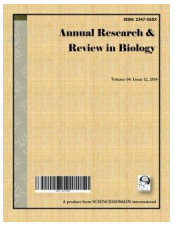

Annual Research & Review in Biology

15(2): 1-7, 2017; Article no.ARRB.34141
ISSN: 2347-565X, NLM ID: 101632869

Determination of Minimum Inhibitory Concentration (MIC) of a PolyHexamethylene Biguanide (PHMB) Solution: A Potential Root Canal Irrigant

Chandki Rita[1*], Vineeta Nikhil[1] and Sai Kalyan Surapaneni[2]

[1]Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.
[2]Department of Conservative Dentistry and Endodontics, Rural Dental College, PIMS, Loni, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author CR designed the study, wrote the protocol and wrote the first draft of the manuscript. Author VN guided the study design and protocol, reviewed the manuscript and made modifications. Author SKS managed the literature searches and execution of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/34141
Editor(s):
(1) Moacir Marocolo Júnior, Physiology, Institute of Biological Sciences, Federal University of Juiz de For a, Brazil.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) M. Jaya Nagendra Krishna, Ntr University of Health Sciences, India.
(2) Fatih Oznurhan, Cumhuriyet University Faculty of Dentistry Department of Pediatric Dentistry, Sivas, Turkiye.
(3) Konda Karthik Roy, Dr. NTR University of Health Sciences, India.
(4) Andrea Scribante, University of Pavia, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history/20271

Short Research Article

Received 15th May 2017
Accepted 26th July 2017
Published 29th July 2017

ABSTRACT

Background: A polymeric biguanide 'PolyHexaMethylene Biguanide' (PHMB) was evaluated for its Minimal Inhibitory Concentration (MIC) against ATCC reference laboratory strain of *Enterococcus faecalis (E. faecalis)*.
Methods: The MIC was determined using criteria's laid down in DIN 58940-7 and 58940-8 and the corresponding supplementary sheets. Serial dilutions of PHMB were tested against *E. faecalis* in 96-well microtitre plates and MIC was read as the minimal concentration that allowed no visible growth.
Results: The MIC for PHMB against tested organism was found to be 2 mg/L.
Conclusion: Lower MIC value (2 mg/L) of PHMB solution against *E. faecalis* is an indicator of its higher potency against the said microorganism and paves way to further research in its potential use as a root canal irrigant.

**Corresponding author: E-mail: chandki.rita@gmail.com;*

Keywords: E. faecalis; Minimum Inhibitory Concentration (MIC); Polyhexamethylene Biguanide (PHMB); Polyhexanide; Root canal irrigant.

1. INTRODUCTION

Root canal disinfection is undoubtedly of paramount importance in Endodontics. Considering the enormous complexities in the root canal system [1] and having known that our instruments have only a very limited reach to the intricacies of root canals [2,3], root canal irrigants have come to the fore as indispensable tools in cleaning of root canals. A vast majority of disinfectant solutions have been researched and numerous papers published in this domain [4-7]. However, owing to the resistant microflora residing in convolutions of root canals [8] and existence of bacteria in form of biofilms [9-10] that collectively shelter the microflora from disinfection procedures, we are yet to find a disinfectant which will achieve near ideal disinfection of root canals with minimal toxic effects on host cells.

E. faecalis, a facultatively anaerobic, gram-positive cocci has omniviously been associated with root canal infections, more commonly with failed root canals than with primary root canal infections [11]. Its ability to form biofilms on root canal surface and survive in harsh environment renders it 1000 times more resistant to destruction as compared to its free floating counterparts [12]. Conventionally, 6% Sodium Hypochlorite (NaOCl) is the only agent capable of both physically eliminating the artificial biofilm and killing bacteria [13] but NaOCl, particularly at high concentration is known to be cytotoxic [14] .

Chlorhexidine, another commonly used root canal irrigant has been found to be inefficient in eradication of *E. faecalis* biofilms [15]. Moreover, It has been found to be more cytotoxic than NaOCl [16].

