REVIEW

On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology

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Abstract

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Antibiotics are a valuable adjunctive to the armamentarium available to health professionals for the management of bacterial infections. During endodontic treatment and when managing trauma to the teeth, antibiotics may be applied systemically (orally and/or parenterally) or locally (i.e. intra-dentally via irrigants and medicaments). Due to the potential risk of adverse effects following systemic application, and the ineffectiveness of systemic antibiotics in necrotic pulpless teeth and the periradicular tissues, the local application of antibiotics may be a more effective mode for delivery in endodontics. The aim of this article was to review the history, rationale and applications of antibiotic-containing irrigants and medicaments in endodontics and dental traumatology. The search was performed from 1981 to 2008 and was limited to English-language papers. The keywords searched on Medline were 'Antibiotics AND endodontics', 'Antibiotics AND root canal irrigation', 'Antibiotics AND intra-canal medicament', 'Antibiotics AND Dental trauma' and 'Antibiotics AND root resorption'. The reference section of each article was manually searched to find other suitable sources of information. It seems that local routes of antibiotic administration are a more effective mode than systemic applications. Various antibiotics have been tested in numerous studies and each has some advantages. Tetracyclines are a group of bacteriostatic antibiotics with antibacterial substantivity for up to 12 weeks. They are typically used in conjunction with corticosteroids and these combinations have anti-inflammatory, anti-bacterial and anti-resorptive properties, all of which help to reduce the periapical inflammatory reaction including clastic-cell mediated resorption. Tetracyclines have also been used as part of irrigating solutions but the substantivity is only for 4 weeks. Clindamycin and a combination of three antibiotics (metronidazole, ciprofloxacin and minocycline) have also been reported to be effective at reducing bacterial numbers in the root canal systems of infected teeth.

Keywords: antibiotics, endodontics, irrigants, medicaments.

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Introduction

The role of microorganisms in the development and perpetuation of pulp and periapical diseases has been demonstrated in animal models and human studies

(Kakehashi *et al.* 1965, Möller *et al.* 1981, Sundqvist 1992). Elimination of microorganisms from infected root canal systems is challenging. Numerous measures have been described to reduce the number of microorganisms in the root canal system, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no definitive evidence in the literature that mechanical instrumentation alone results in a bacteria-free root canal system. Considering the complex anatomy of the root canal system (Hess 1925), this is not surprising. On the contrary, there is laboratory and clinical evidence that mechanical instrumentation leaves significant portions of the root canal walls untouched (Peters *et al.* 2001) and that complete elimination of bacteria from the root canal system by instrumentation alone is not achieved (Byström & Sundqvist 1981, , Dalton *et al.* 1998, Card *et al.* 2002, Trope & Bergenholtz 2002). Therefore, additional methods, such as the use of chemical solutions, are required in order to disinfect the root canal system and kill as many microorganisms as possible. Chemical treatment of the root canal system can be arbitrarily divided into irrigants, rinses and inter-appointment medicaments. Several studies have been conducted on the use antibiotics as root canal irrigants and medicaments. Hence, the purpose of this paper was to review these studies regarding the use of intracanal antibiotics during endodontic treatment and when managing trauma to the teeth. The search was performed from 1981 to 2008 and was limited to English-language papers. The keywords searched on Medline were 'Antibiotics AND endodontics', 'Antibiotics AND root canal irrigation', 'Antibiotics AND intra-canal medicament', 'Antibiotics AND Dental trauma' and 'Antibiotics AND root resorption'. The reference section of each article was manually searched for other suitable sources of information.

History

Antibiotics were first discovered in 1928 but were not routinely used clinically until the early 1940's during the Second World War (Abbott 2000). Prior to this, most wartime deaths were due to bacterial infections of wounds, rather than from the wounds themselves. The use of antibiotics was popularized as a result of the rapid recovery of wounded military personnel and this popularity continued after the end of the war (Abbott 2000). Antibiotics have been an extremely valuable addition to the armamentarium of health practitioners and have doubtless saved many lives that would otherwise have been lost. In endodontics and dental traumatology, antibiotics may be applied systemically (orally or parenterally) and locally (intra-dentally). The first reported local use of an antibiotic in endodontics was in 1951 when Grossman (1951) used a poly antibiotic paste known as PBSC (a mixture of penicillin, bacitracin, streptomycin and caprylate sodium), penicillin targeting Gram-positive organisms, bacitracin for penicillin-resistant strains, streptomycin for Gram-negative organisms and caprylate sodium to target yeasts. Later, Nystatin replaced caprylate sodium as an antifungal agent in a similar medicament, known as PBSN (Weine 2003).

The rationale for local application of antibiotics

Whilst, systemic antibiotics appear to be clinically effective as an adjunct in certain surgical and nonsurgical endodontic procedures, their administration is not without the potential risk of adverse systemic effects, such as allergic reactions, toxicity and the development of resistant strains of microbes. In addition, the systemic administration of antibiotics relies on patient compliance with the dosing regimens followed by absorption through the gastro-intestinal tract and distribution via the circulatory system to bring the drug to the infected site. Hence, the infected area requires a normal blood supply which is no longer the case for teeth with necrotic pulps and for teeth without pulp tissue. Therefore, local application of antibiotics within the RCS may be a more effective mode for delivering the drug (Gilad *et al.* 1999).

