

“3Mix- MP in Endodontics – An overview”

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Abstract: Elimination of the bacteria in the root canal is the key goal in endodontic treatment. Mechanical instrumentation and irrigation alone does not render the canal free of bacteria. Repair of damaged tissue is expected after providing sterile environment. Due to the polymicrobial nature of the infected root canal, combination of antibacterial drug is required. The recent concept of lesion sterilization and repair using triantibiotic paste has suppressed the root canal pathogen to allow healing in large periapical lesion and vital tissue growth in immature teeth with periapical periodontitis. This paper reviews the rationale of antibiotic mixture compared to calcium hydroxide, antibacterial efficiency, current application, tissue reaction and disadvantages.

Key words: 3Mix-MP, triple antibiotic paste, regenerative endodontics, revascularization, periapical lesion, non surgical endodontics

I. Introduction

A tooth with a vital pulp is resistant to microbial invasion. In contrast, a necrotic pulp is rapidly invaded and colonized. Most of the bacteria in an endodontic infection are strict anaerobes. The number of colony forming units in an infected root canal is usually between 10^2 and 10^8 . A polymicrobial ecosystem is produced that selects for anaerobic bacteria over time. This suggests that a symbiotic relationship may lead to an increase in virulence by the organisms in that ecosystem [1]. Therefore the role of endodontic therapy is total disruption of the microbial ecosystem.

Due to the complex anatomy of the root canal, mechanical instrumentation leaves significant portions of the root canal walls untouched [2] and there is no evidence that instrumentation alone can completely eliminate microorganisms from the root canal. Therefore additional irrigants and medicaments are required to disinfect the root canal system to reduce the bacterial load to promote healing.

In recent years, The Cariology Research Unit of the Niigata University has developed the concept of 'Lesion sterilization and tissue repair LSTR' therapy [3] which employs the use of a combination of antibacterial drugs for disinfection of oral infectious lesions, including dentinal, pulpal, and periradicular lesions. Repair of the damaged tissues can be expected if lesions are disinfected. The purpose of this paper is to discuss the current application and efficiency of triple antibiotic paste in sterilization of lesions.

II. Calcium hydroxide

Calcium hydroxide has been used commonly as intracanal medicament. This is mainly due to its high alkaline PH and antibacterial action. The disinfecting action of calcium hydroxide is effective for at least for one week. Further delay in treatment for more than 1 month increases the susceptibility of re-infection [4], as it could be washed by the tissue fluids through open apex. However long-term use of calcium hydroxide can reduce the fracture resistance of the teeth [5].

Traditionally calcium hydroxide has been used in the case of immature teeth with pulp necrosis for apexification [4]. However, it induces calcified barrier when it comes in direct contact with the vital tissue in the root canal and prevent growth of pulp tissue inside the root canal space [6]. The use of triantibiotic paste for disinfection in immature teeth has allowed vital tissue to fill the canal space.

The use of calcium hydroxide has not been recommended [7] to prevent damage to the remaining viable pulp tissue and Stem cells from apical papilla (SCAP). The regenerative procedure suggests the preservation of the any viable tissue in the root canal system that harbors stem cells (i.e SCAP and Dental Pulp Stem Cells). Thus it has been speculated that surviving stem cells after disinfection, differentiates and lead to continue root formation.

III. Rationale of antibiotics

Antibiotics are only considered as an adjunct to definitive non – surgical or surgical endodontic therapy. Removal of etiology is the key goal of treatment. Antibiotics are mainly prescribed primarily to control active microbial infections, not for preventing the possibility of infections, unless the patient is medically compromised. In endodontics, systemic antibiotics are prescribed to suppress the infection in the pulp space and

/ or periapical area. For systemic antibiotic to kill bacteria, it has to be carried by the blood circulation in the pulp space to come in direct contact with the bacteria. Hence in an infected or necrotic pulp due to lack of blood supply, topical antibiotics may be effective in a highly constrained and restricted pulp space [8].

IV. Why combination of antibiotics

For pulp regeneration, both the pulp space and dentinal walls need to be disinfected sufficiently for ingrowth of vital tissue. The level of disinfection needed in regeneration is higher than necessary for nonsurgical endodontic therapy. Due to the polymicrobial nature of infected root canal, single empirical antibiotic is insufficient in disinfection of the root canal. Non specific antibiotic suppress most of the microbial flora and allow residual virulent micro-organisms to repopulate the root canal. Therefore it is essential to use combination of antibiotics to act against all endodontic pathogens and to prevent resistance.