MIC determination is the most commonly employed procedure to evaluate the physiological effects of an anti-microbial agent on microorganisms, and correlation of product concentration and effect [17]. National Committee on clinical laboratory standards [NCCLS] 1997 defined MIC as the lowest concentration that completely inhibits visible growth of the organism, as detected by the unaided eye after an 18-24 hour incubation period, with standard inoculums of approximately 105 colony forming units per milliliter (CFU/ ml) [17]. MICs serve as an important research tool in determining the activity of novel antimicrobials under *in vitro* conditions and the data so obtained can be used to determine MIC breakpoints [18].

Therefore, the aim of this study was to evaluate MIC of a polymeric bisguanide PHMB against *E. faecalis;* the most commonly isolated organism from failed root canals.

2. METHODOLOGY

The MIC was determined using criteria's laid down in DIN 58940-7 and 58940-8 [19,20] and the corresponding supplementary sheets. To summarize, the test organism ATCC laboratory strain (29212) of *E. faecalis* was cultivated on Blood agar at 36°C for 18 hours. Following this, one colony of cultivated *E. faecalis* was transferred into 1 mL of Mueller–Hinton bouillon (Fig. 1) and then diluted to reach 10^5 cfu/mL. 96-well microtitre plates (Fig. 2) were used to perform the test procedure. PHMB in white crystalline form was obtained from Sinobio Chemistry Co., Ltd, China. Hundred mL of defined antiseptic dilution was placed in each well along with 100 mL of test organism suspension. PHMB solution was subjected to serial dilution and a variety of concentrations ranging from 5000 to 1 mg/ L dilutions were made. After a period of 24 h, the turbidity was evaluated. Presence of turbidity was defined as indicator for bacterial growth or viability. After 24 h, plating was done for solutions to confirm their efficacy. (Fig. 3). The MIC was obtained as a single value and hence the results did not involve statistical evaluation.

Fig. 1. Mueller-Hinton broth for culture of *E. faecalis*

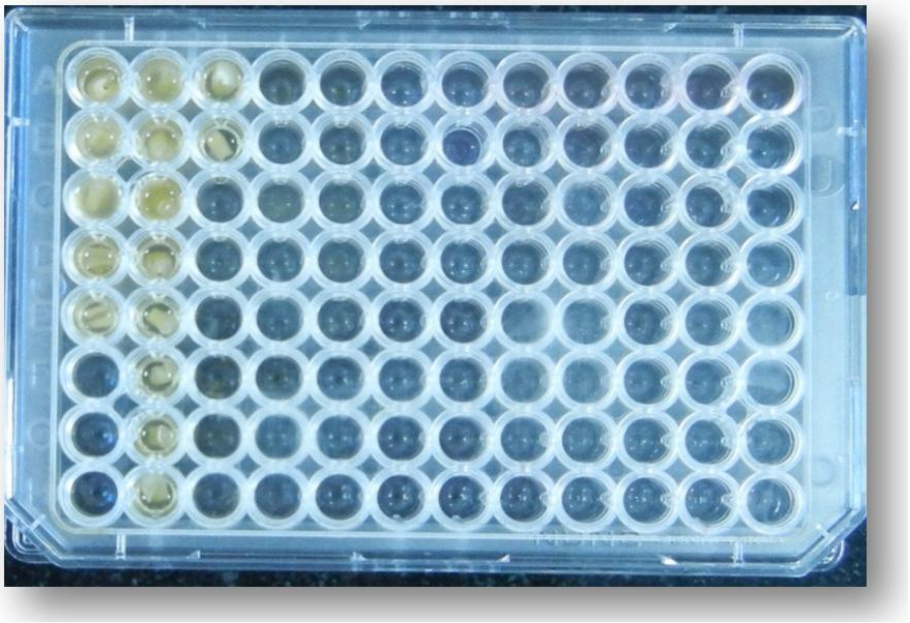

Fig. 2. Serial dilution of 96 well plates

Fig. 3. Plating, incubation and culture of bacteria to confirm antibacterial efficacy

3. RESULTS AND DISCUSSION

A Value of 2 mg/ L was obtained as MIC after 24 hours for PHMB solution against *E. faecalis*.

