
Effect of Intracanal Medicaments On MMP-9 And VIP Levels

PALAK MAYUR SHAH*, LAKSHMI THANGAVELU

Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

*Corresponding Author

Email: lakshmi@saveetha.com

Abstract: Root canal treatment is incomplete without usage of intra canal medicaments. They help in the reduction of bacterial count and its by-products, making canals clean and decreasing postoperative pains. The aim of this study was to evaluate the effect of calcium hydroxide (Ca [OH]₂) and triple antibiotic paste on matrix metalloproteinase-9 (MMP-9) and vasoactive intestinal peptide (VIP). Thirty single rooted teeth with periapical lesions which require root canal treatment were randomly divided into 2 groups; Group 1- calcium hydroxide and Group 2- triple antibiotic paste. After access opening and working length determination, cleaning and shaping of the root canals were done and intracanal dressing was given. In the second appointment, medicaments were removed, and sampling was performed by collecting the interstitial fluid of the apical tissues by paper points to assess the MMP-9 and VIP levels. The MMP-9 and VIP levels were measured by the enzyme-linked immunosorbent assay. The concentration of MMP - 9 on Ca(OH)₂ and TAP was 27.3pg/ml and 29.2pg/ml and VIP on Ca(OH)₂ and TAP was 27.9pg/ml and 28.4pg/ml. There was no statistically significant difference between the intracanal medicament on the MMP-9 and VIP (p>0.05), proving that type of intracanal medicament does not influence MMP 9 and VIP levels.

Keywords: Ca(OH)₂, Triple antibiotic paste, Vasoactive Intestinal peptide, Matrix Metalloproteinase, Intracanal medicament

INTRODUCTION:

Bacteria and toxins in the root canal system activate a local immune response after reaching the periapical tissues through the apical foramen. Several proinflammatory and immunoregulatory cytokines, mediators, chemokines, and neuropeptides are involved in this local immune response,[1,2] during which these molecules degrade extracellular matrix (ECM) components, which are the main components of the connective tissue.[3] Bacterial infection of the dental pulp results in pulpal destruction, and subsequently stimulates an inflammatory cell response and destruction of bone in the periapex. Bacterial components, including lipopolysaccharides, induce the production of many polypeptide mediators, or cytokines, by inflammatory cells. These cytokines, which include macrophage-derived interleukin-1 beta, interleukin-1 alpha and tumor necrosis factor, and lymphocyte-derived lymphotoxin, have been shown to potentially stimulate bone resorption and to inhibit reparative bone formation in vitro and in vivo. [4]

During an immune response, immune cells and dental pulp neurons release vasoactive intestinal peptide (VIP), which has potent immunomodulatory properties. [5] VIP may regulate the growth of apical periodontitis lesions by inhibiting bone resorption through the suppression of osteoclast functions. In addition, during an immune response, the immune cells release matrix metalloproteinases (MMPs) that degrade all ECM components, including the bone matrix. Several studies have shown that MMPs participate in the pathogenesis of pulp and periapical inflammation. [6] Matrix metalloproteinase-9 (MMP-9) plays an important role in the development of periapical lesions and is highly expressed in apical periodontitis. MMPs are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense. [7] A recent study suggested that the epigenetic modulation of the MMP-9 gene may contribute to the pathogenesis of periapical granulomas and radicular cysts. [8] There is no solid evidence in the literature that mechanical instrumentation alone results in a bacteria-free root canal system. Considering the complex anatomy of root canal pulp space, this is not surprising. [9]

Bacteria and their byproducts in the root canal system are the primary causes of apical periodontitis, it is important to develop an optimal root canal disinfection protocol. Intracanal medicaments such as calcium hydroxide (Ca[OH]₂) and Triple Antibiotic Paste (TAP) are widely used in root canal treatments because of their antimicrobial and anti-inflammatory properties. Calcium hydroxide exerts antibacterial effects in the root canal system as long as a high pH is maintained. It was shown that a 7-day application of a calcium hydroxide medicament was sufficient to reduce canal bacteria to a level that gave a negative culture.[10] It has also been

shown that an aqueous calcium hydroxide paste and a silicone oil-based calcium hydroxide paste are effective in the elimination of enterococcus faecalis in dentinal tubules. [11] Triple antibiotic paste is a combination of three antibiotics such as ciprofloxacin, metronidazole, and minocycline. [12] Previous studies have evaluated the anti-inflammatory properties of Ca(OH)₂ and TAP, no study has investigated the effects of these two intracanal medications on the secretion of VIP and MMP-9.[13]