The use of antibiotic in endodontics was first reported in 1951 by Grossman which was known as polyantibiotic paste (PBSC). PBSC is a mixture of penicillin, bacitracin, streptomycin and caprylate sodium. Penicillin was used for targeting against Gram-positive organisms, bacitracin for penicillin-resistant strains, streptomycin for Gram-negative organisms and caprylate sodium to target yeasts [8]. Recently triantibiotic paste containing ciprofloxacin, metronidazole and minocycline has been introduced for lesion sterilization and repair.

Metronidazole is a nitroimidazole compound that exhibits broad spectrum of activity against protozoa and anaerobic bacteria. Metronidazole is selectively toxic to anaerobic microorganisms. Its nitro group is reduced by certain redox proteins to highly reactive nitro radical after entering the cell. It binds to the DNA, disrupts the helical structure and lead to rapid cell death [9]. Tetracycline, which includes doxycycline and minocycline are primarily bacteriostatic, inhibiting protein synthesis by binding to 30S ribosomes in susceptible organism. They exhibit broad spectrum of activity against gram positive and gram negative microorganisms. Minocycline is a semisynthetic derivative of tetracycline with a similar spectrum of antibacterial activity. Ciprofloxacin is a synthetic fluoroquinolone with rapid bactericidal action. It inhibits the enzyme bacterial DNA gyrase, which nicks the double stranded DNA, introduces negative supercoil and then reseals the nicked end. The bactericidal action probably results from digestion of DNA by exonucleases whose production is signaled by the damaged DNA. It exhibits very potent activity against gram negative bacteria but very limited activity against gram positive bacteria [9]. Most of the anaerobic bacteria are resistant to ciprofloxacin. Hence it is often combined with metronidazole in treating mixed infections.

V. Composition of 3Mix-MP

V.I According to Hoshino et al [10]

- Antibiotic (3Mix) – ratio 1:1:1
- Ciprofloxacin 200mg, Metronidazole 500mg, Minocycline 100mg
- Carrier (MP) – ratio 1:1
- Macrogol ointment, Propylene glycol

3Mix is incorporated into MP using the following

- 1:5 (MP:3Mix)
- 1:7(standard mix)

V.II According to Takushige T et al [11]

The drugs are powdered and mixed in a ratio of 1:3:3 (3 Mix) and added either with macrogol-propylene glycol (3 Mix-MP) or a canal sealer (3 Mix-sealer).

VI. Vehicle/carrier

The ideal or optimum vehicle for delivery of antibiotics in root canal should have ability to facilitate better diffusion of medicament through dentinal tubules and anatomical aberrations like fins, isthmuses and blocked canals. Therefore diffusion of antibiotic into cementum and periradicular tissue may be advantageous.

Hoshino et al [10] used propylene glycol and macrogol for delivery of triple antibiotic paste. Cruz EV et al [12] investigated the penetration effect of propylene glycol into root dentine. The area and the depth of penetration of Safranin O dye in propylene glycol were reported to be significantly greater than dye with distilled water into root dentine. The presence of smear layer significantly delayed the penetration of the dye in this study indicating the need for their removal for better diffusion of medicament. The penetration of the drug mixture is enhanced efficiently through the prepared canal especially after ultrasonic irrigation [13]. Thus propylene glycol is a useful vehicle for delivering intracanal medicaments in root canal system.

In an in vitro study the antimicrobial action of ciprofloxacin, metronidazole and polyethylene glycol and natrosol vehicles were assessed against 23 strains [14]. It was concluded that ciprofloxacin presented antimicrobial action against all tested bacterial strains, and its association with metronidazole was reported to be synergistic. The vehicle polyethylene glycol showed antimicrobial effect and the ciprofloxacin/polyethylene

glycol association was the most effective combination for reducing the tested bacteria and yeasts. Thus delivery of the drug with ideal vehicle into root canal reduces the bacterial loads in the infected root canal.

