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RESEARCH ARTICLE

EFFECT OF INTRACANAL MEDICAMENTS USED IN ENDODONTIC REGENERATION PROCEDURES ON MICROHARDNESS AND CHEMICAL STRUCTURE OF DENTIN

Shalu Krishan, Anurag Aggarwal and *Saloni Gupta

National Dental College and Hospital, Dera Bassi, India

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ABSTRACT

Objectives: This study was performed to investigate the effects of different intracanalmedicaments on chemical structure and microhardness of dentin.

Materials and Methods: Twenty Four human dentin discs were obtained from intact third molars and randomly assigned into three treatment groups. The threetreatment groups were irrigated with NaOCl, treated for four weeks with either 1 g/mL triple antibiotic paste (TAP), 1 g/mL - modified triple antibiotic paste (MTAP), or calcium hydroxide [Ca(OH)2] and finally irrigated with EDTA. Aftertreatment, one half of each dentin disc was subjected to Vickers microhardness (n = 10per group) and the other half was used to evaluate the chemical structure (phosphate/amide I ratio) of treated dentin utilizing attenuated total reflection Fourier transforminfrared spectroscopy (n = 4 per group). One-way ANOVA followed by Fisher's leastsignificant difference were used for statistical analyses.

Results: Dentin discs treatedwith different intracanal medicaments showedsignificant reduction in microhardness ($p < 0.0001$) and phosphate/amide I ratio ($p< 0.05$). Furthermore, dentin discs treatedwith TAP had significantly lower microhardness ($p < 0.0001$) and phosphate/amideI ratio ($p < 0.0001$) compared to all other groups.

Conclusions: Within the limitations of this study, the use of TAP in ER caused significantly higher dentin demineralization and reduction in dentin microhardness compared to MTAP or Ca(OH)2.The use of MTAP or Ca(OH)2 rather than TAP as intracanalmedicaments during ER may minimize the reduction in dentin microhardness and the change in chemical structure of superficial dentin.

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INTRODUCTION

The endodontic management of necrotic immature teeth is one of the most challenging treatments due to their thin fragile roots and blunderbuss apices. Calcium hydroxide [Ca(OH)2] apexification or artificial apical plug application using mineral trioxide aggregate (MTA) has been traditionally used to treat necrotic teeth with incomplete apices (Yassen, 2012; El-Meligy, 2006). However, these approaches require a long time to complete treatment and do not reinforce the thin and weak roots of immature teeth (Trope, 2010). Indeed, it has been suggested that the long term use of Ca(OH)2 during apexification procedures significantly reduces fracture resistance of the root (Andreasen, 2006 and Yassen, 2006), Endodontic regeneration (ER, pulp revascularization) has been used as a treatment option to manage necrotic immature teeth (Diogenes, 2013). In a recent study, ER was proposed to significantly increase the thickness and length of immature roots compared to teeth treated with Ca(OH)2 apexification or MTA (Jeeruphan, 2012). However, some cases treated with ER have been associated with suboptimal clinical outcomes where there is an apical closure with no increase in immature root thickness (Nosrat, 2012 and Nosrat, 2013). Root canal disinfection using intracanal medicaments is an essential requirement for endodonticregeneration (Fouad, 2011). Triple antibiotic paste (TAP), a mixture of metronidazole, ciprofloxacin, and minocycline, is themost widely used intracanal medicament in endodonticregeneration (Diogenes, 2011). However, crown discoloration has beenassociated with TAP (Kim, 2010; Petrino *et al*., 2010; Miller, 2012 and Nagata, 2014). Therefore, recent studies havesuggested substituting minocycline with another antibiotic (Diogenes, 2013 and McTigue, 2013). Clindamycin has been found to be effective againstvarious endodontic pathogens (Skucaite, 2010 and Gomes, 2011). A modified triple antibiotic paste (MTAP) composed of metronidazole, ciprofloxacin, and clindamycin was successfully used as an intracanal medicament to disinfect necrotic immature teeth during an endodontic regeneration procedure (McTigue, 2013).

Corresponding author: Saloni Gupta
National Dental College and Hospital, Dera Bassi, India

Sample preparation: Twenty four caries-free human third molars were selected forthis study. The molars were stored at 4ºin0.1% thymol and used within six months after extraction. A 1.5 mm coronal dentin disc was cross-sectioned fromeach molar using a low-speed saw (IsoMet, Buehler, Lake Bluff, IL, USA) with a diamond disc (15LC Diamond Wafering Blade, Buehler) under deionized running water (Figure 1). The non-pulpal outer sides of the dentin discswere flattened to a uniform thickness using a automaticpolishing machine (Struers Rotopol 31, Struers, Cleveland, OH, USA) with 500-grit silicon carbide grinding paper (Struers). The deep pulpal side of each dentin disc was polished with 1,200, 2,400 and 4,000 grit papers (Struers) and finally using a 1 µm diamond polishing suspension (Struers). As a final cleaning step, the polished specimens were sonicated in de-ionized water for 3 minutes.

