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ANTIMICROBIAL EFFICACY OF TRIPLE ANTIBIOTIC PASTE IN ROOT CANAL PATHOSIS. AN INVITRO STUDY

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ABSTRACT

Background: There is incidence of microorganisms in the progression of pulp and periapical diseases and complete removal of these microorganisms from the infected root canals is a complicated . The bacteria in primary endodontic infections are mixed in origin, and usually are Gram-negative anaerobic rods, whereas Enterococcus faecalis is associated with secondary infection. The main goal of endodontic treatment is removal of these microorganisms and their by-products from the root canal space by using various antimicrobial agents to provide an environment free of microorganisms. Systemic antibiotics as an adjunct appear to be clinically effective in various surgical and nonsurgical endodontic cases. Their administration comes with the potential risk of adverse side effects and the development of resistant strains of microbes. Hence, in Root canal system local application of antibiotics isa more effective mode for delivering the drug. Poly-antibiotic paste was the first local antibiotic used which is a mixture of minocycline, (a tetracycline), ciprofloxacin and metronidazole. **Objective:** The aim of this study is to evaluate the antibacterial efficiency of Triple antibiotic paste in root canal pathosis. **Material and Methods:** Crowns of 45 extracted upper central incisor teeth were sectioned at the cemento-enamel junction. The samples were stored temporarily in physiological saline solution. The canals were shaped using crown down technique upto F4 protaper file. Enterococcus faecalis was inoculated on Brain heart infusion (BHI) agar plate and incubated anaerobically at 37 °C for 24 h. After incubation, the specimens were removed and then rinsed. In the negative group A (n=15), there was no treatment. In the other group B (n=15), the sections were treated with ledermix paste. The third group C (n=15) is treated with triple antibiotic paste. After disinfection with the medicaments the samples were subjected to CFU-counting evaluations. **Results:** After treatment, the elimination of bacteria and the smear layer in the medicaments and the positive groups B and C were significantly superior, compared with the negative group A ($p<0.01$). All the medicaments used in this study exerted antibacterial efficacy against *E. faecalis*. Triple antibiotic paste had the highest antimicrobial activity compared with ledermix paste. **Conclusion:** Both medicaments groups, especially the triple antibiotic group, showed satisfactory bactericidal effects in experimentally contaminated root canals.

INTRODUCTION

The development and progression of endodontically induced periapical lesion is clearly associated with the presence of microorganisms in the root canal system.[1] The infection of the root canal system is considered to be a polymicrobial infection, consisting of both aerobic and anaerobic bacteria.[2] The most prevalent organisms among the samples of secondary root canal infections are Enterococcus faecalis that are more prevalent organisms in the failed root canals.[3] The prevalence of complex root canal system results in sheltering the microorganisms from the effect of medicaments. A single antibiotic is insufficient to eradicate all polymicrobial flora; hence, triple antibiotic paste (TAP) is used to achieve complete disinfection. The combination that appearsto be most promising consists of metronidazole, ciprofloxacin, and minocycline.[4,5] Since penicillin discovery by Alexander Fleming in 1928, health care has entirely been revolutionized with the introduction of antibiotics.[6] In 1951, Grossman – Father of Endodontics – proposed the use of polyantibiotic paste – in a combination of penicillin, streptomycin, bacitracin, and caprylate sodium suspended in silicon vehicle to be used as an antibioticlocally.[7] The rationale of intracanal medicaments in modern endodontics is to reduce bacterial regrowth and possibly improve bacterial suppression.[8] Intracanal medicament is generally recommended when treatment cannot be completed in one appointment and there are chances that surviving intracanal bacteria often proliferate between the appointments.[9] There is no reason to counsel against single-visit endodontics. However, if multiple-visit

endodontics is chosen, an intracanal medicament is strongly recommended. Triple antibiotics paste was first tested for its effectiveness against *Escherichia coli*‐infected dentin *in vitro*.[10] The clinical effectiveness of the triple antibiotic paste in the disinfection of immature teeth with apical periodontitis has been reported.[11] Metronidazole (2%) has been shown to be superior to calcium hydroxide in inhibiting *E. faecalis*. However, in spite of its good antibacterial efficacy to eliminated intracanal bacterial flora, one potential concern of using an intracanal antibiotic paste is that it may cause bacterial resistance. In addition, intracanal use of minocycline can cause tooth discoloration, creating potential cosmetic complications. To overcome this disadvantage, double antibiotic paste eliminating minocycline has been advocated.[12]

MATERIAL AND METHOD

Fourty five extracted upper central incisor teeth were stored in 5.2% NaOCl solution for 30 min to remove organic residues and left in saline solution until the procedure began. The crowns were sectioned at the cemento‐enamel junction using high‐speed diamond disk to obtain root canal length of 15 mm. Access opening was done proglider path file and working length was established. The canals were shaped using crown down technique upto F4 protaper file by using X SMART plus endomotor system.

The strains of microorganisms used for the study were standard strains of E.faecalis which were sub‐cultured in agar plate (selective medium) and was incubated at 37°C for 24 h.