VII. Antibacterial efficiency

In vitro antibacterial efficiency of a mixture of ciprofloxacin, metronidazole and minocycline (3Mix), with and without the addition of rifampicin (100 µg each/ml) (4Mix), against oral bacteria of children was assessed by Sato et al. The antibiotic combinations were observed to be effective against both carious and endodontic lesions in vitro [15]. Hoshino et al determined that 25 µg each/ml of ciprofloxacin, minocycline and metronidazole antibiotic mixture to be effective in sterilizing the infected root dentin in vitro [10]. Sato et al studied the ability of a mixture of ciprofloxacin, minocycline and metronidazole (0.5mg of each) in an in vitro study to eliminate experimental infection in deep layers of root dentin by E.coli [13]. In other in vitro study the minimal inhibitory combination for ciprofloxacin and minocycline against E. faecalis and E. faecium were found to be 5 and 20 µg respectively and metronidazole was reported to have no inhibitory effect. However, as combination (100µg each /ml) they inhibited the growth of every strain completely [16]. Thus this suggests 3mix may be effective in persistent endodontic infection.

Windley et al assessed the efficiency of triple antibiotic paste in disinfection of immature dog teeth with apical periodontitis. The samples were assessed before and after irrigation with 1.25% NaOCl and after dressing with triple antibiotic mixture. Of the 30 samples that were cultured before treatment, 90% remained positive following irrigation with 10ml of NaOCl. This level dropped to 30% following application of triple antibiotic paste for 2 week signifying the efficiency of triple antibiotic mixture [17]. Madhubala MM et al compared the antimicrobial activity of calcium hydroxide, triantibiotic mixture, and an ethanol extract of propolis as intracanal medicaments on Enterococcus faecalis-infected root canals. The percentage reduction in colony forming unit was reported to be highest for propolis showing 100% reduction on day 2 followed by triantibiotic mixture showing 82.5%, 92.2%, and 98.4% of reduction on days 1, 2, and 3, respectively. Calcium hydroxide showed a gradual increase in antibacterial activity with a maximum of 59.4% on day 7 [18]. The ciprofloxacin and metronidazole combination with calcium hydroxide was reported to be effective against all the four species (S. aureus, Pseudomonas aeruginosa, Enterococcus faecalis and B. fragilis) tested when compared with calcium hydroxide, iodine potassium iodide and iodoform. This suggests the drug combination can be effective in mixed bacterial flora as in infected root canal [19].

VIII. Avulsion scenario

Various studies on traumatic injuries to immature teeth have shown promising results for revascularization in replanted avulsed immature teeth. In these cases, the uninfected necrotic pulp may act as a scaffold for ingrowth of vital tissue. The absence of the bacteria is the key determinant in success for revascularization in avulsed teeth. In immature teeth with apical periodontitis, the disinfection of the necrotic infected root canal can render the scenario similar to avulsion. Therefore disinfection of the necrotic infected root canal system can create an environment conducive to revascularization.

In a study with experimentally avulsed immature dog teeth, they reported that prior treatment with topical minocycline before replantation resulted in revascularization of 91% of the teeth whereas topical doxycycline resulted in revascularization in 73% of the teeth. The use of topical minocycline and doxycycline resulted in higher success in revascularization compared to the control teeth soaked in saline before replantation which resulted in 33% success[20]. Therefore by creating aseptic environment, there is ingrowth of vital tissue from periapical region.

IX. Application in regeneration/ revascularization

In recent years the popularity of regenerative procedure has led to shift towards less invasive procedure in endodontics. The clinical disinfection protocol in revascularization procedure followed in these studies can be summarized as: The antimicrobial paste is placed in the canal to a depth slightly short of the remaining vital tissue with a lentulo spiral. The accessed cavity is sealed with 2 mm-thick cavit and then glass ionomer cement to create double seal. After 2 weeks, the patient is recalled for review. If the tooth is asymptomatic with lack of clinical signs of pathology in the next visit, the canal is reentered and irrigated with NaOCl and chlorhexidine followed by saline and dried. Then bleeding is induced inside the canal by over instrumentation. After blood clot is formed, MTA is placed 3mm below the level of CEJ. The access cavity is sealed with bonded restoration and tooth is reviewed periodically. Table 1 shows successful revascularization of case reports using triantibiotic paste. Thus triple antibiotic paste is effective for disinfection of the infected necrotic tooth to create a favourable environment for regeneration of vital tissue.