Antimicrobial activity plays a central role in any root canal irrigant. Knowledge of MIC of an antiseptic is of utmost significance in clinical applications. Little is known regarding the use of sub-MIC of intracanal irrigants and their contribution to the switch to pathogenicity in *E. faecalis* [21]. It has been established by several studies that the common antimicrobial agents used at sub-MICs, may upregulate the expression of some biofilm-related genes in the planktonic or biofilm state [22,23].

This study was carried out to determine MIC of a broad spectrum antimicrobial PHMB against *E. faecalis*, the most commonly associated microorganisms with failed root canals.

PHMB is a known antiseptic for treatment of acute and chronic wounds, particularly when the wound is critically colonised and locally infected [24]. In its Polymeric form, it has been found to be an effective broad spectrum antimicrobial with efficacy against some fungi and protozoa in addition to gram positive and gram negative bacteria [25]. In addition, Its properties like prolonged duration of action [26] and abilty to remove biofilms [27] coupled with its established higher safety margin [28,29] may implicate its potential use as a root canal irrigant, particulary in infected and chronic cases.

Mechanism of action of PHMB has been described by Ikeda et al. [30]. PHMB acts on negatively charged species in the bilayer composed of neutral and acidic phospholipids. After its adsorption into Phosphatidyl glycerol bilayer, its biguanide groups interact with the polar headgroups of the lipids and hexamethylene groups with the hydrophobic interior. The Phosphatidyl glycerol bilayer hence becomes disorganized with resulting greater fluidity, lateral expansion and raised permeability of the bilayer.

No evidence of microbial resistance against PHMB can be found in literature. This in part can be attributed to its superficial interaction with the membrane in polymeric form which merely involves physical bridging of the molecule in the phospholipids. Lack of any chemical interaction avoids any possibility of generation of any mechanism for reduced susceptibility [31].

Traditionally, a plethora of methods have been employed for determining susceptibility to antimicrobials; Broth microdilution test [32,33], disk diffusion test [34,35] and automated generated systems [36] to name a few. Amongst these, Agar dilution and Broth microdilution are the popular methods for quantitative determination of antimicrobial activity in terms of MIC.

Broth Microdiluton test was chosen for determination of MIC in this study because it is reproducible, easy to perform as channels are prepared, cost-effective and saves reagents and space [37].

Polyhexanide showed an MIC of 2 mg/L in this study. Our results were in agreement with the results of Koburger and Colleagues [38] who employed a microdilution test and a quantative suspension test to determine MIC values of a variety of antiseptic solutions at 24 h and 48 h and Minimum bactericidal concentration (MBC) at 24 h. They found octenidine and polyhexanide to be most efficacious with equally low MIC and MBC values at all times. However, CHX after a period of 24 h was found to eliminate *E. faecalis* at 16 mg/ L [38]. Moreover it has high toxicity and low tissue tolerability [6]. On the contrary, Polyhexanide has been shown to have better safety profile [39,40].

Muller and Kramer in 2008 introduced a Biocompatibiity Index (BI) as a measure of microbicidal activity of Antimicrobials against cytotoxicity. A BI greater than 1 indicates good antiseptic efficacy with a relatively low cytotoxicity, whereas antiseptics with BI less than 1 present a relatively high cytotoxicity. Polyhexanide was found to have BI greater than 1 [41].

Hirsch and colleagues tested a variety of antiseptics for antimicrobial activity and cytotoxicity against primary human keratinocytes, primary human fibroblasts and human keratinocyte cell line. Their results were in favour of Lavasept and Prontosan which showed best antimicrobial efficacy with low or no cytotoxicity on different cell lines [42].