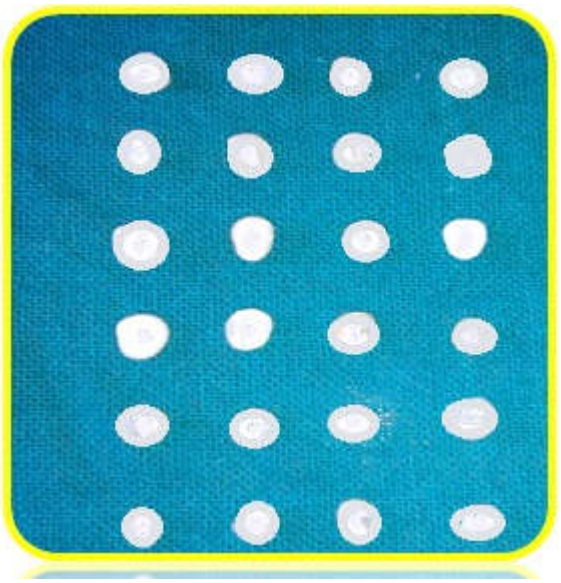

Figure 1. Sample preparation

Preparation of medicaments used in the study

To prepare the clinically used concentration of TAP (1 g/ mL), 1 g of United States Pharmacopeia grade antibioticpowders compounded of equal portions of metronidazole, ciprofloxacin and minocycline (Champs Pharmacy, SanAntonio, TX, USA) was mixed with 1 mL of sterile water (Ruparel, 2012). To prepare1 g/mL MTAP, 1 g of USP-grade antibiotic powderscomprising ciprofloxacin 14%, metronidazole 43% andclindamycin 43% (Skywalk Pharmacy, Wauwatosa, WI, USA) were mixed with 1 mL of sterile water. A commercial Ca(OH)2 intracanalmedicament (UltraCal XS, Ultradent, South Jordan, UT, USA) was also used in this study.

Treatment procedure

The dentin discs were randomized into three treatmentgroups (Figure 2). For each of thethree treatment groups, the pulpal side of each dentindisc was slowly irrigated with 20 mL of 1.5% NaOCl for5 minutes. Then, 0.1 mL of TAP, MTAP, or Ca(OH)2 paste was applied on the pulpal side of each specimen using a disposable syringe (BD, Franklin Lakes, NJ, USA). The treated dentin disks were kept for four weeks at 37ºin asealed 2 mL conical sample cup containing a cotton pellet saturated with distilled water. After four weeks, the treated side of each dentin specimen in all treatment groups was irrigated with 20 mL of 17% EDTA for 10 minutes. Theamount of intracanal medicaments applied in the three treatment groups (0.1 mL) was just enough to cover thepulpal surface of dentin discs with the medicament to avoid application of excessive amount of paste. The four weeks of intracanal medication and the time and volume of both EDTA and NaOCl irrigation used in this study were based on the current ER protocol recommended by AAE (http://www.aae.org/clinical-resources/regenerative-endodontics/considerations-for-regenerative-procedures.aspx).

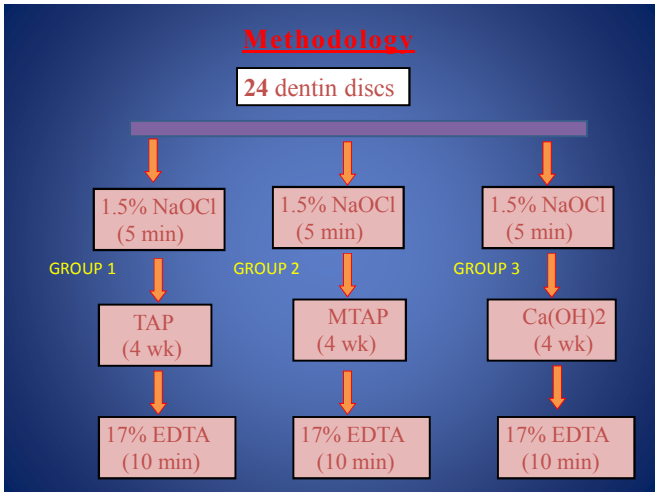

Figure 2. Treatment groups

Microhardness measurement

The treatment side of each dentin disc was bisected byan imaginary line into two halves and labeled (Figure 3).One half of each treatment side was randomly selected and subjected to micro hardness measurements using a Vickersmicrohardness tester (LM247, Leco, St. Joseph, MI, USA).Three indentations, spaced 200 µm apart, were made on each specimen using a 50 g load and a 10 second dwell time. The indentations were precisely measured using an optical microscope equipped with a digital camera and image analysis software. The representative hardness value for each specimen was obtained as the mean of the results from the three indentations.

