($p<0.05$) and has less antibacterial activity when compared to MTAD, sodium hypochlorite and chlorhexidine [Figure 1] and is in agreement with the findings of the earlier researchers [28, 36]. This minimal antibacterial effect of EDTA may be due to bactericidal effects as a result of chemical chelation involving the external bacterial membrane. Chlorhexidine (2%) has shown significantly higher antimicrobial activity as compared to saline, 17% EDTA and 1.0% sodium hypochlorite ($p<0.05$), however, its antimicrobial efficacy was less than that of 5.25% sodium hypochlorite and MTAD. Zehnder Matthias [4] has reported chlorhexidine to show higher negative to positive culture reversals. This may be explained to its incapability to solubilize necrotic pulp tissue remnants and lack of effective cleansing of the canal system chemically. Recently introduced irrigant MTAD contains doxycycline (3%), citric acid (4.25%) and polysorbate (0.5%) a detergent (Tween 80) in the aqueous solution. MTAD has been reported to be a biocompatible material and an effective root canal irrigant (37). In this study, MTAD remain the most effective medicament against E.faecalis showing significantly higher zones of inhibition ($p<0.05$) compared to 2% chlorhexidine, 5.25% NaOCl, 1.0% NaOCl, 17% EDTA and saline.

In terms of eradicating bacteria from the infected root canals, MTAD has been considerably more efficient than NaOCl (5.25%). It has been confirmed [10, 27, 33, 38] that the key difference between EDTA and MTAD is its high binding affinity due to the presence of doxycycline which may allow a prolonged antibacterial effect, further the concentration of doxycycline is relatively high and its effect may be due to its low pH (2.15) [33, 38], anti-collagenous activity, its capability of binding to dentinal tissues and its gradual release.

MTAD contains a detergent [Tween80] that reduces its surface tension and facilitates the penetration in to the dentinal tubules. The better penetration in to dentinal tubules enhances its antibacterial effects against E.faecalis. It is claimed that its prolonged influence of MTAD is due to bacteriostatic effects produced by tetracycline. The tetracycline works by restricting the process of protein synthesis in bacterial cells and inhibits the bacterial growth. It has also been claimed that the low pH of citric acid and its chelating property is effective in destroying E. faecalis although the exact mechanism is not known [15].

Role of pH: In our study, the rationale behind selecting pH of 6.4, 6.8 and 7.4 was to check whether the normal variation of pH within the oral cavity has any influence on the antibacterial efficiency of the commonly used irrigants. Previously, the irrigants have been found to be more effective at a neutral pH [39]. For the range of pH investigate [6.4, 6.8 and 7.4] showed that MTAD has the highest antibacterial efficacy against E.faecalis [Figure 1]. Individual irrigants showed different antibacterial effectiveness at different pH values however no specific trend and are of no clinical significance. In the recent past, only a few studies have been conducted to assess the significance of pH in determining the antibacterial efficiency of irrigants against E.faecalis. However, further exploration over the wide pH range [3.0-10.5] is required to establish its association to the antibacterial effectiveness of endodontic irrigants.

CONCLUSIONS

Within the limitations of this study, it can be concluded that MTAD is effective for the eradication of E.faecalis. Around neutral pH, the effective inhibition of E.faecalis is better than other root canal irrigators investigated in this study. However it will be interesting to study such irrigators under extreme conditions of pH. Another limitation of this study was that it was conducted under static conditions and lack of flow of irrigator. Further studies under dynamic conditions of flow mimicking closely to clinical conditions can provide better understanding as flow of irrigator on its own can provide cleansing effects and enhance the antimicrobial activity.

Conflict of Interest: The authors declared no conflict of interest for conducting this research.