Tetracyclines

Tetracyclines, including tetracycline-HCl, minocycline, demeclocycline and doxycycline, are a group of broad-spectrum antibiotics that are effective against a wide range of micro-organisms (Torabinejad *et al.* 2003a). Tetracyclines are bacteriostatic in nature (Torabinejad *et al.* 2003b). This property may be advantageous because, in the absence of bacterial cell lysis, antigenic by-products such as endotoxin are not released (Barkhordar *et al.* 1997). Tetracyclines also have many additional properties other than their antimicrobial action, such as the inhibition of mammalian collagenases, which prevent tissue breakdown (Pierce & Lindskog 1987, Venillo *et al.* 1994), and the inhibition of clastic cells (Pierce & Lindskog 1987, Pierce *et al.* 1988,

Bryson *et al.* 2002), which results in anti-resorptive activity (Bryson *et al.* 2002). Inflammatory diseases such as periodontitis include an excess of tissue collagenases, which may be blocked by tetracyclines, thus leading to enhanced formation of collagen and bone (Barkhordar *et al.* 1997).

In endodontics, tetracyclines have been used to remove the smear layer from instrumented root canal walls (Barkhordar *et al.* 1997, Haznedaroglu & Ersev 2001), for irrigation of apical root-end cavities during periapical surgical procedures (Barkhordar & Russell 1998), and as intracanal medicaments (Molander & Dahlen 2003).

Barkhordar *et al.* (1997) showed that doxycycline–HCl eliminated smear layer in a concentration dependent manner with 100 mg mL^{-1} doxycycline being more effective than lower concentrations. In another investigation, Haznedaroglu & Ersev (2001) reported that tetracycline was as effective as citric acid in removing the smear layer. Barkhordar & Russell (1998) evaluated the effect of doxycycline on the apical penetration of dye through the margins of root-end fillings. The teeth with IRM or amalgam fillings placed following doxycycline irrigation had significantly less dye penetration than those that were not irrigated with doxycycline.

Pinheiro *et al.* (2004) evaluated the antibiotic susceptibility of *Enterococcus faecalis* isolates from canals of root filled teeth with periapical lesions. The antibiotics were benzylpenicillin, amoxicillin, amoxicillin with clavulanic acid, erythromycin, azithromycin, vancomycin, chloramphenicol, tetracycline, doxycycline, ciprofloxacin and moxifloxacin. The vast majority (85.7%) of the isolates were susceptible to tetracycline and doxycycline.

Based on the hypotheses that microorganisms can reach the apical area of recently replanted teeth from the oral cavity (or from contaminated root surfaces during the extra-oral time), and that tetracyclines can potentially inhibit this route of bacterial contamination, Cvek *et al.* (1990) developed a protocol for the topical treatment of exposed roots with doxycycline before replantation. The aim was to eliminate the microorganisms from the root surface of an avulsed tooth via direct local application of the antibiotic to decrease the frequency and severity of the inflammatory response. They showed that topical doxycycline significantly increased the chances of successful pulp revascularization and decreased the number of microorganisms that could be isolated from the root canals. They also reported a decreased frequency of ankylosis,

external replacement resorption and external inflammatory resorption. The beneficial effect of soaking a tooth in doxycycline has also been confirmed by Yanpiset & Trope (2000).

Ritter *et al.* (2004) investigated the effect of topical antibiotic treatment on pulp revascularization in replanted dog teeth by using laser Doppler flowmetry (LDF), radiography and histology. After extraction, the teeth were kept dry for 5 min and either covered with minocycline, soaked in doxycycline, or soaked in saline before reimplantation. Teeth in the positive control group were not extracted. Postoperative radiographs and LDF readings were obtained for 2 months after replantation. After sacrifice of the animals, the jaws were collected and processed for light microscopy. Pre- and post-replantation LDF readings and radiographs, and the histological findings were analysed to assess revascularization. Pulp revascularization occurred in 91% of the teeth treated with minocycline, 73% of those soaked in doxycycline, and only 33% of the teeth soaked in saline.

Bryson *et al.* (2003) evaluated the effect of minocycline on the healing of replanted dog teeth after extended dry times of 60 min. Their results indicated that the roots with and without minocycline treatment showed no significant differences in the remaining root mass or the percentage of favourably healed root surfaces. In addition, no benefit was found from the use of topically applied minocycline in the attenuation or prevention of external root resorption. The lack of significant differences is likely to have been a result of the extended dry period before replantation as most of the periodontal ligament cells would have died within this time period and therefore external replacement resorption is the typical result.

Substantivity of tetracyclines

Tetracyclines readily attach to dentine and are subsequently released without losing their antibacterial activity (Torabinejad *et al.* 2003a). This property creates a reservoir of active antibacterial agent, which is then released from the dentine surface in a slow and sustained manner. In an *in vivo* periodontal study, Stabholz *et al.* (1993) compared the antibacterial substantivity of two concentrations of tetracycline HCl (50 mg mL^{-1}, 10 mg mL^{-1}) and 0.12% chlorhexidine. Their findings showed that both concentrations of tetracycline demonstrated residual antibacterial activity and the antibacterial substantivity of the three solutions in descending order was: 50 mg mL^{-1} tetracycline >10 mg mL^{-1} tetracycline >0.12% CHX.

Abbott *et al.* (1988) demonstrated that tetracyclines form a strong reversible bond with the dental hard tissues and that they exhibit slow release and diffusion through dentine over an extended period of time up to at least 12 weeks. Khademi *et al.* (2006) compared the antibacterial substantivity of 2% CHX, 100 mg mL^{-1} doxycycline–HCl and 2.6% NaOCl in bovine root dentine over five experimental periods of 0, 7, 14, 21 and 28 days *in vitro*. Their findings indicated that after 7 days, the NaOCl and doxycycline groups had the lowest and the highest number of colony forming units (CFU), respectively. However, after the longer time periods, the CHX group had the lowest number of CFU's.