A pure, single E. fecalis colony was isolated from the same cultured plate and Gram's staining was done to confirm its growth, which was observed under microscope and then inoculated with a brain heart infusion (BHI) broth. The BHI broth was incubated at 37°C for a 24‐h period and checked for bacterial growth by changes in turbidity. A drop of BHI containing E.fecalis was placed into saline solution and checked for correct bacterial concentration.

The teeth were randomly assigned to three groups and placed into nutrient broth containing bacterial suspension.

Group A received no intracanal medicament. Specimens of Groups B and C were treated with ledermix paste and triple antibiotic paste respectively. After treatment with medicament the teeth were placed in vials, which contained 2 mL of the nutrient broth.

The vials were incubated at 37°C for 24 h. The vials were checked for turbidity after 24 h incubation. Overall 6 mL of broth from all the samples together was collected and seeded on a Petri dish containing UTI Hichrome agar in order to count the CFUs. Incubation lasted 48 h at 37°C. Grown colonies were seen in all groups and were identified by standard methods.

RESULT

Colony counts were done with the aid of a magnifying lens. Statistical analysis used was the simple proportion. The mean numbers for CFUs/mL of groups were: Group A: 187/153, Group B: 63/153 and Group C: 11/153. Higher mean CFU/mL is recorded in Group A followed by Group B and Group C, respectively.

DISCUSSION

To increase the efficiency of instrumentation, root canal irrigating solutions and intracanal medicaments are used to eliminate the bacteria from the root canals.[13] Antibiotics are used in dentistry both systemically and topically. During systemic administration of antibiotics, negligible concentrations reach the root canal, whereas during the local administration of antibiotics, greater concentrations can be used as intracanal medicaments, to decrease systemic consequences and complications. Because of the complexity of root canal infection, single irrigant or a medicament or an antibiotic could not result in effective sterilization of the root canal. Combination of irrigants or medicaments decreases the development of resistant bacterial strains and produces synergistic effect, whose antimicrobial action lasts longer and also sustains release of medicaments occurs.[14] Different studies have used E. faecalis to assess antibacterial effectiveness of intracanal medications. This is due to the strain being found in failed root canal treatment with persistent periapical pathosis and is highly resistant against antibacterial agents.[15-18] In the present work, contrary to the results found in the triantibiotic paste, the variability of live cells found in ledermix paste and control group was higher. This probably could be explained by the effect of neutralizing substances that can be found in the biofilms as dead cells or an exopolymeric matrix.

However, the antimicrobial effect of ledermix paste was superior to control group which does not show a significant effect against the biofilm. According to study conducted by Hoshino et al.[19] and Sato et al.[20] where triple antibiotic paste was shown to be effective even at high dilutions, suggesting that low concentrations of antibiotics may be enough to attain the required antibacterial effect. Another study done by Mehta et al.[21] which demonstrated significant inhibition of E. faecalis and C. albicans at 24, 48, and 72 hours by triple antibiotic paste. At all the time intervals, triple antibiotic paste achieved least optical density at 1:20 dilution (1.25 µg/mL)against E. faecalis; however, maximum inhibition of growth was seen only at highest concentration [(25 µg/mL at 24 h, 1:10 dilution, (2.5 µg/mL) at 48 hours, and 1:20 dilution (1.25 µg/mL) at 72 hours] for C albicans. Pinheiro *et al*. reported most of the 21 microbial isolates from root canals of filled teeth with persistent periapical lesions were susceptible to tetracycline and doxycycline. According to Nakornchai *et al.*, the 3-Mix antibiotic paste containing

Ciprofloxacin, Minocycline and Metronidazole is superior to vitapex for root canal treatment of pulpally involved primary molars.[22] But Kim *et al.*, and Lenherr et al, identified the discoloration caused by Minocycline used in tri-antibiotic paste.[23,24] Thomson and Kahler substituted Amoxicillin for Minocycline in their case report to avoid this discoloration.[25] Other medicaments such as Ledermix paste have been recommended as routine intracanal medicaments. Ledermix paste has been advocated as an initial dressing, particularly if the patient presents with endodontic symptoms.[26] It is a corticosteroid and antibiotic paste. Ledermix paste contains triamcinolone acetonide as an anti-inflammatory agent, at a concentration of 1%. Ledermix paste is a nonsetting, water-soluble paste material for use as root canal medicament or as a direct or indirect pulp capping agent. Studies have shown that triamcinolone is released from Ledermix paste in the root canal and can reach the systemic circulation via diffusion through dentinal tubules, lateral canals, and the apical foramen.[27] After the first 24 h, 30% of the triamcinolone was released. By the end of 14 weeks, the remaining 70% had been released. In a recent study, the groups treated with Ledermix, had significantly more favourable healing and more remaining root structure than the group filled with gutta-percha and sealer.[28] In the present study, triple antibiotic paste showed a considerable decrease in microbial pathogens from root canal of the sample teeth, the results of the present study were concordance with various studies conducted in the past.

CONCLUSION

Triple antibiotic paste was evaluated for its ability to remove smear layer and kill bacteria in vitro. The proprietary medicament demonstrated the ability to completely remove the smear layer and kill Enterococcus faecalis. The antimicrobial efficacy of triantibiotic paste and ledemix paste were effective, comparable, and reliable. Triple antibiotic paste a novel intracanal medicament, demonstrated potential remedy as for root canals due to its advantages of easy manipulation and efficient antimicrobial properties.

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