Bose et al [32] in a retrospective study used geometric image program to calculate continued development of root length and dentin wall thickness from radiographs from 54 published and unpublished endodontic regenerative cases and 40 control cases (20 apexification and 20 nonsurgical root canal treatments). The results

showed regenerative endodontic treatment with triple antibiotic paste and Ca(OH)₂ produced significantly greater increases in root length than either the MTA apexification or non surgical root canal treatments control groups. The triple antibiotic group showed the highest percentage increase in the dentin wall thickness compared with the Ca(OH)₂ or formocresol groups. The position of Ca(OH)₂ also influenced the outcome. When Ca(OH)₂ was radiographically restricted to the coronal half of the root canal system, it produced better results than when it was placed beyond the coronal half. Lovelace et al [33] demonstrated that the evoked-bleeding step in regenerative procedures after disinfection with triantibiotic paste triggers the accumulation of undifferentiated stem cells into the canal space from periapical region. These cells might contribute to the regeneration of pulpal tissues after effective disinfection. Thus teeth subjected to disinfection with sodium hypochlorite associated with the triple antibiotic paste has been reported to show significant reduction of periapical lesions, gain in root length, and increased wall thickness [34].

X. Application in Large periradicular cyst like lesion

Large periradicular cyst like lesion that has direct communication with the root canal system responds favourably to nonsurgical root canal treatment. Researchers have reported that proper infection control and sterilization of root canal system and periradicular region favor good healing of periradicular lesion. Table 2 shows case reports of successful healing of periradicular region after triantibiotic paste intracanal medication. Thus from the literature it is clear that triantibiotic paste was changed every month until elimination of symptoms to facilitate periapical bone healing.

On the contrary, EndoVac system has been suggested to be more effective than conventional irrigation and triantibiotic paste dressing. Histological evaluation of apical negative pressure irrigation (EndoVac system) was reported to present more exuberant mineralized formations, more structured periapical connective tissue and a more advanced repair process than conventional irrigation and triantibiotic paste [40]. Cohenca N et al assessed the in vivo efficacy of two root canal disinfection techniques in immature dog teeth with apical periodontitis. The microorganisms were absent in 88.6% of root canals treated with EndoVac system and 78.28% of root canals treated with conventional irrigation and triantibiotic paste[41]. Thus following effective root canal disinfection protocol reduces periapical lesion size and improves root development.

XI. Application for primary teeth

The clinical studies in infected primary teeth suggest 3Mix to be an effective root canal treatment material as shown in table 3. Both 3Mix and vitapex have been suggested to be effective root canal treatment material for primary teeth as there was no statistical significance in radiographic success. Both groups showed 100% and 96% clinical success at 6 and 12 months, respectively [42]. Pinky C et al replaced metronidazole with Ornidazole as it had longer duration of action, better efficacy and slower metabolism when compared with metronidazole. The study concluded that there was no statistical difference between metronidazole and ornidazole groups, group containing orindazole was reported to exhibit better results during the evaluation period of 3, 6 and 12 months in infected primary teeth [43].

Though the various studies have been reported the effectiveness of triantibiotic paste, Trairatvorakul C et al suggested that non instrumentation endodontic treatment using 3Mix –MP cannot replace conventional root canal filling material as a long term therapy in primary teeth. In this clinical study non-instrumentation endodontic treatment using 3Mix-MP in primary mandibular molars showed good clinical success but had a low success rate based on radiographic evaluation at 2-year follow-up [44].

XII. Root fracture

Er K et al described the treatment of a horizontal root fracture in a maxillary central incisor using a triple antibiotic paste and mineral trioxide aggregate. In this case report, the coronal root fragment was irrigated with 1% sodium hypochlorite and triple antibiotic paste was used as intracanal medicament. The coronal fragment was obturated with MTA. At follow-up examination after 12 months, the tooth was asymptomatic and radiographically showed repair of the fracture region. [45].

XIII. GIC + triantibiotic paste

Yesilyurt C et al [47] evaluated the antibacterial effects, physical properties and bonding strengths of conventional glass-ionomer cements (GICs) containing antibiotics and determined the optimal concentration of antibiotics addition for use with the ART approach. Three antibiotic mixtures, ciprofloxacin, metronidazole and minocycline, were added to powdered GIC (Fuji IX) to obtain concentration ratios of 1.5, 3.0 and 4.5% w/w. The GIC-containing antibiotics were effective in inhibiting S Mutans and L Casei. The addition of a 1.5% antibiotic mixture was optimal for adequate physical and bonding properties.

XIV. Tissue reaction

JE Gomes-Filho et al [46] evaluated the response of 30 rat subcutaneous tissues to implanted polyethylene tubes that were filled with triantibiotic paste or calcium hydroxide and another empty tube as control. 30 additional rats received 2 individual implants consisting of polyethylene tubes filled with dressing material carriers (macrogol and propylene glycol) and a sham procedure. Both dressing materials induced moderate reactions at 7 and 15 days. These reactions were similar to the control and reduced in intensity (to mild) from day 30 onward. The carriers did not interfere with the reaction of the dressing materials. The study concluded that the both triantibiotic paste and calcium hydroxide paste are biocompatible .