Mohammadi *et al.* (2007) evaluated the antibacterial substantivity of three concentrations of doxycycline–HCl (100 mg mL^{-1}, 50 mg mL^{-1} and 10 mg mL^{-1}) in bovine root dentine over five experimental periods of 0, 7, 14, 21 and 28 days. At 7 days, the 100 mg mL^{-1} group and the 10 mg mL^{-1} group showed the lowest and highest numbers of CFU's respectively. In each group, the numbers of CFU's increased significantly over time.

BioPure (MTAD)

BioPure (Dentsply, Tulsa Dental, Tulsa, OK, USA), otherwise known as MTAD (mixture of tetracycline, acid and detergent), is a relatively new root canal irrigant, which was introduced by Torabinejad & Johnson (2003). This solution contains doxycycline (at a concentration of 3%), citric acid (4.25%) and a detergent, Polysorbate 80 (0.5%) (Torabinejad & Johnson 2003). Several studies have evaluated the effectiveness of MTAD for the disinfection of root canals. It has been shown that MTAD is able to remove the smear layer (Torabinejad & Johnson 2003) and is effective against *E. faecalis* (Shabahang & Torabinejad 2003, Shabahang *et al.* 2003, Torabinejad *et al.* 2003b).

Shabahang *et al.* (2003) compared the antibacterial efficacy of a combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse with that of 5.25% NaOCl. Their findings showed that using MTAD in addition to 1.3% NaOCl was more effective at disinfecting root canals than using 5.25% NaOCl alone. However, Tay *et al.* (2006) found that when MTAD was applied to 1.3% NaOCl-irrigated dentine, its antimicrobial substantivity was reduced. They attributed this to the oxidation of MTAD by NaOCl in a manner similar to the peroxidation of tetracycline by reactive oxygen species.

In another study, Shabahang & Torabinejad (2003) compared the antibacterial effects of MTAD with those of NaOCl and EDTA by standard *in vitro* microbiological techniques and reported that MTAD was significantly more effective against *E. faecalis.* On the other hand, Kho & Baumgartner (2006) compared the antimicrobial efficacy against *E faecalis* of 1.3% NaOCl/MTAD with that of the combined alternate use of 5.25% NaOCl and 15% EDTA for root canal irrigation. Bacterial samples taken early in the canal cleaning process revealed growth in none of the 20 samples irrigated with the 5.25% NaOCl/15% EDTA combination but 8 of the 20 samples irrigated with 1.3% NaOCl/MTAD had bacterial growth. Further samples taken after additional canal enlargement revealed growth in none of 20 samples when 5.25% NaOCl/ 15% EDTA were used, but there was still growth in 10 of the 20 samples when 1.3% NaOCl/MTAD was used. This investigation showed consistent disinfection of infected root canals when a combination of 5.25% NaOCl and 15% EDTA was used. However, the combination of 1.3% NaOCl/ MTAD left nearly 50% of the canals contaminated with *E. faecalis.*

Krause *et al.* (2007) compared the antimicrobial effect against *E. faecalis* of MTAD, two of its components (doxycycline and citric acid) and NaOCl in two *in vitro* models using two different methods. In the tooth model, NaOCl and doxycycline were more effective than the control in killing *E. faecalis* at shallow bur depths into dentine, but at deeper depths, the NaOCl was superior. In the agar diffusion model, NaOCl produced less inhibition of bacteria than MTAD or doxycycline. Ghoddusi *et al.* (2007) evaluated the effect of MTAD as a final irrigant on bacterial penetration into the root canal system, and its interaction with two conventional root canal cements (AH-Plus and Rickert's cement). They reported that it took longer for bacteria to penetrate the canals when either EDTA or MTAD had been used for smear layer removal.

Davis *et al.* (2007) investigated the antimicrobial action of Dermacyn (Oculus Innovative Sciences, Petaluma, CA, USA), MTAD, 2% CHX and 5.25% NaOCl against *E. faecalis* using a zone of inhibition test. MTAD showed significantly larger zones of inhibition than 5.25% NaOCl, 2% CHX and Dermacyn. Newberry *et al.* (2007) investigated the *in vitro* antimicrobial effect of MTAD as a final irrigant on eight strains of *E. faecalis* and they also measured the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MLC) of MTAD. After irrigating with 1.3% NaOCl, the root canals and the external root surfaces

were exposed to MTAD for 5 min. This treatment regimen was effective in completely eliminating growth of seven of the eight strains of *E. faecalis.* The MIC/MLC tests showed that MTAD inhibited growth of most strains of *E. faecalis* when diluted 1 : 8192 times and it killed most strains of *E. faecalis* when diluted 1 : 512 times.

Shabahang *et al.* (2008) evaluated the effect of the addition of chlorhexidine to MTAD and the substitution of the doxycycline in MTAD with chlorhexidine to create a solution they named MCAD. They compared the effectiveness of these formulations at disinfecting extracted human teeth that had been infected with *E. faecalis.* None of the samples treated with standard MTAD or with the MTAD/chlorhexidine mixture showed the presence of residual bacteria. In contrast, seven of the 10 samples treated with MCAD (doxycycline substituted by chlorhexidine) showed positive cultures of *E. faecalis.* These results clearly showed that, although the addition of chlorhexidine did not negatively impact on the efficacy of MTAD, the substitution of doxycycline with chlorhexidine significantly reduced the efficacy of the resultant solution.

