



EVALUATION OF THE STABILITY AND ANTI BACTERIAL ACTIVITY OF VARIOUS CONCENTRATIONS OF TRIPLE ANTIBIOTIC PASTE AGAINST STREPTOCOCCUS MUTANS – AN INVITRO STUDY

Divya Subramanyam and Sujatha somasundaram

Department of Pedodontics, Saveetha Dental College, Chennai- 600077

ARTICLE INFO

Article History:

Received 27th February, 2017
Received in revised form 10th
March, 2017
Accepted 6th April, 2017
Published online 28th May, 2017

Key words:

Antibacterial activity, Stability,
Triple Antibiotic Paste

ABSTRACT

Aim: The Aim of this study is to evaluate the antibacterial effect of various concentrations of triple antibiotic paste against Streptococcus mutans by antimicrobial susceptibility testing.

Material and Methods: The stability and antimicrobial effect of triple antibiotic paste were tested by antibiotic susceptibility testing against Streptococcus mutans by using Agar disc diffusion method over the period of one day, 3 days and 7 days. Freshly prepared triple antibiotic past the samples were divided into three groups: Group 1, 1% TAP; Group 2, 2% TAP; Group 3, 3%; Group 4, Chlorhexidine (control). The zone of inhibition was measured after 24 hours and recorded in millimeters and the same procedure was repeated after 3 days and 7 days using triple antibiotic paste which was prepared on day one.

Result: Higher concentration of triple antibiotic paste (3%) showed superior antibacterial effect compared to 1%, 2% TAP and chlorhexidine. The antibacterial efficacy increased more rapidly after 7 days compared to 24hours and 3 days.

Conclusion: Under the limitations of this invitro study, higher concentrations of triple antibiotic paste can be used as an effective intracanal medicament.

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INTRODUCTION

The successful endodontic treatment depends upon completely eliminating the potential source of microorganisms causing persistent periradicular infections. Bacterial byproducts are principal factors responsible for periapical lesions and failure of endodontic therapy². Bacteria may invade the root canal system by diffusion through the dentinal tubules and elicit the inflammatory changes in the pulp-dentin complex³. In spite of adequate chemo-mechanical preparation, there is presence of various microorganisms surviving in the complex root canal system. Root canals harbor various aerobic and facultative anaerobes. Studies have shown the greater prevalence of streptococcus mutans in the root canals contaminated with direct exposure to the oral cavity⁴. Streptococcus mutans is a gram-positive, facultative anaerobe found in the infected root canals associated with apical periodontitis⁵. The coronal portion of the root canal exposed to the oral cavity is dominated by streptococci because it obtains energy from fermentable carbohydrates⁶. The literature shows the presence of 85% streptococcus in the root canals of deciduous teeth with pulp necrosis⁷. When pulpal involvement occurs due to carious lesion which consist more of streptococcus mutans, it may penetrate in to the root canals and can cause periapical infection. Various intracanal medicaments have been over

many years like formocresol, glutaraldehyde, calcium hydroxide, CMCP etc. Calcium hydroxide is a common intracanal dressing material used in endodontic therapies due to long term success. The systemically delivered antibiotics reaching the root canals is difficult compared to direct topical application of antibiotics in the root canals². A single antibiotic does not provide adequate sterilization and eliminate the microbes present in the root canals. Over the past decades the resistance of microorganisms to antibiotics has raised due to regular use of antibiotics for various infections⁸. Therefore to eliminate the variety of microbes present in the complex root canal system, a mixture of antibiotics like ciprofloxacin, metronidazole and doxycycline introduced by Hoshino *et al.* are used as a non surgical endodontic therapy to eradicate the resistant microbes. A periapical lesion responds to non surgical endodontic therapy by proper disinfection of the root canal system with the help of intracanal medicament. Antibiotics are essential for inhibition of bacterial infections. The cariology research unit of the niigata university School of Dentistry introduced the concept of "Lesion sterilization and tissue repair therapy". Non surgical endodontic therapy such as local application of antibiotics in the form of "LSTR" has been on rise over the recent years to avoid the possible side effects of systemic antibiotics⁹. Literature has shown that a mixture of Ciprofloxacin, Metronidazole and Doxycycline produce