REFERENCES

1. Ingle, J.I., 2002. Endodontics. 5th ed. Hamilton, Ont. ; London: BC Decker.
2. Ørstavik, D. and M. Haapasalo, 1990. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Dental Traumatology; 6(4): 142-149.
3. Singla, M.G., A. Garg, S. Gupta, 2011. MTAD in endodontics: an update review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 9;112(3): e70-e76.
4. Zehnder, M., 2006. Root canal irrigants. J Endod; 32(5): 389-398.
5. Baumgartner, J.C., S. Johal and J.G. Marshall, 2007. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod; 33(1): 48-51.
6. Hülsmann, M., M. Heckendorff and A. Lennon, 2003. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J; 36(12): 810-830.
7. Lynne, R.E., F.R. Liewehr, L.A. West, W.R. Patton and T.B. Buxton, 2003. McPherson III JC. In Vitro Antimicrobial Activity of Various Medication Preparations on< i> E. faecalis</i> in Root Canal Dentin. J Endod; 29(3): 187-190.
8. Zafar, M.S., 2013. Effects of Surface Pre-Reacted Glass Particles on Fluoride Release of Dental Restorative Materials. World Applied Sciences Journal; 28(4): 457-462.
9. Berber, V., B. Gomes, N. Sena, M. Vianna, C. Ferraz, A. Zaia, *et al*., 2006. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J; 39(1): 10-17.
10. Shabahang, S., M. Pouresmail and M. Torabinejad, 2003. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. J Endod; 29(7): 450-452.
11. Shabahang, S., M. Pouresmail and M. Torabinejad, 2003. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. J Endod; 29(7): 450-452.
12. Gomes, B., C. Ferraz, V. ME, V. Berber, F. Teixeira and F. Souza-Filho, 2001. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J; 34(6): 424-428.
13. Law, A. and H. Messer, 2004. An evidence-based analysis of the antibacterial effectiveness of intracanal medicaments. J Endod; 30(10): 689-694.
14. Podbielski, A., A. Spahr and B. Haller, 2003. Additive antimicrobial activity of calcium hydroxide and chlorhexidine on common endodontic bacterial pathogens. J Endod; 29(5): 340-345.
15. Portenier, I., T. Waltimo, D. Ørstavik and M. Haapasalo, 2006. Killing of< i> Enterococcus faecalis</i> by MTAD and Chlorhexidine Digluconate with or without Cetrimide in the Presence or Absence of Dentine Powder or BSA. J Endod; 32(2): 138-141.
16. Schäfer, E. and K. Bössmann, 2005. Antimicrobial Efficacy of Chlorhexidine and Two Calcium Hydroxide Formulations Against< i> Enterococcus faecalis</i>. J Endod; 31(1): 53-56.
17. Sena, N., B. Gomes, M. Vianna, V. Berber, A. Zaia, C. Ferraz, *et al*., 2006. In vitro antimicrobial activity of sodium hypochlorite and chlorhexidine against selected single-species biofilms. Int Endod J; 39(11): 878-885.
18. Royal, M.J., A.E. Williamson and DR. Drake, 2007. Comparison of 5.25% sodium hypochlorite, MTAD and 2% chlorhexidine in the rapid disinfection of polycaprolactone-based root canal filling material. J Endod; 33(1): 42-44.
19. Zafar, M.S. and E. Javed, 2013. Extraoral Radiography: An Alternative to Intraoral Radiography for Endodontic (Root Canal System) Length Determination. European Scientific Journal; 9(15): 51.
20. Siqueira Jr, J.F., I.N. Rôças, S.R. Santos, K.C. Lima, F.A. Magalhães and M. de Uzeda, 2002. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod; 28(3): 181-184.
21. Spangberg, L., B. Engström and K. Langeland, 1973. Biologic effects of dental materials: 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surgery, Oral Medicine, Oral Pathology; 36(6): 856-871.
22. Goldman, L.B., M. Goldman, J.H. Kronman and P.S. Lin, 1981. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surgery, Oral Medicine, Oral Pathology; 52(2): 197-204.
23. Heling, I. and N. Chandler, 1998. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J; 31(1): 8-14.

24. Grawehr, M., B. Sener, T. Waltimo and M. Zehnder, 2003. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J; 36(6): 411-415.
25. Kanisavaran, Z.M., 2008. Chlorhexidine gluconate in endodontics: an update review. Int Dent J; 58(5): 247-257.
26. Torabinejad, M. and W. Johnson, 2003. Irrigation solution and methods for use, United States Patent Application 20030235804.
27. Machnick, T.K., M. Torabinejad, C.A. Munoz and S. Shabahang, 2003. Effect of MTAD on flexural strength and modulus of elasticity of dentin. J Endod; 29(11): 747-750.
28. Torabinejad, M., A.A. Khademi, J. Babagoli, Y. Cho, W.B. Johnson, K. Bozhilov, *et al.* 2003. A new solution for the removal of the smear layer. J Endod; 29(3):170-175.
29. Zafar, M.S. and N. Ahmed, 2013. Nano-Mechanical Evaluation of Dental Hard Tissues Using Indentation Technique. World Applied Sciences Journal; 28(10): 1393-1399.
30. Torabinejad, M., S. Shabahang, R.M. Aprecio and J.D. Kettering, 2003. The antimicrobial effect of MTAD: an in vitro investigation. J Endod; 29(6): 400-403.
31. Krause, T.A., F.R. Liewehr and C. Hahn, 2007. The Antimicrobial Effect of MTAD, Sodium Hypochlorite, Doxycycline and Citric Acid on< i> Enterococcus faecalis</i>. J Endod; 33(1): 28-30.
32. Giardino, L., E. Ambu, E. Savoldi, R. Rimondini, C. Cassanelli and E.A. Debbia, 2007. Comparative Evaluation of Antimicrobial Efficacy of Sodium Hypochlorite, MTAD and Tetraclean Against< i> Enterococcus faecalis</i> Biofilm. J Endod; 33(7): 852-855.
33. Beltz, R.E., M. Torabinejad and M. Pouresmail, 2003. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite and EDTA on bovine pulp and dentin. J Endod; 29(5): 334-337.
34. Andrews, J.M., 2005. BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 4). J Antimicrob Chemother; 56(1): 60-76.
35. Dametto, F.R. and C.C.R. Ferraz, 2005. de Almeida Gomes, Brenda Paula Figueiredo, Zaia AA, Teixeira FB, de Souza-Filho FJ. In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against< i> Enterococcus faecalis</i>. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology; 99(6): 768-772.
36. Abdullah, M., Y. Ng, K. Gulabivala, D.R. Moles and DA. Spratt, 2005. Susceptibilties of Two< i> Enterococcus faecalis</i> Phenotypes to Root Canal Medications. J Endod; 31(1): 30-36.
37. Park, D.S., M. Torabinejad and S. Shabahang, 2004. The effect of MTAD on the coronal leakage of obturated root canals. J Endod; 30(12): 890-892.
38. Zhang, W., M. Torabinejad, Y. Li, 2003. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod; 29(10): 654-657.
39. Serper, A. and S. Çalt, 2002. The demineralizing effects of EDTA at different concentrations and pH. J Endod; 28(7): 501-502.