XV. Disadvantage

XIV.I Discolouration

Even though the triple antibiotic paste initially introduced by Hoshino et al eradicates bacteria in the root canal, it had a disadvantage of causing aesthetic problem leading to tooth discolouration. Kim et al 2010 identified the discolouration was caused by minocycline in an in vitro study [48]. Ledermix an intracanal medicament containing tetracycline has also shown to cause greater discolouration in immature teeth than mature teeth.

Lenherr et al investigated the discolouration potential of various endodontic materials in bovine tooth model. The most severe discolouration was reported to be detected after 12 months in triple antibiotic paste group followed by ledermix paste indicating the effect of tetracycline [49].

Some variation of the antibiotic paste have been tried to prevent discolouration. Either an antibiotic alternate to minocycline is used or it is omitted (biantibiotic paste). Trope et al suggested that Arestin can be used as substitute for minocycline to markedly reduce discoloration [4]. Thomson A and kahler B substituted amoxicillin for minocycline in his case report to avoid discoloration [26].

Other alternate measure is to eliminate or reduce discoloration through internal bleaching. Miller EK et al performed internal bleaching to remove the cervical discolouration from the triple antibiotic paste six months later. During follow up period of 18 months, the tooth remained sensitive to vitality test and showed evidence of continued root development [50]. A novel approach to seal dentinal tubules of the pulp chamber to avoid crown discolouration has been described by Reynolds K et al [25]. The inner surface of the coronal access was etched, rinsed, bonding agent applied and cured. A canal projector with size 20 K file was placed inside the prepared access to maintain patency. The space between the projector and the coronal dentine was sealed with flowable composite and light cured for 30 sec. The projector was then removed by engaging it with a Hedstrom file. Then tri-antibiotic paste was introduced into the canal using backfill approach with 20G needle.

XIV.II Bacterial resistance

Another concern regarding the use of antibiotic is development of bacterial resistance.

XIV.III Safety

Although the volume of the drug used in LSTR therapy is small and there were no reports of side effects, care should be taken if patients are sensitive to chemicals or antibiotics.

XVI. Conclusion

- The combination of irrigation and disinfection with triple antibiotic paste protocol allow apical root closure in regenerative endodontic procedure and periapical lesion healing in nonsurgical endodontic therapy.
- The addition of triple antibiotic paste with propylene glycol allows efficient delivery deep into dentinal tubules and beyond cementum, thereby enhancing the healing of large peri-radicular lesion.
- 3Mix can be used as root canal filling material in primary endodontic teeth.

Thus from the literature, it is clear that triple antibiotic paste is effective in disinfection of root canal and successful healing of large periradicular lesions.

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Table 1 shows successful revascularization of case reports using triantibiotic paste.

Author & year	Treatment protocol	Irrigation	Intracanal medicament	Treatment outcome (clinically and radiographically)
Iwaya et al 2004 [21] No of cases :1 Tooth no : 45 Age / sex :13/F Diagnosis: Apical periodontitis with intraoral sinus tract	1 st visit access cavity prepared but left patent for drainage 5 weekly visit- access prepared to orifice level, no mechanical instrumentation medicated 6 weekly visit- vitapex placed close to vital tissue. sealed with glass ionomer and adhesive composite resin	5% sodium hypochlorite (NaOC) & 3% hydrogen peroxide	Metronidazole and ciprofloxacin	5 month – first sign of apical closure 15 month- access cavity reopened, dentin bridge formation on clinical evaluation, positive response to electric pulp testing. 30 month – complete closure of apex and thickening of root wall on radiographic evaluation
Banchs F & Trope M 2004 [7] No of cases :1 Tooth no : 45 Age / sex :11/M Diagnosis: Apical periodontitis with intraoral sinus tract	1 st visit- access, no instrumentation, disinfection and sealed with cavite 26 days– irrigation and bleeding induced with endo explorer. After 15 min, MTA, wet cotton pellet and cavite placed. 2 weeks – composite restoration	10ml of 5.25% of NaOCl & 0.12 % chlorhexidine (CHX)	3Mix (Hoshino et al) spun down the canal to a depth of 8mm with lentulospiral (for 26 days)	6 month- resolution of periapical radiolucency 1 – 18 month- continued development of apex 2 year- closure of apex and thickening of dentinal wall. Positive response to cold test.
Thibodeau B and Trope M 2007 [22] No of cases :1 Tooth no : 11 Age / sex :9/M Diagnosis: Acute apical abscess	1 st visit- incision and drainage 2 nd visit- access, no instrumentation, disinfection and sealed with cavite 11 weeks- irrigation, bleeding induced with file till CEJ. MTA placed over blood clot. 1hour later bonded composite placed	1.25 % NaOCl	Metronidazole, ciprofloxacin & cefaclor paste placed with lentulospiral (11 weeks)	3 months- asymptomatic . no response to pulp test (CO ₂ ice) 6- 12 months- gradual apical closure. Diffuse radiopacity in the root canal
Petrino et al 2007 [23] No of cases :1 Tooth no : 11 Age / sex :8/M Diagnosis: Chronic periradicular abscess	1 st visit- access, no instrumentation , irrigation, medicated and sealed 2 nd visit- bleeding induced with endoexplorer till 3mm below CEJ	5.25% NaOCl and 0.12% CHX	3Mix	8 months- apical closure with thickening of dentinal wall on radiographic examination.