Substantivity of MTAD

As stated above, tetracyclines (including doxycycline) readily attach to dentine and are subsequently released without losing their antibacterial activity (Torabinejad *et al.* 2003a). The presence of doxycycline in MTAD suggests that MTAD may have some substantive antimicrobial action (Torabinejad *et al.* 2003a). In an *in vitro* study, Mohammadi & Shahriari (2008) evaluated the substantivity of NaOCl, CHX and MTAD using a human dentine tube model. Dentine chips were removed from the walls of root canals with sequential sterile low-speed round burs with increasing diameters of sizes: 025, 027, 029, 031 and 033 at time intervals of 0, 7, 14, 21 and 28 days following irrigation with the test solution. The authors concluded that the substantivity of MTAD was significantly greater than CHX and NaOCl. In another study, Mohammadi (2008) assessed the substantivity of three concentrations (100%, 10% and 1%) of MTAD using a bovine dentine tube model. As described, dentine chips were removed from the canals with sequential sterile low-speed round burs with increasing ISO sizes of 025, 027, 029, 031 and 033 at 0, 7, 14, 21 and 28 days. Results showed that in the first culture, MTAD 100% and MTAD 1% had the lowest and highest number of CFUs, respectively. In each group, the number of CFUs increased

significantly over time. In conclusion, the substantivity of 100% MTAD was significantly greater than the two lower concentrations of MTAD.

Tetraclean

Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy), like MTAD, is a mixture of an antibiotic, an acid and a detergent. However, the concentration of the antibiotic, doxycycline (50 mg mL^{-1}) and the type of detergent (polypropylene glycol) differ from those of MTAD (Giardino *et al.* 2006). Giardino *et al.* (2006) compared the surface tension of 17% EDTA, Cetrexidin, Smear Clear, 5.25% NaOCl, MTAD and Tetraclean. The NaOCl and EDTA had the highest surface tensions, whereas Cetrexedin and Tetraclean had the lowest values. In another study, they compared the antimicrobial efficacy of 5.25% NaOCl, MTAD and Tetraclean against an *E. faecalis* biofilm generated on cellulose nitrate membrane filters. Only the NaOCl could disaggregate and remove the biofilm at every time interval tested although treatment with Tetraclean caused a high degree of biofilm disaggregation at each time interval when compared with MTAD (Giardino *et al.* 2007).

Ledermix paste

Ledermix is a glucocorticosteroid-antibiotic compound that was developed by Schroeder & Triadan in 1960, and released for sale in Europe by Lederle Pharmaceuticals in 1962 (Athanassiadis *et al.* 2007). The primary interest in developing Ledermix paste was based on the use of corticosteroids to control pain and inflammation associated with pulp and periapical diseases (Athanassiadis *et al.* 2007). The sole reason for adding the antibiotic component to Ledermix was to compensate for what was perceived at the time to be a possible corticoid-induced reduction in the host immune response. The developers initially incorporated chloramphenicol in their first trials but when Lederle Pharmaceuticals became the manufacturer, the antibiotic was changed to demeclocycline–HCl. Today, Ledermix paste remains a combination of the same tetracycline antibiotic, demeclocycline–HCl (at a concentration of 3.2%), and a corticosteroid, triamcinolone acetonide (concentration 1%), in a polyethylene glycol base (Athanassiadis *et al.* 2007).

The two therapeutic components of Ledermix paste (i.e. triamcinolone and demeclocycline) are capable of diffusing through dentinal tubules and cementum to reach the periradicular and periapical tissues (Abbott

1990). Abbott *et al.* (1988) showed that the dentinal tubules were the major supply route by which the active components could diffuse to the periradicular tissues, whilst the apical foramen was not a significant supply route. Various factors can affect the supply of the active components to the periradicular tissues – these include the presence or absence of a smear layer (Abbott *et al.* 1989a), the presence or absence of cementum (Abbott *et al.* 1989a), and the presence of other materials within the canal, for example, calcium hydroxide (Abbott *et al.* 1989b).

The concentration of demeclocycline within Ledermix paste itself (i.e. as it would be when placed within the root canal) is high enough to be effective against susceptible species of bacteria. However, within the peripheral parts of the dentine and in the periradicular tissues, the concentration achieved through diffusion is insufficient to inactivate bacteria, especially over time (Abbott *et al.* 1990). In the dentine immediately adjacent to the root canal, inhibitory levels of demeclocycline are achieved for all reported bacteria within the first day of application of the paste but this level drops to about one 10th of the initial level after 1 week in both the mid-root and the apical third levels. Further away from the root canal towards the cementum, the concentration of demeclocycline after 1 day is not high enough to inhibit growth of 12 of the 13 strains of commonlyreported endodontic bacteria (Abbott *et al.* 1990). Heling & Pecht (1991) reported that both Ledermix paste and 3% tetracycline in a hydrous base were effective in reducing the amount of *Staphylococcus aureus* in the dentinal tubules after 7 days of incubation and also after recontamination. However, they were not effective after just 24 h.

Although concerns were raised in the 1960's and 1970's (Athanassiadis *et al.* 2007) about the possible systemic side effects of using corticosteroids and tetracyclines in teeth, Abbott (1992) showed that the intradental use of Ledermix paste is unlikely to result in any such effects as the amount of these components that reach the periradicular tissues is too small. In addition, the large dilution that occurs as soon as the components reach the periradicular tissues further negates any possibility of systemic side effects.