sterilization of root canal dentin by penetrating and completely eradicating the resistant bacteria. Various studies evaluated the antimicrobial effectiveness of TAP against *S.mutans* but there is a lacunae of research regarding the stability of triple antibiotic paste at different concentrations against *S.mutans* over a period of 3 days. This study aims in determining the stability of TAP by evaluating the antibacterial effect of various concentrations of *S.mutans* by antimicrobial susceptibility testing.

MATERIAL AND METHODS

The study approval was obtained from the institutional review board, Saveetha Dental College. Triple antibiotic paste made of metronidazole, ciprofloxacin, doxycycline (1:1:1) were prepared by using sterile mortar and pestle at different concentrations using propylene glycol as a carrier in the ratio of 1:1 and stored under sterile conditions. The antimicrobial bacterial effect of triple antibiotic paste which was prepared on day one was tested by antimicrobial susceptibility testing against *Streptococcus mutans* by using Agar disc diffusion method over the period of one day, after 3 days and 7 days. The samples were divided into three groups: Group 1, 1% TAP; Group 2, 2% TAP; Group 3, 3%; Group 4, Chlorhexidine (control). Mueller- Hinton agar plates were used and inoculated with *S.mutans* and sterile discs with different concentrations of triple antibiotic paste were placed over it and incubated for 24 hours at 37 °C for 24 hours¹⁰. The zone of inhibition was measured after 24 hours and recorded in millimeters and the same procedure was repeated after 3 days. The zone of inhibition was measured after 24 hours and recorded in millimeters. The sterile discs (5 mm diameter) were dipped aseptically in different extracts for one minute and placed over nutrient agar plates seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 16 hours and observed for zone of inhibition. The diameter of inhibition zones was measured in millimeters.

Statistical Analysis

The results were analyzed statistically by the Kruskal-Wallis test at 5% significance level.

RESULTS

The inhibitory zones at various concentrations of triple antibiotic paste and Chlorhexidine against *S.mutans* are shown in [Table 1] and [Figure 1]. From the disc diffusion method, it was observed that the zone of inhibition for [Group 1; 1% TAP] was 30mm, 38 mm and 27mm at 24 hours, 3days and 7 days [Group 2; 2% TAP] was 34mm,42mm and 34mm [Group 3; 3% TAP] was 37mm ,45 mm and 39mm at 24h, 3 days and 7 days respectively. [Group 4; Chlorhexidine] showed a zone of inhibition of 17mm, 25mm and 29mm at 24h, 3 days and 7 days respectively. The greater antimicrobial effect of TAP was seen for higher concentration and over higher time period i.e after 7 days compared to other groups [Table 1]. The antimicrobial effect of triple antibiotic paste increased after 7 days. But chlorhexidine showed a greater zone of inhibition after 7 days compared to 24 h or 3 days of incubation against *S.mutans*.

Table 1 Comparison of Zone of Inhibition of 1%, 2%, 3% Triple antibiotic paste and Chlorhexidine against *S.Mutans*

Sample	Zone of inhibition <i>S.Mutans</i>		
	24 hours	3days	7days
1% Triple Antibiotic Paste	30mm	38mm	27mm
2% Triple Antibiotic Paste	34mm	42mm	34mm
3% Triple Antibiotic Paste	37mm	45mm	39mm
Chlorhexidine	17mm	25mm	29mm