This is a critical gap in the literature, because VIP and MMP-9 may play an important role in mediating the apical diseases. Therefore, it can be hypothesized that the level of VIP and MMP-9 secretion could be affected by the type of intracanal medication. The present study investigated the effects of these intracanal medicaments on MMP-9 and VIP secretion. The null hypothesis of this study was that the use of Ca(OH)₂ paste and TAP for intracanal medication would not affect VIP and MMP-9 secretion levels. [14,15]

We have numerous highly cited publications on well designed clinical trials and lab studies [16–31]. This has provided the right platforms for us to pursue the current study. Our aim of the present study was to evaluate the effects of calcium hydroxide and triple antibiotic paste on MMP-9 and VIP levels.

MATERIALS AND METHODS:

• Patient selection:

Total of 30 patients who underwent root canal treatment with periapical lesions were taken in the study. The study protocol received institutional approval from the Ethics Committee of the university. The study group consisted of patients aged between 25-60 years undergoing root canal treatment with periapical lesions for single rooted teeth. Exclusion criteria include medically compromised patients, pregnancy, steroid therapy, metabolic disorder patients. All the patients who were included in the study were informed about the treatment procedure and possible complications were also explained to them. Informed consent was taken from them.

• Sample collection:

The study consists of two groups - Group 1: Treatment with calcium hydroxide paste Group 2: Treatment with Triple antibiotic paste. Teeth were isolated and disinfected using 30% H₂O₂ v/v and 2.5% NaOCl for 30sec then 5% sodium thiosulphate was used to inactivate the 2.5% NaOCl. Access cavity preparation was done, working length determined with 15 K hand files using radiographs. During instrumentation, root canals were irrigated with 2ml 1% NaOCl. The canals were then irrigated again with saline for 1min after root canal instrumentation and the medicaments were placed and access cavity sealed with temporary filling. Three days later the root canals medication was removed and cleaned with 17% EDTA and then with distilled water. The interstitial fluid was collected from the apical tissue through the paper points that were used to dry the root canal and stored.

• Estimation of VIP by ELISA

Pipetted out 100µl of standards and test samples into the appropriate wells and incubated the plate at room temperature for 1 hour, centrifuge at 300 rpm and then washed the wells. Added 100 µl of VIP Antibody into each well. Incubated the plate at room temperature 25°C for 1 hour, centrifuge at 300rpm on an orbital micro plate shaker. Added 100 µl of biotinylated VIP working solution into each well. After washing, 100 µl of substrate Solution was added into each well. The incubation time may be extended up to 20 minute if the reaction temperature is below 20°C. Absorbance was read within 5 minutes using a microplate reader set to 450 nm.

• Estimation of MMP-9 by ELISA

About 100µl of antibody was coated on a microtiter plate and incubated overnight Then, 100 µl of anti-MMP9 and standards were added to appropriate wells and covered with adhesive strips followed by incubation for two hours at room temperature. Later, 100 µL (each) of working dilution of biotinylated MMP9 and substrate solution were added to each well, after washing the plate three times with a wash buffer between each addition, and incubated for 20 minutes at room temperature. Finally, 50 µL of stop solution was added to each well to stop enzyme reaction and the color generated was read at 450 nm. The concentration of OPG in the tested samples was calculated using the standard curve plotted using the optical density values with the standards. The data was collected and subjected to statistical analysis using independent t test and the significance level was set at 0.05

RESULTS AND DISCUSSION:

VIP level was found to be estimated as 27.9pg/ml on the usage of Ca(OH)₂. The MMP-9 level was found to be estimated as 27.3pg/ml on the usage of Ca(OH)₂. Similarly when the VIP was found to be 28.4pg/ml with the usage of TAP. This shows a slight deviation of 0.5 pg/ml. Similarly the MMP-9 level was found to be 29.2pg/ml with the usage of TAP. Here the deviation is found to be upto 1.9 pg/ml. There was no statistical significant difference seen between the intracanal medicaments in terms of MMP-9 and VIP levels (p>0.05).

According to the results of the present study, the type of medication did not affect the amount of VIP and MMP-9 secretion. VIP is a neuropeptide that promotes new bone formation, and MMP helps in promoting bone growth. Ca(OH)₂ is used for intracanal medication between treatment visits.