Pierce *et al.* (1988) demonstrated histologically that Ledermix paste prevented experimentally induced external inflammatory root resorption *in vivo*. They also found that Ledermix paste had no damaging effects on the periodontal membrane and that this paste was an effective medication for the treatment of progressive root resorption in traumatically injured teeth.

Taylor *et al.* (1989) evaluated the effects of Ledermix paste and Pulpdent paste on the mitosis in and survival of mouse fibroblasts and survival of *Lactobacillus casei* and *Streptococcus mutans in vitro*. Ledermix paste was found to reversibly inhibit mitosis whilst present in the concentration range of 10^{-3}–10^{-6} mg mL^{-1}. Mixing the Ledermix paste with Pulpdent did not modify this antimitotic effect. Ledermix paste killed mouse fibroblasts at concentrations of 10^{-3} mg mL^{-1} and above, whilst Pulpdent killed at 1 mg mL^{-1} and above. The toxic effect of Pulpdent was slightly inhibited by mixing it with the Ledermix paste. Ledermix killed *S. mutans* at approximately the same concentration at which it killed mammalian cells, but required a 1000-fold greater concentration to kill *L. casei.* Pulpdent killed both *L. casei* and *S. mutans* at approximately one-fifth the concentration at which it killed mammalian cells.

Thong *et al.* (2001) found that periodontal ligament inflammation and inflammatory root resorption were markedly inhibited by both the calcium hydroxide (Pulpdent paste) and corticosteroid-antibiotic pastes relative to untreated controls. Replacement resorption was the lowest in the corticosteroid-antibiotic group, and significantly more normal periodontal ligament was present in this group than in the calcium hydroxide and control groups.

Wong & Sae-Lim (2002) evaluated the effect of immediate placement of intracanal Ledermix paste on root resorption of delayed-replanted monkey teeth. Ledermix paste was placed in the root canals of the experimental teeth prior to extraction and replantation after 1-h of being left dry on the bench. The positive control teeth were root filled and replanted after 1 h whilst the negative control teeth were root filled and replanted immediately. As expected, the negative control group produced significantly more favourable healing and less unfavourable healing compared to the Ledermix group, and the Ledermix group showed significantly more complete healing (35.46%) compared with the positive control group (16.58%) but there were no significant differences in the amount of inflammatory root resorption and replacement resorption. Nevertheless, when the latter two unfavourable healing patterns were combined, there was a significantly lower amount of overall unfavourable healing in the Ledermix group (64.54%) compared with the positive control group (83.43%). This unfavourable healing outcome in the Ledermix group, however, was not significantly different from the favorable healing outcome with the same treatment modality. The results of the Ledermix-treated group in this study are likely to

have been affected by the 1 h of dry storage as such conditions predispose to replacement root resorption as very few periodontal ligament cells would survive these conditions. Furthermore, inflammatory resorption was not very likely to occur in any of the experimental or control groups as the root canals were filled with either the medicament or a filling prior to extraction. This reduces the chances of the roots becoming infected, which in turn reduces the chances of external inflammatory resorption occurring.

Bryson *et al.* (2002) evaluated the effect of placing Ledermix paste and Ca(OH)$_2$ in the root canals of dog teeth immediately after replantation, which followed extended dry times of 60 min. Their findings demonstrated that the Ledermix paste-treated roots had statistically significantly more healing and less resorption than the roots treated with Ca(OH)$_2$. Root filling with Ledermix paste also resulted in significantly less loss of root mass due to resorption compared to the roots filled with Ca(OH)$_2$.

Chen *et al.* (2008) evaluated the individual influence of triamcinolone and demeclocycline on external root resorption after extended extra-oral dry time of 60 min. The teeth treated with Ledermix paste, triamcinolone and demeclocycline had statistically significantly more favorable healing than the group filled with gutta percha and replanted after 60 min dry time (i.e. the positive controls). There was no statistically significant difference between the Ledermix group and the triamcinolone group, whilst the demeclocycline group showed less favourable healing than the negative control, the Ledermix group and the triamcinolone group. They concluded that the corticosteroid and tetracycline, as anti-inflammatory and anti-resorptive agents, shut down or minimized the inflammatory reaction including clastic-cell mediated resorption, and thus promoted more favorable healing than the positive control group, which had no intracanal medicaments. Furthermore, they forecasted that in severe traumatic injuries, where a large surface area of periodontal ligament inflammation is expected, removing the pulp and placing corticosteroids into the canal at the emergency visit would become a standard protocol (Chen *et al.* 2008).

Trope (1990) evaluated the relationship of intracanal medicaments to so-called 'endodontic flare-ups'. Formocresol, Ledermix and calcium hydroxide were placed in strict sequence irrespective of the presence or absence of symptoms and radiographic signs of apical periodontitis. He found no significant difference in the flare-up rate amongst these three intracanal medica-

ments. However, the study did not distinguish between the different pulp and periapical diseases and therefore it is not possible to determine how many cases had infected canals and whether the anti-microbial action of these medicaments played a role or not. By contrast, Ehrmann *et al.* (2003) investigated the relationship of postoperative pain associated with three different treatment regimes for infected teeth with acute apical periodontitis after complete biomechanical debridement of the root canal system in patients presenting for emergency relief of pain. They reported that the patients with teeth dressed with Ledermix paste had less pain than that experienced by patients who had teeth dressed with calcium hydroxide or no dressing at all. In addition, although the teeth were randomly assigned to each treatment group, the teeth that were treated with Ledermix paste had more preoperative pain – that is, this group of teeth started with more pain but had less pain following treatment, which further emphasises the value of this medicament in relieving pain associated with apical periodontitis.