4. CONCLUSION

We believe that this *in vitro* study will be a valuable guide for determining the optimal first-line drug at higher concentrations but at lower toxicity levels for endodontic irrigation,

particularly in retreatment cases. Therefore, based on this study results confirming antimicrobial activity of PHMB against *E. Faecalis*, we suggest that PHMB may be useful as an effective endodontic irrigant. However, the limitation of this study was that MIC was determined against pure cultures without load of debris/ proteins which might be an interfering factor. Further research should be carried out to determine its antimicrobial activity against various other microorganisms found in infected root canals and its effect on removal of root canal biofilm.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hess W, Zurcher E. The anatomy of root canals of the teeth of the permanent and deciduous dentitions. NewYork: William Wood & Co; 1925.
2. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89:321–328.
3. Gutmann JL, Dumsha T. Cleaning and shaping the root canal system. In: Cohen S, Burns RC, eds. Pathways of the Pulp. 4th ed, St Louis: Mosby. 1987;156-82.
4. Salgar A, Chandak M, Dass A, Saxena A, Bhatia C, Chandak R. Comparison of different irrigating solutions on root canal disinfection after mechanical preparation by using scanning electron microscope: An *in vitro* study. J Interdiscip Dentistry. 2015;5:65-70.
5. Zehnder M. Root Canal Irrigants. J Endod. 2006;32:389–98.
6. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod. 1998;24:472–6.
7. Vatkar NA, Hegde V, Sathe S. Vitality of *Entercoccus faecalis* inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19:445-9.
8. Tikku AP, Pragya Pandey W, Ivy Shukla. Intricate internal anatomy of teeth and its clinical significance in endodontics - A review. Endodontology. 2012;24(2):160-169.
9. Chavez de Paz LE. Redefining the persistent infection in root canals: Possible role of biofilm communities. J Endod. 2007;33:652–62.
10. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: Study of prevalence and association with clinical and histopathologic findings. J Endod. 2010; 36:1277–88.
11. Rocas IN, Siqueira JF, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod. 2004;30(5):315-20.
12. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod. 2002;28(10):689-93.
13. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms *in vitro*. J Endod. 2006; 32(5):434-7.
14. Brown DC, Moore BK, Brown CE, Newton CW. An *in vitro* study of apical extrusion of sodium hypochlorite during endodontic canal preparation. J Endod. 1995;21(12) :587–91.
15. Arias-Moliz MT, Baca P, Ordóñez-Becerra S, González-Rodríguez MP, Ferrer-Luque CM. Eradication of enterococci biofilms by lactic acid alone and combined with chlorhexidine and cetrimide. Med Oral Patol Oral Cir Bucal. 2012;17(5):e902-6.
16. Vouzara T, Koulaouzidou E, Ziouti F, Economides N. Combined and in dependentent cytotoxicity of sodium hypochlorite, ethylenediaminetetraacetic acid and chlorhexidine. Int Endod J. 2016;49(8):764-73.
17. PHMB and its potential contribution to wound management. Wounds UK, Aberdeen; 2010.
18. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48(1):5-16.
19. Deutsches Institut fu¨ r Normung. DIN EN 1040: Chemical disinfectants and antiseptics—quantitative suspensiontest for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics—test method and requirements (phase 1). Berlin, Germany: Beuth; 2005.
20. Deutsches Institut fu¨ r Normung. DIN EN 1275: Chemical disinfectants and

antiseptics-quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics—test method and requirements (Phase 1). Berlin, Germany: Beuth; 2005.