The ability of Ca(OH)₂ to reduce osteoclast-like cell differentiation may have shifted these lesions to the healing stage and increased VIP levels. [32] Moreover, Ca(OH)₂ increases Th-2-type cytokine levels, and the stimulation of Th-2-type cytokines induces VIP expression. However, there are several factors that influence the immune response. Therefore, it is more relevant to conclude that the medications were effective in reducing intracanal bacteria, and the host immune response subsequently changed. [33] Most commonly used intracanal medicament used is calcium hydroxide. Calcium hydroxide has been widely used as an intracanal medicament. Several studies have reported that due to alkaline pH of calcium hydroxide there is difficulty in eliminating resistant microorganisms. [34]

EDTA was used to remove Ca(OH)₂ because a previous study reported that EDTA is effective at removing Ca(OH)₂ from root canals. EDTA reacts with calcium ions and forms soluble calcium chelates, subsequently becoming easier to remove from root canal walls. The chelating effect of EDTA is self-limiting because equilibrium is formed when all ions have been bound. [35,36] In addition, EDTA has minimal antimicrobial effects. It was previously reported that EDTA has no significant effect on biofilm viability and was ineffective against *E. faecalis* even after 60 min of contact. In the present study, a 1-min EDTA application was used to remove the Ca(OH)₂. Therefore, it is unlikely that removing the Ca(OH)₂ using EDTA interfered with the results of the Ca(OH)₂ group. [37]

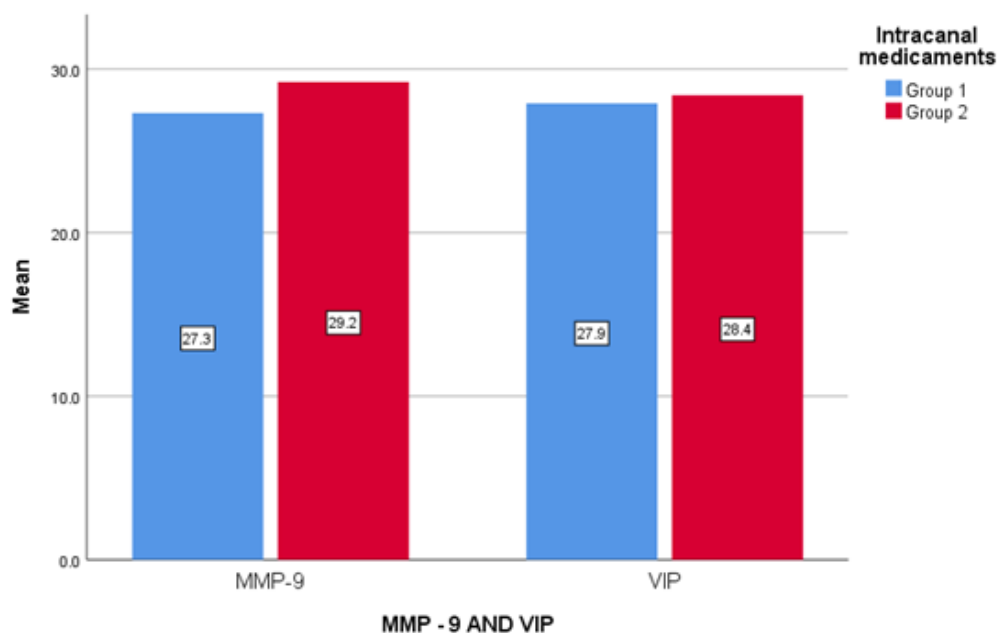


Fig.1: Bar chart represents the MMP -9 and VIP levels in group 1 and group 2. X represents the VIP and MMP-9 levels in accordance with different groups. Y axis shows the mean of VIP and MMP-9 levels. It is found that the MMP-9 and VIP was slightly at a higher level in group 2 (red) compared to group 1 (blue), however it was not statistically significant ($p>0.05$).

CONCLUSION:

Within the limitations of the present study it was found that there was no significant difference in levels of VIP and MMP-9 when calcium hydroxide or triple antibiotic paste were used as intracanal medicaments. Therefore it can be concluded that usage of intracanal medicaments does not influence the MMP -9 and VIP levels. However further studies need to be performed to substantiate these findings.