Kim *et al.* (2000a,b) investigated whether Ledermix paste used as an intracanal medicament caused discolouration of teeth, and whether the discolouration was related to the method of application or the effects of sunlight. These studies demonstrated that after 12 weeks, sunlight exposure had caused dark grey–brown staining of both mature (Kim *et al.* 2000a) and immature (Kim *et al.* 2000b) teeth in the Ledermix groups, and the discolouration was more severe in the immature teeth because of the wider dentinal tubules. The teeth did not discolour if they were kept in the dark away from sunlight. Staining of the crowns of the teeth only occurred when the Ledermix paste filled the pulp chamber and not when the paste was only placed in the tooth root. Hence, they suggested that if placement of the Ledermix was restricted to below the gingival margin, then such effects could be minimized.

Combination of Ledermix and calcium hydroxide

The combination of Ledermix paste with calcium hydroxide was originally advocated by Schroeder, initially for the treatment of pulpless infected teeth with incomplete root development (Athanassiadis *et al.* 2007). A 50 : 50 mixture of Ledermix paste and calcium hydroxide has also been advocated as an intracanal dressing in cases of infected root canals, pulp necrosis and infection with incomplete root formation (as an initial dressing prior to using calcium hydroxide

alone for apexification), perforations, inflammatory root resorption, inflammatory periapical bone resorption, and for the treatment of large periapical radiolucent lesions (Abbott 1990). It has been shown that the 50 : 50 mixture results in slower release and diffusion of the active components of Ledermix paste, which makes the medicament last longer in the canal (Abbott *et al.* 1989b). This in turn helps to maintain the asepsis of the canal for longer and also maintains a higher concentration of all components (Abbott *et al.* 1989b) within the canal.

The 50 : 50 mixture of Ledermix paste and calcium hydroxide paste does not alter the pH to any noticeable extent (Taylor *et al.* 1989) and therefore it is expected that the mixture will act in a similar manner when calcium hydroxide is used alone. Taylor *et al.* (1989) also showed that for two indicator micro-organisms, *Lactobacillus casei* and *Streptococcus mutans* (which are cariogenic), the 50 : 50 mixture was marginally more effective than either paste used alone. However, Seow (1990) showed that for *Streptococcus sanguis* and *Staphylococcus aureus*, the addition of only 25% by volume of Calyxl (a calcium hydroxide in saline paste) (Otto and Co., Frankfurt, Germany) to Ledermix converted the zone of complete inhibition originally seen in Ledermix to one of only partial inhibition. This latter study suggested that some medicaments should not be used in combination, and that when two medicaments with strong antimicrobial activity are combined there may be no additive or synergistic effects against some organisms (Seow 1990).

Chu *et al.* (2005) compared the efficacy of disinfection of root canals with periapical radiolucencies when treated with a antibiotic/corticosteroid medicaments - either Ledermix paste or Septomixine Forte paste (a mixture of Neomycin, polymixine B sulphate, dexamethasone) (Septodont, Saint-Maur, France), or a calcium hydroxide paste (Calasept). Their findings showed that in the Ledermix group, 38 strains of bacteria were recovered. The Septomixine Forte group had 25 strains, and the Calasept group had 25 strains. Gram-positive facultative anaerobic cocci (including staphylococci and streptococci) were more prevalent than the Gram-negative obligate anaerobic rods after treatment in all three groups.

Septomixine Forte

Septomixine Forte contains two antibiotics – neomycin and polymixin B sulphate. Neither of these can be considered as ideal or suitable for use against the commonly reported endodontic bacteria because of their limited spectra of activity (Abbott *et al.* 1990). Neomycin is bactericidal against Gram-negative bacilli but it is ineffective against *Bacteroides* and related species, as well as against fungi. Polymyxin B sulphate is ineffective against Gram-positive bacteria, as shown by Tang *et al.* (2004) who demonstrated that a routine 1-week application of Septomixine Forte was not effective in inhibiting residual intracanal bacterial growth between appointments. In addition, although the anti-inflammatory (corticosteroid) agent, dexamethasone (at a concentration of 0.05%), is clinically effective, triamcinolone is considered to have less systemic side effects.

Clindamycin

Clindamycin is effective against many of the usual endodontic pathogens, including Actinomyces, Eubacterium, Fusobacterium, Propionobacterium, microaerophilic Streptococci, Peptococcus, Peptostreptococcus, Veillonella, Prevotella and Porphyromonas. It is particularly effective *in vitro* against black-pigmented Prevotella and Porphyromonas species (Gilad *et al.* 1999).

Molander & Dahlen (2003) investigated the effect of clindamycin on root canal infections and apical periodontitis when placed as an intracanal dressing. They used a 150 mg clindamycin capsule that was mixed with sterile saline. Following initial bacteriological sampling and routine instrumentation of the canals, the clindamycin paste was placed in the root canals for 14 days. The presence or absence of bacteria was determined in samples taken immediately after removal of the dressing, and also after a further period of 7 days during which the canals were filled with a sampling fluid. The results indicated that clindamycin offered no advantage over conventional root canal dressings, such as calcium hydroxide. However, no negative controls were used and the concentration of the drug was not stated. The ability of clindamycin to penetrate deeply into the root canal system was also not investigated. Nonetheless, the clindamycin paste was successful in eliminating bacterial growth in 21 of the 25 teeth tested by the 14th day. In the four remaining teeth, *Enterococci* were the dominant species.