<p>Jung IY et al 2008 [24] No of cases :8 Tooth no : 35 and 45 Age: 9 – 14 yrs Diagnosis: Pulpal necrosis and apical periodontitis</p>	<p>1st visit- access, disinfection and sealed with cavit 2nd visit(1 -2 week later)- 4 cases treated without induction of blood clot, while other treated with blood clot and MTA placed. 3rd visit(2 -3 weeks later)- bonded resin restoration</p>	<p>2.5 - 5.25 % NaOCl replaced every 3 minutes for total 30 minutes</p>	<p>6 cases – 3Mix paste with lentulospiral (1- 2 weeks) as described by Hoshino et al. 2 cases - Ca(OH)₂</p>	<p>6- 12 months- asymptomatic, complete resolution of radiolucency and canal space narrowed 1 - 2 years- continued thickening of the dentinal wall with apical closure.</p>
<p>Reyonlds K et al 2009 [25] No of cases : 1 Tooth no : 35 and 45 Age/ Sex: 11/F Diagnosis: Pulpal necrosis with chronic supprative periradicular periodontitis (dens evaginatus)</p>	<p>1st visit- access, no instrumentation, irrigation, coronal dentin sealed with flowable composite, medicated and sealed with cavit 2nd visit (1 month later)- bleeding induced with 20 size k file. Sealed with MTA and cavit 3rd visit (2 weeks later) – bonded resin restoration</p>	<p>6 % NaOCl followed by saline and 2 % CHX</p>	<p>(250 mg /each) 3Mix mixed with sterile saline. (250 mg /each) 20G needle placed 2mm short of working length and filled with backfill approach upto CEJ . (for 1 month)</p>	<p>18 month- asymptomatic Positive response to cold test (1,1,1,2 tetrafluoroethane). Periradicular bone healing and significant root development with maturation of root</p>
<p>Thompson A and Kahler B 2009 [26] No of cases : 1 Tooth no : 35 Age/ Sex: 12/F Diagnosis: Pulpal necrosis with chronic periapical abscess</p>	<p>1st visit – access, disinfection and sealed with cavit and Fuji IX 2nd visit (3 weeks later)- redresses due episode of pain. 20ml of NaOCl was ultrasonically agitated with 10 size k file for 1 minute for each 5ml. redressed with triple antibiotic paste 3rd visit (3 weeks later)- ultrasonic irrigation, bleeding induced with D11T NiTi spreader. Sealed with MTA, Fuji IX and composite restoration</p>	<p>30ml of 1% NaOCl</p>	<p>(20mg/ each) ciprofloxacin, metronidazole and amoxicillin mixed with 1ml of sterile saline. Placed with lentulospiral. Left for 3 weeks</p>	<p>18 month – asymptomatic Positive response to electric pulp testing, Osseous healing with maturation of root formation</p>
<p>Ding RY et al 2009 [27] No of cases : 12 Tooth no : 5 incisors and 7 premolar Age : 8 - 11 years Diagnosis: Periapical periodontitis</p>	<p>1st visit – canal left patent for abscess cases for 3 days 2nd visit -access , disinfection and sealed with Coltosol F 3rd visit (1 week) - bleeding induced with 40 size k file. MTA placed and sealed 4th visit (1 week) - bonded composite restoration</p>	<p>20ml of 5.25% NaOCl</p>	<p>Mixture of ciprofloxacin, metronidazole and minocycline as described by Hoshino et al for 1 week</p>	<p>Six patient dropped out of the study (due to pain or failure to induce bleeding) and standard apexification procedure performed 3 patient did not return for recall appointment 12 – 15 month - remaining 3 patient exhibited positive response to electric pulp testing Apical closure and normal periradicular architecture on radiographic examination</p>
<p>Petrino A et al 2010 [28] No of cases : 3(5 teeth) Tooth no : 11, 21, 41, 45,21 Age : 8 - 11 years Diagnosis: Pulp necrosis with apical periodontitis- 3 teeth chronic periapical abscess – 2 teeth</p>	<p>2nd visit – access, disinfection and sealed with cavit 3rd visit- irrigation, bleeding induced with endo explorer till 3 mm below DEJ. Sealed with MTA and cavit 4th visit- cavit replaced with bonded restoration</p>	<p>10ml of 5.25% NaOCl and 0.12 % CHX</p>	<p>Metronidazole, minocycline and ciprofloxacin (100mg of each drug in a 0.5ml total volume)</p>	<p>6 month- 1year – asymptomatic Apical closure with thickening of dentinal wall and root length Healing of apical radiolucency. 1 case showed no increase in thickness of root wall and length</p>