21. Tong Z, Huang L, Ling J, Mao X, Ning Y, et al. Effects of intracanal irrigant MTAD combined with Nisin at sub-minimum inhibitory concentration levels on *Enterococcus faecalis* growth and the expression of pathogenic genes. PLoS ONE. 2014;9(3):e90235.
22. Wu Q, Wang Q, Taylor KG, Doyle RJ. Subinhibitory concentrations of antibiotics affect cell surface properties of Streptococcus sobrinus. J Bacteriol. 1995; 177:1399–401.
23. Liping Dong, Zhongchun Tong, DakeLingh, Yuan Lin, Rui Tao, Jun Liu, Yu Tian, Longxing Ni. Effects of sub-minimum inhibitory concentrations of antimicrobial agents on *Streptococcus mutans* biofilm formation. International Journal of Antimicrobial Agents. 2012;39:390–395.
24. Dissemond J, Gerber V, Kramer A, Riepe G, Strohal R, Vasel-Biergans A, Eberlein T. A practiceoriented recommendation for treatment of critically colonised and locally infectedwounds using polihexanide. J Tissue Viability. 2010;19(3):106-15.
25. Allen MJ, Morby kAP, White GF. Cooperativity in the binding of the cationic biocide polyhexamethylene biguanide to nucleic acids. Biochem. Biophys. Res. Commun. 2004;318:397–404.
26. Rosin M, Welk A, Kocher T, Majic-Todt A, Kramer A, Pitten FA. The effect of a polyhexamethylene biguanide mouthrinse compared to an essential oil rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque regrowth. J Clin Periodontol. 2002;98:392e9.
27. Harbs N, Siebert J. *In vitro* efficacy of octenidine and polihexanide against biofilms composed of *Pseudomonas aeruginosa*. GMS Krankenhaushyg Interdiszp. 2007;2(2). Doc.45(20071228).
28. Ikeda T, Tazuke S, Watanabe M. Interaction of biologically active molecules with phospholipid membranes. I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. Biochem Biophys Acta. 1983; 735:380e6.
29. Mulder GD, Cavorsi JP, Lee DK. Feature: Polyhexamethylene biguanide (PHMB): An addendum to current topical antimicrobials. Wounds. 2007;19(7):173e82.
30. Ikeda, T, Ledwith A, Bamford CH, Hann RA. Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochim. Biophys. Acta. 1984;769:57–66.
31. Wessels S, Ingmer H. Modes of action of three disinfectant active substances: A review. Regul Toxicol Pharmacol. 2013; 67(3):456-67.
32. European committee for antimicrobial susceptibility testing (EUCAST) of the European society of clinical microbiology and infectious diseases (ESCMID). Determination of minimum Inhibitory concentrations (MICs) of antibacterial agents by Broth dilution. Clinical Microbiology and Infection. 2003;9(8):1-7.
33. Tong Z, Dong L, Zhou L, Tao R, Ni L. Nisin inhibits dental caries-associated microorganism *in vitro*. Peptides. 2010; 31(11):2003-8.
34. Graham DR, Dixon RE, Hughes JM, Thornsberry C. Disk diffusion antimicrobial susceptibility testing for clinical epidemiologic purposes. Am J Infect Control. 1985;13(6):241-9.
35. Poggio C, Colombo M, Scribante A, Sforza D, Bianchi S. *In vitro* antibacterial activity of different endodontic irrigants. Dent Traumatol. 2012;28(3):205-9.
36. Forry SP, Madonna MC, López-Pérez D, Lin NJ, Pasco MD. Automation of antimicrobial activity screening. AMB Express. 2016;6(1):20.
37. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing:A reviewof general principles and contemporary practices. Clin Infect Dis. 2009; 49(11):1749-55.
38. Koburger T, Hübner NO, Braun M, Siebert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP–iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. J Antimicrob Chemother. 2010;65(8):1712-9.
39. Gilliver S. PHMB: A well-tolerated antiseptic with no reported toxic effects. Journal of Wound Care/ Activa Health Care Supplement. 2009:9-14.
40. Kaehen K. Polihexanide: A safe and highly effective biocide. Skin Pharmacol Physiol. 2010;23Suppl:7-16.

41. Müller G, Kramer A. Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity. J Antimicrob Chemother. 2008;61(6): 1281-7.

42. Hirsch T, Koerber A, Jacobsen F, Dissemond J, Steinau HU, Gatermann S, Al-Benna S, Kesting M, Seipp HM, Steinstraesser L. Evaluation of toxic side effects of clinically used skin antiseptics *in vitro*. J Surg Res. 2010;164(2):344-50.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20271