Gilad *et al.* (1999) evaluated the efficacy of clindamycin in an ethylene vinyl acetate (EVA) vehicle in reducing bacterial growth *in vitro*. Clindamycin fibres were manufactured as follows: 0.075 g of calcium phosphate monobasic was combined with 10 mL of

distilled water, and then added to a solution consisting of 0.05 g of clindamycin phosphate and 10 mL of distilled water. The combined solution was then lyophilized for 24 h, and the resultant powder was filtered to achieve a uniform particle size of 45 μ. The powder (125 mg) was combined with 375 mg of EVA particles and then processed through an extrusion plastometer at diameters of 2 mm, 1 mm and 0.5 mm. The final extrusion produced a 250 mm-long fibre, with a calculated approximate dose of 0.2 mg of clindamycin/mm of fibre. Results of the bacterial sensitivity test demonstrated that at the concentration of 10 μgmL^{-1}, all bacteria tested showed varying degrees of inhibition, especially *P. intermedia*, followed by *F. nucleatum*, *P. micros* and *S. intermedius*. They also found that the clindamycin/EVA fibres significantly reduced the number of bacteria present in extracted human teeth. Furthermore, clindamycin/EVA fibres demonstrated the ability to release the drug for at least 2 weeks.

Lin *et al.* (2003) compared the antibacterial effect of clindamycin and tetracycline in a bovine dentinal tubule model as well as with an agar diffusion test. They showed that the clindamycin significantly reduced the amount of viable bacteria in each dentine layer compared with the tetracycline. The agar diffusion test, wherein dilutions in increments of 1 : 3 and 1 : 9 were used, revealed that both medicaments had antibacterial activity, but clindamycin was significantly better. At the 1 : 27 dilution, clindamycin had a minor effect and tetracycline had no effect at all.

Triple antibiotic paste

Infections of the root canal system are considered to be polymicrobial consisting of both aerobic and anaerobic bacterial species. Because of the complexity of root canal infections, the use of a single antibiotic may not result in effective disinfection of the RCS. A combination of antibiotics may be needed to address the diverse flora encountered. A combination of antibiotics might also decrease the likelihood of the development of resistant bacterial strains. The combination that appears to be most promising consists of metronidazole, ciprofloxacin and minocycline (Windley *et al.* 2005). Sato *et al.* (1996) evaluated the potential of this mixture to kill bacteria in the deep layers of root canal dentine *in situ*. No bacteria were recovered from the infected dentine of the root canal wall 24 h after application of the drug combination, except in one case in which a few bacteria were recovered. Hoshino *et al.*

(1996) investigated the antibacterial effect of this same mixture, with and without the addition of rifampicin, on bacteria taken from the dentine of infected root canals. The efficacy was also determined against bacteria of carious dentine and infected pulps which may the precursory bacteria for an infected RCS. None of the individual drugs resulted in complete elimination of bacteria. However, in combination, these drugs were able to consistently disinfect all samples.

Iwaya *et al.* (2001) presented a case report of an immature mandibular second premolar tooth with a pulpless, infected RCS with periapical involvement and a sinus tract. Instead of following the standard root canal treatment protocol and apexification, two antibiotics (metronidazole and ciprofloxacin) were placed in the canal, after which the canal was left empty. Radiographic examination showed the commencement of apical closure 5 months after the completion of the antimicrobial protocol. Thickening of the root dentine and complete apical closure was confirmed 30 months after the treatment, indicating the revascularization potential of a young permanent tooth pulp into a bacteria-free root canal space.

Takushige *et al.* (2004) evaluated the efficacy of a poly antibiotic paste consisting of ciprofloxacin, metronidazole and minocycline, on the clinical outcome of so-called 'Lesion Sterilization and Tissue Repair' (LSTR) therapy in primary teeth with periradicular lesions. They reported that the clinical symptoms (such as gingival swelling, sinus tracts, induced dull pain, spontaneous dull pain and pain on biting) disappeared after treatment in all but four cases. The four cases that did not resolve after initial treatment had resolution of the clinical signs and symptoms after further treatment using the same procedures again. Thus, gingival abscesses and draining sinuses, if present, disappeared after a few days. Successor permanent teeth erupted without any disorders, or were found radiographically to be normal and in the process of eruption. All cases were evaluated as being successful. The mean functional time of the primary teeth was 680 days (range: 68–2390 days), except for one case in which the successor permanent tooth was congenitally missing.

Windley *et al.* (2005) assessed the efficacy of a triple antibiotic paste in the disinfection of immature dog teeth with apical periodontitis. The canals were sampled before (S1) and after (S2) irrigation with 1.25% NaOCl and after dressing with a triple antibiotic paste (S3) consisting of metronidazole, ciprofloxacin and minocycline. At S1, 100% of the samples had a positive culture result for bacteria with a mean CFU count of

1.7×10. At S2, 10% of the samples were bacteria-free with a mean CFU count of 1.4×10, and at S3, 70% of the samples were bacteria-free with a mean CFU count of only 26. Reductions in mean CFU counts between S1 and S2 as well as between S2 and S3 were statistically significant.

Metronidazole

Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Known for its strong antibacterial activity against anaerobic cocci as well as Gram-negative and Gram-positive bacilli, it has been used both systemically and topically in the treatment of periodontal disease. Metronidazole readily permeates bacterial cell membranes and it then binds to DNA, disrupting its helical structure, which leads to rapid cell death (Windley *et al.* 2005). Roche & Yoshimori (1997) investigated the antibacterial activity of metronidazole against clinical isolates from odontogenic abscesses *in vitro*. They showed that metronidazole had excellent activity against anaerobes but it had no activity against aerobes.