Nosrat A et al 2011 [29] No of cases : 2 Tooth no : 36, 46 Age / Sex : 8/F, 9/M Diagnosis: pulpal necrosis with apical periodontitis	1 st visit- access, instrumented to increase apical diameter 1- 2 mm, medicated and sealed with glass ionomer cement 3 week later- bleeding induced, calcium enriched mixture placed	5.25% NaOCl for 20 minutes	3 weeks of metronidazole, ciprofloxacin, and minocycline paste dressing.	Asymptomatic, and functional, periapical radiolucencies were healed, and roots continued to develop.
Torabinejad M and Turman M 2011 [30] No of cases : 1 Tooth no : 15 Age / Sex :12/M Diagnosis: pulpal necrosis with apical periodontitis	1 st visit- access, irrigation, medicated and sealed 22 days later- platelet rich plasma injected upto the level below the CEJ and MTA was placed. 3days later – double seal restoration placed	5.25% NaOCl	Triple antibiotic paste mixed with distilled water and left in canal for 22 days	5 ½ months - resolution of the periapical lesion, further root development, and continued apical closure
Kim DS et al 2012 [31] No of cases : 3 Tooth no : 35 Age / Sex : 10-12/M Diagnosis: pulpal necrosis with apical periodontitis	1 st visit – access, irrigation, medicated and sealed with caviton 2weeks later – bleeding induced with 10 size K file below CEJ MTA was placed and sealed with caviton 3 rd visit- caviton replaced with composite	3% NaOCl	Triple antibiotic paste mixed with distilled water and placed with lentulospiral. Left in canal for 2 weeks.	24- 48 months- increase in root thickness and periapical radiolucency disappeared completely radiographically

Table 2 shows case reports of successful healing of periradicular region after triantibiotic paste intracanal medication.

Author & year	Radiographic finding	Treatment protocol	Irrigation	Intracanal medicament	Treatment outcome
Ozan et al 2005 [35] No of cases :1 Tooth no : 46 Age / sex : 15/M Diagnosis: Radicular cyst	Well circumscribed radiolucency involving apices of 45, 46, 47	1 st visit – access, instrumented with size 15 – 40 size k files using step back technique, drainage performed through the canal until drainage ceased, sealed with zinc oxide eugenol No medicament placed. Continued for next 15 days. When drainage ceased, triantibiotic paste placed, sealed 2 months later irrigated, obturated with AH plus sealer and gutta percha using lateral condensation technique, sealed with amalgam	2.5% NaOCl with 27 gauge needle	Mixture of ciprofloxacin, metronidazole and minocycline as described by Takushige et al spun down with lentulospiral. Medicament placed for 2 months	2 month – bone expansion stopped 5- 7 month- gradual bone healing 7 month- absent radiolucent lesion with well defined trabeculae
Akgun OM et al 2009 [36] No of cases :1 Tooth no : 11 Age / sex : 8/F Diagnosis: Apical periodontitis	Open apex with periapical radiolucency	1 st visit- access, instrumented to increase apical diameter 1- 2 mm, medicated and sealed with glass ionomer cement 1 -12 month- Medicament changed every 1 month for 12 month After 12 month – conventional endodontic treatment with ca(OH) ₂ based sealer, obturated with vertical condensation, Coronal fragment reattached with adhesive composite restoration	2.5% NaOCl	Mixture of ciprofloxacin, metronidazole and minocycline as described by Hoshino et al for 1month. Dressing changed every month for 12 month until healing	4 month – asymptomatic Complete resolution of radiolucency and continued development of root apex 1 year – closure of apex 2 year- no sign of radiolucency