Fourier transform infrared spectroscopy measurement

The chemical integrity of treated dentin was evaluated using Fourier transform infrared (FTIR) spectrophotometer (Jasco Inc., Tokyo, Japan) with a diamond attenuated total reflectance accessory. Five dentin disc samples were randomly selected from each group for FTIR measurement. Three different areas were selected from the other half, not subjected to micro hardness testing (Figure 3). FTIR spectra were then collected from the three selected areas in each sample between 800 and 2,000 cm-1 at 4 cm-1 resolution using 100 scans. Each obtained spectrum was processed by smoothing, baseline correction and normalization to the amide I peak using Spectra manager software (Jasco Inc.). The ratio of integrated areas of the phosphate v1 and v3peaks to the amide I peak was quantified to calculate the relative contents of these inorganic and organic components. The representative final ratio allocated for each dentin sample was obtained as the mean of the ratios obtained from the three single scans. Larger phosphate/amide I ratios compared to untreated dentin samples corresponded to a higher collagen deproteinization, whereas smaller phosphate/amide I ratios compared to untreated dentin samples corresponded to a higher dentin demineralization.

Statistical Analysis

All data were checked for normality using the Kolmogorov-Smirnov test and the normality assumptions were satisfied. The differences in microhardness and phosphate/amide Iratios among experimental groups were examined usingone-way ANOVA followed by Fisher's least significantdifference. Pearson correlation coefficients overall andby group were calculated to determine the correlations between phosphate/ amide I ratios and micro hardness measurements. A 5% level of statistical significance was applied for all analyses.

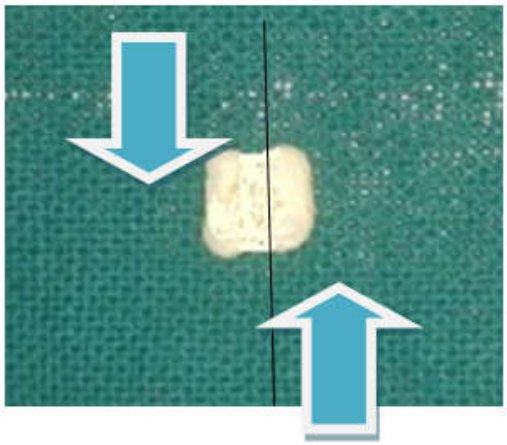

Figure 3. Dentin Disc divided in two halves

Figure 4. showing the Vickers microhardness of dentin treated with various intracanal medicaments and thatof the control group

TAP	Ca(OH)2	MTAP
15.20	35.32	31.43
10.59	28.45	29.63
17.34	32.89	32.34
19.46	38.89	31.78
15.72	38.93	28.37
10.38	33.21	35.21
11.11	35.89	29.12
14.28	35.63	31.22
15.50	35.60	32.45

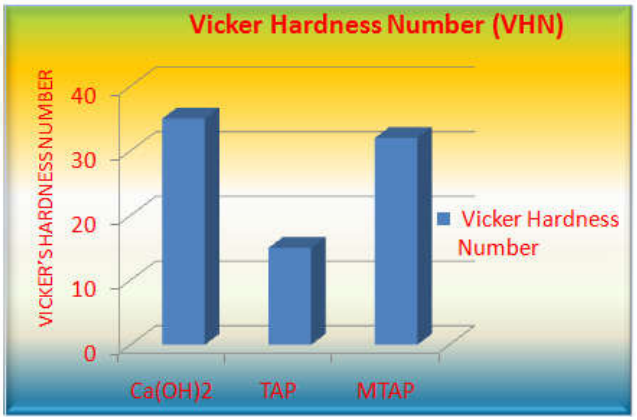

Figure 4. Bar graph showing the Vickers micro hardness of dentin treated with various intracanal medicaments and that of the control group

RESULTS

Figure 4 shows a significant reduction in meanmicrohardness values in NaOCl + EDTA treated dentinas well as dentin treated with TAP, MTAP and Ca(OH)2compared to untreated dentin ($p < 0.0001$). Furthermore, dentin treated with TAP had lowest micro hardness values compared to the other groups ($p < 0.0001$). However, no significant difference in mean micro hardness values was found between MTAP and Ca(OH)2 treated dentin. Figure 5 illustrates that untreated dentin had significantly higher mean phosphate/amide I ratio compared to dentin treated with NaOCl + EDTA ($p = 0.003$), TAP ($p < 0.0001$), DTAP ($p = 0.006$) and Ca(OH)2 ($p = 0.002$).