Siqueira & de Uzeda (1997) evaluated the antibacterial activity of 0.12% chlorhexidine gel, 10% metronidazole gel, calcium hydroxide plus distilled water, calcium hydroxide plus camphorated paramonochlorophenol (CPMC) and calcium hydroxide plus glycerin using an agar diffusion test. The calcium hydroxide/CPMC paste and chlorhexidine were effective against all bacterial strains tested. Metronidazole caused inhibition of growth of all obligate anaerobes tested and was more effective than calcium hydroxide/CPMC against two of the strains. In another study, Lima *et al.* (2001) evaluated the effectiveness of chlorhexidine-based or antibiotic-based medications in eliminating *E. faecalis* biofilms. They found that there were significant differences between the formulations tested. The association of clindamycin with metronidazole significantly reduced the number of cells in 1-day old biofilms. However, of all the medications tested, only 2% chlorhexidine-containing medications were able to thoroughly eliminate most of both the 1-day and 3-day *E. faecalis* biofilms.

Wang *et al.* (2003) evaluated the effect of a metronidazole–chlorhexidine solution on the treatment of chronic apical periodontitis. They reported that 97.6% of the cases healed. Yu *et al.* (2000) evaluated the effect of a paste made of erythromycin ethylsuccinate, metronidazole and camphorated paramonochlorophenol (CP) to sterilize the root canal system. The clinical observation of 180 patients with fully developed root apices and acute or chronic apical periodontitis showed that there was no significant difference in root canal disinfection when the erythromycin–ethylsuccinate–metronidazole–CP mixture was compared with formocresol. Therefore, the irritability and toxicity of the treatment could be reduced by using the erythromycin–ethylsuccinate–metronidazole–CP mixture instead of formocresol. They concluded that root canal disinfection with erythromycin–ethylsuccinate–metronidazole–CP was a safe and effective method to promote the healing of periapical diseases (Yu *et al.* 2000).

Gao *et al.* (2004) investigated a sustained release delivery gutta-percha point containing metronidazole (SRDGM) for root canal disinfection, and determined the drug concentration *in vitro* and the time that the device maintained an effective drug concentration. Their study showed that the SRDGM contained 2013 micrograms of metronidazole and it could release 68.24% of the drug over a period of 24 h when tested *in vitro*. An effective metronidazole concentration was released over more than 10 days. On the 10th day, $33.13 \, \mu g \, mL^{-1}$ of metronidazole was released, which was more than minimum inhibitory concentration of metronidazole.

Hoelscher *et al.* (2006) evaluated the antimicrobial effects against *E. faecalis* of five antibiotics (amoxicillin, penicillin, clindamycin, metronidazole and doxycycline) when added to Kerr Pulp Canal Sealer EWT *in vitro*. They found that all of these antibiotics except metronidazole could enhance the antimicrobial efficacy of the sealer.

Krithikadatta *et al.* (2007) evaluated the disinfection of dentinal tubules using 2% chlorhexidine gel, 2% metronidazole gel, bioactive glass (S53P4) and calcium hydroxide. Their findings demonstrated that the overall percentage inhibition of bacterial growth (at depths of $200 \, \mu$ and $400 \, \mu$ into the dentine) was 100% with the chlorhexidine gel whereas metronidazole gel (86.5%), bioactive glass (62.8%) and calcium hydroxide (58.5%) were less effective.

Conclusions

1. The local application of antibiotics within the root canal system may be a more effective mode for delivering such drugs than systemic routes of administration.

2. Tetracyclines have been used to remove the smear layer from instrumented root canal walls, for irrigation of apical root-end cavities during periapical surgical procedures, and as an intracanal medicament.

3. Substantivity of tetracyclines has been shown for up to at least 12 weeks.

4. BioPure (MTAD) is effective in removing the smear layer. However, the antimicrobial efficacy against *E faecalis* of 1.3% NaOCl/MTAD compared with that of the combined alternate use of 5.25% NaOCl and 15% EDTA is still controversial.

5. Substantivity of MTAD has been shown to last for up to 4 weeks. Furthermore, application of MTAD to 1.3% NaOCl-irrigated dentine may reduce its substantivity.

6. Tetraclean, is a mixture of an antibiotic (doxycycline), an acid and a detergent (like MTAD), with a very low surface tension and high degree of efficacy against bacterial biofilms.

7. Ledermix, a glucocorticosteroid-antibiotic compound, has anti-inflammatory, anti-bacterial and anti-resorptive properties, all of which help to reduce the periapical inflammatory reaction including clastic-cell mediated resorption. This material has been shown to significantly lower the incidence of inflammatory and replacement resorption, and thus prompts more favorable healing in replanted teeth.

8. A 50 : 50 mixture of Ledermix paste and calcium hydroxide has been advocated as an intracanal dressing in cases of pulpless infected root canals, pulp necrosis and infection with incomplete root formation (apexification), perforations, inflammatory root resorption, inflammatory periapical bone resorption and for the treatment of large periapical radiolucent lesions.

9. Clindamycin alone or in an ethylene vinyl acetate (EVA) vehicle can reduce the bacterial load inside the root canal system (including dentinal tubules) significantly.

10. A triple antibiotic paste consisting of metronidazole, ciprofloxacin and minocycline, has been reported to be very effective in the disinfection of the root canal system.

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