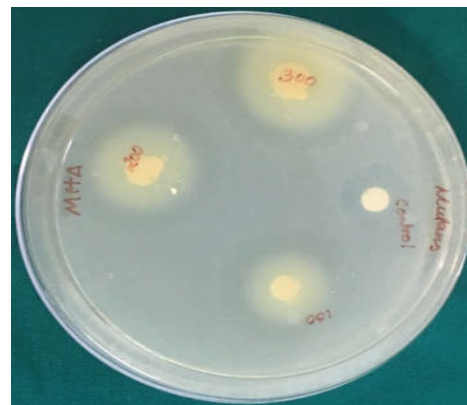


Figure 1 Zone of Bacterial Inhibition against *Streptococcus Mutans*

DISCUSSION

Coronal and apical sealing of root canal system in addition to complete debridement is necessary for successful prognosis of endodontic infections¹¹. Antimicrobial agents are necessary to provide complete disinfection over a period of time to remove the residual bacteria which remained untouched after mechanical instrumentation over a period of time¹². *S.mutans* was chosen as the test organism because studies have reported the presence of these species commonly associated with symptomatic apical periodontitis in root canal treated teeth and it shown to have resistance to calcium hydroxide¹³. *S.mutans* has the role in producing resistant biofilm which makes it more persistent in the root canals¹⁴. Numerous intracanal medicaments have been used over the years, but calcium hydroxide was the most commonly used which effectively disinfects the root canals, but it is said to have decreased antibacterial effect after mechanical removal¹⁵. Due to its alkalinity, it was seen to have less effect against resistant bacteria. In this study, chlorhexidine has been used as a control as it has many properties of an ideal intracanal medicament like superior antimicrobial activity, substantivity, lower cytotoxicity and its clinical effectiveness compared to other medicaments as it has the capacity to inhibit matrix metalloproteinase¹⁶. It removes the smear layer produced during mechanical instrumentation^{17, 18}. Chlorhexidine has better antibacterial property compared to Ca(OH) against resistant bacteria present in the root canal¹⁹. In dentistry, over the past many years antibiotics are used both systemically as well as topically to combat endodontic infections.

During systemic administration, the adequate concentrations of antibiotics reaching the complex root canal system are difficult. To overcome these complications, topical application of antibiotics into the root canals has been used in the form of intracanal medicaments to deliver appropriately high concentrations over short period of time¹⁷. Early research investigated the effectiveness of grossman's polyantibiotic paste (penicillin, bacitracin or chloramphenicol and streptomycin), it was found that it less efficacy when used as a root canal medicament²⁰. To overcome this hurdle triple

antibiotic paste has been used recently as an intracanal medicament during regenerative therapy¹⁵. The stability of TAP is tested by inoculating freshly prepared medicament over the period of 1 and 3 days. From this study, it is observed that 3% TAP has shown more antibacterial effect compared to 1% and 2% TAP at 3 days and 7 days. This indicates that if higher concentration of intracanal medicaments used, higher will be the antibacterial effect. This study clearly indicated that TAP when used in lower concentration (1% TAP) had less antibacterial effect compared to higher concentration (3%TAP). This was in agreement in other invitro studies which showed favourable effect of TAP²¹. The findings of this present study indicate that both TAP and chlorhexidine have substantiative antibacterial activity which lasts upto to 7 days. A study by Rosental *et al*, showed that chlorhexidine was effective upto 12 weeks. In this invitro study, the antibacterial effect of chlorhexidine was lower against S.mutans at all the time period compared to various concentrations of triple antibiotic paste. Study by Daniel B. Jenks *et al*, 2016 has shown that when double antibiotic paste when used for 4 weeks, it showed a significant greater antibiofilm effect compared to 1 week. In this study 1, 2 and 3% TAP was used because it has been reported that higher concentration of TAP may cause adverse effects on the stem cells of dental papilla²². In future more invivo and invitro studies are advocated to determine the stability and antimicrobial effect of TAP under proper oral conditions against s.mutans for a longer period of time to confirm the findings obtained from this study.

CONCLUSION

Under the limitations of this invitro study, 3% TAP exhibited greater antimicrobial effect when compared to low concentration and chlorhexidine which proves that triple antibiotic paste can effectively eliminate s.mutans from the root canals compared to chlorhexidine.

Acknowledgement

We thank the Department of Microbiology for their guidance and support.

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