ACKNOWLEDGEMENT:

The authors are grateful to the authorities of Saveetha Dental College and Hospitals, Chennai for the facilities.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Lundberg P, Lie A, Bjurholm A, Lehenkari PP, Horton MA, Lerner UH, et al. Vasoactive intestinal peptide regulates osteoclast activity via specific binding sites on both osteoclasts and osteoblasts. *Bone*. 2000 Dec;27(6):803–10.

2. De la Fuente M, Delgado M, Gomariz RP. VIP modulation of immune cell functions. *Adv Neuroimmunol.* 1996;6(1):75–91.
3. Danin J. Factors Associated with Healing of Periradicular Lesions. 2003. 56 p.
4. Márton II, Kiss C. Overlapping protective and destructive regulatory pathways in apical periodontitis. *J Endod.* 2014 Feb;40(2):155–63.
5. Colić M, Lukić A, Vučević D, Milosavljević P, Majstorović I, Marjanović M, et al. Correlation between phenotypic characteristics of mononuclear cells isolated from human periapical lesions and their in vitro production of Th1 and Th2 cytokines. *Arch Oral Biol.* 2006 Dec;51(12):1120–30.
6. Rajendra Jangde. Matrix metalloproteinase: An overview. *Research J. Science and Tech.* 2011; 3(6): 301-307 .
7. Herrera DR, Silva EJNL, Santos CC, Zaia AA, Ferraz CCR, Almeida JFA, et al. Root canal content from primary endodontic infection and upregulation of gelatinases in fibroblast cells. *Int Endod J.* 2015 Dec;48(12):1168–74.
8. G. Lokhasudhan, Ajitha. P. Role of Antibiotics as Intracanal Medicament – A Literature Review. *Research J. Pharm. and Tech* 2018; 11(4): 1691-1696. doi: 10.5958/0974-360X.2018.00315.3
9. SJÖGREN U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing [Internet]. Vol. 24, *International Endodontic Journal.* 1991. p. 119–25. Available from: <http://dx.doi.org/10.1111/j.1365-2591.1991.tb00117.x>
10. Han G, Park S, Yoon T. Antimicrobial Activity of Ca(OH)₂ Containing Pastes with *Enterococcus faecalis* In Vitro [Internet]. Vol. 27, *Journal of Endodontics.* 2001. p. 328–32. Available from: <http://dx.doi.org/10.1097/00004770-200105000-00004>
11. Lavanya. E, Jayalakshmi, S Delphine Priscilla Antony. Intracanal Medicaments in Revascularization-A Review. *Research J. Pharm. and Tech* 2018; 11(6): 2672-2676.
12. Paula-Silva FWG, da Silva LAB, Kapila YL. Matrix metalloproteinase expression in teeth with apical periodontitis is differentially modulated by the modality of root canal treatment. *J Endod.* 2010 Feb;36(2):231–7.
13. Ballal V, Rao S, Bagheri A, Bhat V, Attin T, Zehnder M. MMP-9 in Dentinal Fluid Correlates with Caries Lesion Depth. *Caries Res.* 2017 Aug 22;51(5):460–5.
14. Barbosa-Ribeiro M, Arruda-Vasconcelos R, de-Jesus-Soares A, Zaia AA, Ferraz CCR, de Almeida JFA, et al. Effectiveness of calcium hydroxide-based intracanal medication on infectious/inflammatory contents in teeth with post-treatment apical periodontitis. *Clin Oral Investig.* 2019 Jun;23(6):2759–66.
15. Ramarao S, Sathyanarayanan U. CRA Grid - A preliminary development and calibration of a paper-based objectivization of caries risk assessment in undergraduate dental education. *J Conserv Dent.* 2019 Mar;22(2):185–90.
16. Poorni S, Srinivasan MR, Nivedhitha MS. Probiotic strains in caries prevention: A systematic review. *J Conserv Dent.* 2019 Mar;22(2):123–8.
17. Manohar MP, Sharma S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. *Indian J Dent Res.* 2018 Nov;29(6):716–20.
18. Azeem RA, Sureshabu NM. Clinical performance of direct versus indirect composite restorations in posterior teeth: A systematic review. *J Conserv Dent.* 2018 Jan;21(1):2–9.
19. Jenarathanan S, Subbarao C. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: A randomized controlled clinical trial. *J Conserv Dent.* 2018 May;21(3):297–301.
20. Nandakumar M, Nasim I. Comparative evaluation of grape seed and cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. *J Conserv Dent.* 2018 Sep;21(5):516–20.
21. Malli Sureshabu N, Selvarasu K, V JK, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. *Case Rep Dent.* 2019 Jan 22;2019:7046203.
22. Siddique R, Nivedhitha MS, Jacob B. Quantitative analysis for detection of toxic elements in various irrigants, their combination (precipitate), and para-chloroaniline: An inductively coupled plasma mass spectrometry study. *J Conserv Dent.* 2019 Jul;22(4):344–50.
23. Teja KV, Ramesh S, Priya V. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. *J Conserv Dent.* 2018 Nov;21(6):592–6.
24. Rajakeerthi R, Ms N. Natural Product as the Storage medium for an avulsed tooth – A Systematic Review. *Cumhuriyet Dental Journal.* 2019 Jun 11;22(2):249–56.
25. Siddique R, Nivedhitha MS. Effectiveness of rotary and reciprocating systems on microbial reduction: A systematic review. *J Conserv Dent.* 2019 Mar;22(2):114–22.
26. Janani K, Sandhya R. A survey on skills for cone beam computed tomography interpretation among endodontists for endodontic treatment procedure. *Indian J Dent Res.* 2019 Nov;30(6):834–8.