Kusgoz A et al 2009 [37] No of cases :1 Tooth no : 32, 33 Age / sex : 12/M Diagnosis: Previously root canal treated teeth with periapical abscess	Periapical radiolucency with well defined margin	1 st visit- access, root canal material retrieved with Gates Glidden and k file, canal instrumented with step back technique, Ca(OH) ₂ dressing placed 2 nd and 3 rd visit- Ca(OH) ₂ dressing changed every 2 weeks for total of 3 times. Failure to eliminate symptoms- Triple antibiotic medication 3 months later- irrigated obturated with gutta percha and AH sealer using lateral condensation for 33, apical MTA placed in 32 and obturated 24 hours later	2.5% NaOCl with 27G needle	Initial visit – Ca(OH) ₂ . Persistent symptom- changed to triple antibiotic paste as described by Takushige et al (0.5mg of each). Medicament changed every month for 3 month until elimination of symptoms	2 – 4 weeks – Ca(OH) ₂ dressing failed to eliminate symptoms 1 year- complete healing periapical radiolucency 30 month – radiographic examination showed no pathology
Taneja S and Manju K 2010 [38] No of cases :1 Tooth no : 11, 12 Age / sex : 20/M Diagnosis: Chronic periapical abscess	Large periradicular radiolucency around apices of 12 and 11 measuring about 13 X 17 mm	1 st visit- access, pus discharge, canal instrumented to 60-100 size k file for 11 and 80- 110 size k file for 12 using step back technique, disinfected and sealed with cavit 2 nd visit (1 -3 weeks later)- Ca(OH) ₂ dressing changed 3 months later- irrigated, obturated with AH plus sealer and gutta percha using lateral condensation technique. Sealed with cavit 6 month later – sealed with composite resin	3% NaOCl with 27 gauge, EDTA, then again NaOCl	Initial 3 weeks- Ca(OH) ₂ dressing. Failure to eliminate pus discharge & swelling re-occurred, Ca(OH) ₂ removed & triple antibiotic paste placed with lentulospiral (100mg/ each in total volume of 0.5ml. carriers- propylene glycol & polyethylene glycol). Antibiotic paste changed every month for 3 month until asymptomatic	6 month – asymptomatic 15 month- complete bone healing with defined trabeculae
Taneja S et al 2010 [39] No of cases :3 Tooth no : 36, 11,13,11,12 Age / sex : 20/M, 17/M, 9/M Diagnosis: Chronic periapical abscess	Well circumscribed lesion measuring 10- 14 mm in diameter	1 st visit- access, purulent discharge instrumented using step back technique 1 – 3 weeks- Ca(OH) ₂ dressing changed every week. Discharge did not cease completely 3weeks later- triple antibiotic paste placed every month for 3 month until asymptomatic 3 month later- irrigated, obturated with gutta percha and AH plus sealer using lateral condensation	3% NaOCl with 27 gauge needle	Ca(OH) ₂ dressing changed weekly for 3 weeks. Persistent discharge from canal- Medicament changed to triple antibiotic paste (100mg of each in total volume of 0.5ml) placed with lentulospiral for 3 months and changed every month.	12 – 16 months – complete bone healing with well defined trabeculae

Table 3 shows clinical study of triantibiotic paste as endodontic filling material in primary teeth

Author & year	No of primary teeth	Method	Method of evaluation	Result	Follow up period
Takushige et al 2004 [11] Type of study: clinical Age : 4- 18 yrs No of patients: 56	87 (81 cases had physiological resorption, 54 had periapical radiolucency)	Access prepared, root canal orifice enlarged (medication cavity), 3Mix-MP/3Mix – sealer placed and sealed with glass ionomer cement. Further reinforced with composite inlay	Clinical and radiographic	Clinically 83 cases – swelling (52 cases), sinus tracts (22 cases), induced dull pain (3 cases), spontaneous dull pain (26 cases), pain on biting (46 cases)- disappeared after treatment. 4 cases required retreatment with 3Mix-MP to resolve symptoms	Mean observation period – 680 days Maximum – 2390 days