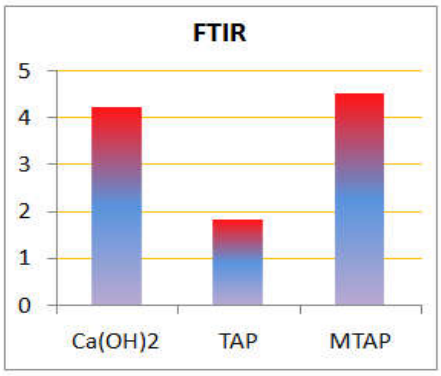

Figure 4. Bar graphs showing the phosphate/Amide I ratios of dentin treated with various intracanal medicaments and that of the control group

Additionally, dentin treated with MTAP, and Ca(OH)2 had significantly higher mean phosphate/amide I ratios compared to dentin treated with TAP ($p < 0.0001$).However, no significant differences in mean phosphate/amide I ratios were found between dentin treated with MTAP and Ca(OH)2 (Figure 5).

DISCUSSION

ER has gained popularity in the last 15 years (Diogenes, 2013). However; it is still questionable to get optimal treatment outcomessuch as continued root development with normal pulpdentincomplex (Geisler, 2012). Therefore, it is essential to preserve the mechanical, physical and chemical properties of root dentin from any negative effects of chemical agents used in ER. In this study, dentin treated with MTAP, and Ca(OH)2 caused significant reduction in microhardnesscompared to untreated control dentin. However, dentin treated with TAP caused significant reduction in micro hardness compared to all other groups. This could be explained by the strong demineralization effect of TAP reported in a previous study as well asthe negative effect of both NaOCl and EDTA irrigants on micro hardness of dentin (Yassen *et al.*, 2013). Hardness testing is not usually used to predict root fracture. However, the main advantage of performing a hardness test on dentin is that it is a well standardized indentation test that might be related to other mechanical properties such as tensile strength, compressive strength and modulus of elasticity (Kinney, 2003). A recent study found that a significant decrease in micro hardness of roots treated with TAP for one month was followed by significant reduction in root fracture resistance after three months treatment with TAP.[18] Our study also showed no significant differences in micro hardness between dentin treated with NaOCl +EDTA, MTAP, and Ca(OH)2. This indicates that both MTAP and Ca(OH)2 medicaments can be used in ER without causing any additional reduction in micro hardness compared to the use of the essential irrigation solutions alone. However, additional mechanical tests should be performed in future studies to confirm the findings of this study. It is also noteworthy to mention that bothCa(OH)2 and

lower concentrations of TAP (0.1 – 1 mg/mL) has been recommended in the recent ER literatures toavoid the toxic effect of the currently used 1,000 mg/m LTAP on stem cells of the apical papillae (Ruparel, 2012 and Althumairy, 2014). Additionally, these low concentrations of TAP were also found to be effective against endodontic pathogens (Sabrah, 2013). One of the limitations of studying the chemical structure of dentin with FTIR approach isthat the depth of penetration of infrared radiation in the FTIR technique is limited to a few microns. Therefore, the spectral data and the phosphate/amide I ratios reported inour study may only represent the net chemical change of superficial dentin after various treatment protocols rather than the whole chemical change across the total thickness of the dentin specimens. The overall high correlation between phosphate/amide I ratios and micro hardness values of dentin implies that the reported reduction indentin micro hardness among all treatment groups could be explained by the superficial demineralization effect following the ER protocols (reduction in phosphate/amideI ratios).

These findings generally agree with previous studies, which also suggested that dentin microhardness depends on mineral concentration (Kinney, 2003 and Featherstone, 1983). It is worth noting that the 10minutes irrigation with 20 mL of EDTA used in this studyhas been recommended by AAE during ER procedures (http://www.aae.org/clinical-resources/regenerative-endodontics/considerations-for-regenerative-procedures.aspx). This relatively long EDTA irrigation time was proposed to washout the remaining intracanal medicaments, open dentinal tubules and expose various growth factors during the ERprocedure (Geisler, 2012). However, prolonged EDTA irrigation for 10minutes was suggested to cause excessive dentin erosionand shorter EDTA irrigation time is usually recommended during routine endodontic therapy (Ozdemir, 2012 and Ozdemir, 2006). In this study, deep coronal dentin were used instead ofradicular dentin to guarantee enough dentin surface areato perform both micro hardness and FTIR measurement onthe same samples and obtain a valid correlation analysesbetween the two outcomes. A recent report suggested nosignificant differences in tubular density and tubular crosssection area between deep coronal and radicular dentin, regardless of the acidic challenge used (Caiado, 2010).

Conclusions

Within the limitations of this study, the use of TAP in ER caused significantly higher dentin demineralization and reduction in dentin microhardness compared to MTAP or Ca(OH)2. The use of MTAP or Ca(OH)2 rather than TAP as intracanal medicaments during ER may minimize the reduction in dentin microhardness and the change in chemical structure of superficial dentin.

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