27. Siddique R, Sureshababu NM, Somasundaram J, Jacob B, Selvam D. Qualitative and quantitative analysis of precipitate formation following interaction of chlorhexidine with sodium hypochlorite, neem, and tulsi. *J Conserv Dent.* 2019 Jan;22(1):40–7.
28. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. *Pesqui Bras Odontopediatria Clin Integr.* 2019;19(1):1–10.
29. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. *Clin Oral Investig.* 2017 Mar;21(2):567–71.
30. Khandelwal A, Palanivelu A. Correlation Between Dental Caries And Salivary Albumin In Adult Population In Chennai: An In Vivo Study. *BDS.* 2019 Apr 30;22(2):228–33.
31. Caviedes-Bucheli J, Azuero-Holguín MM, Moreno GC, González IL, Mateu E, Salazar JF, et al. Vasoactive intestinal peptide receptor expression in chronic periapical lesions [Internet]. Vol. 40, *International Endodontic Journal.* 2007. p. 521–5. Available from: <http://dx.doi.org/10.1111/j.1365-2591.2007.01250.x>
32. Jiang J, Zuo J, Chen S-H, Shannon Holliday L. Calcium hydroxide reduces lipopolysaccharide-stimulated osteoclast formation [Internet]. Vol. 95, *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 2003. p. 348–54. Available from: <http://dx.doi.org/10.1067/moe.2003.18>
33. Comparative Evaluation of Stability and Anti Bacterial Activity of various concentrations of triple antibiotic paste against *Streptococcus salivarius* – An in-vitro study. *Research J. Pharm. and Tech* 2018; 11(4):1327-1330.
34. Zehnder M, Wegehaupt FJ, Attin T. A First Study on the Usefulness of Matrix Metalloproteinase 9 from Dentinal Fluid to Indicate Pulp Inflammation [Internet]. Vol. 37, *Journal of Endodontics.* 2011. p. 17–20. Available from: <http://dx.doi.org/10.1016/j.joen.2010.10.003>
35. Vassiliou E, Jiang X, Delgado M, Ganea D. TH2 Lymphocytes Secrete Functional VIP upon Antigen Stimulation [Internet]. Vol. 109, *Archives of Physiology and Biochemistry.* 2001. p. 365–8. Available from: <http://dx.doi.org/10.1076/apab.109.4.365.4245>
36. Ferraz C, Dealmeidagomes B, Zaia A, Teixeira F, Desouzafilho F. In Vitro Assessment of the Antimicrobial Action and the Mechanical Ability of Chlorhexidine Gel as an Endodontic Irrigant [Internet]. Vol. 27, *Journal of Endodontics.* 2001. p. 452–5. Available from: <http://dx.doi.org/10.1097/00004770-200107000-00004>
37. Buck R, Cai J, Eleazer P, Staat R, Hurst H. Detoxification of Endotoxin by Endodontic Irrigants and Calcium Hydroxide [Internet]. Vol. 27, *Journal of Endodontics.* 2001. p. 325–7. Available from: <http://dx.doi.org/10.1097/00004770-200105000-00003>