REVIEW ARTICLE

ANTIMICROBIAL AGENTS USED IN ENDODONTIC TREATMENT

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***Summary*:** Biomechanical preparation alone does not completely eradicate microorganisms from the root canal, hence the next logical step is to perform root canal procedures in conjunction with antimicrobials. The use of an antimicrobial agent improves the efficacy and prognosis of endodontic treatment. This review enumerates the most widely used antimicrobial agents, their mechanism of action and their potential use in reducing the microbial load.

***Key words**: Antimicrobial agent; Microbial resistance; Antimicrobial irrigation; Endodontics*

Introduction

Antimicrobials have a long heritage of usage for prevention of oral diseases (75). One of the ardent proponents of antiseptics has been W. D. Miller. He advocated the use of antiseptics for preventing caries as he had recognised the infectious character of tooth decay (79). In the subsequent years, following various research findings on the microbial etiology of oral diseases, there was a renewed interest in antimicrobial agents (6). The normal oral microflora comprises numerous bacterial species. However, the root canal environment is highly selective due to the limited availability of nutrients, complex bacterial interactions, and differences in oxygen potential in root canals with necrotic pulp. Hence the number of bacterial species which can survive in this harsh environment is comparatively less than that found in the oral cavity. The majority of the root canal microbiota therefore comprises facultative and strict anaerobic microorganisms which cause infections that stimulate periapical bone resorption, and are recalcitrant to endodontic treatment (104). Acute periradicular inflammation is mainly caused by anaerobes, especially black-pigmented Gram negative anaerobes (88, 121, 138). The absence of bacteria during root canal filling enhances the endodontic prognosis (45, 46).

Root canal debridement and antimicrobial irrigation reduce the endodontic microbial load (15). Saline reduces the bacterial count during the manual instrumentation of canals. However, it does not result in negative cultures in a single visit (15), thereby emphasizing the significance of an antibacterial agent.

Definition

Antimicrobial agents may be disinfectants and antiseptics that destroy or inhibit the growth of microorganisms and thereby prevent infection by pathogenic or potentially pathogenic microorganisms. Disinfectants are used on inanimate objects or surfaces, whilst antiseptics are used on living tissues (10, 78).

Classification

Antimicrobial agents can be broadly classified into two groups: conventional antiseptics and chemotherapeutics (56).

Tab. 1: Classification of antimicrobial agents.

Conventional antiseptics	Chemotherapeutics
1. Alcohols – Ethyl alcohol, Isopropylalcohol	Antibiotics
2. Phenolic Compounds – Camphorated phenol, Monochlorophenol, Thymol, Cresol, Creosote	
3. Heavy Metal Salts	
4. Cationic Detergents – Quarternary ammonium compounds	
5. Halogens – Hypochlorite, Chloramine T, Iodine, Iodophores	

Adapted from Ingle JI, Bakland LK, 1994

General mechanism of action of antimicrobial agents

The mechanism of action of antimicrobial agents is varied as they have multiple sites of action except for antibiotics, which have very specific sites of action. The nature of the organism, antimicrobial agent and the concentration determine the response of the microorganisms to the antimicrobials. Furthermore, with the involvement of multiple cell structures causing primary and secondary effect and cell lysis, it is difficult to determine the precise mode of action of these antimicrobial agents. The cell wall, cytoplasmic membrane and ribosomes of vegetative cells, the coat and cortex of bacterial spores, envelope and capsid of viruses and proteins (structural proteins, enzymes), nucleic acids and polysaccharides are some of the sites of action of antimicrobial agents. These antimicrobial actions eventually result in the loss of important cell functions like protein synthesis and metabolism, replication, transcription and destruction of cell membranes with leakage of cell contents (103).

Efficacy of antimicrobial agents

The two most important features which determine the efficacy of antimicrobial agents are the killing and the cleaning potential of the agent. The antimicrobial activity may vary from inhibition of metabolism to destruction of the microorganisms. The specific target of action of antimicrobials is difficult to elucidate as antimicrobial agents act on multiple cell components, resulting in both primary and secondary effects, which in turn is hard to distinguish. However, a combination of several techniques can help solve this problem. For example if enzyme inactivation and/or if damage of the cell wall, respiratory apparatus, ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) are involved, then it implies that the antimicrobial inhibits the metabolic activity of the microbe. Thus the target site of activity of the antimicrobial can be elucidated.

Culture-based methods are used to measure the number of surviving bacteria. However, viable but, non culturable, "VBNC"(65) are not represented. Hence the numbers of viable organisms measured by culture are less than their true population. These methods are still useful in measuring the microorganisms in the sample. These include modified cultivation-based methods (12), application of fluorescent dyes, analysis of respiratory activity using the tetrazolium salt 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (100) and staining with the fluorochromes contained in the Live/Dead BacLight Bacterial Viability Kit, which measures the metabolic activity of antimicrobial-treated bacteria (12). Some of the other techniques are measurement of the transmembrane potential with Rhodamine 123 or DiBAC4 (3), which is used to determine the antimicrobial-treated biofilms to utilise nutritional substrates (73) and indirect measurement of the metabolic activity of antimicrobial-treated bacteria by (49) using microelectrodes to determine the redox potential in the biofilm. Methods like image analysis (132) are used to detect surface-associated, stained bacteria, while a confocal laser scanning microscope (CLSM) detects changes in the morphology of the biofilm resulting from antimicrobial treament. Scanning electron microscopy (SEM) is often used to analyse the surface associated biofilm before and after antimicrobial treatment. All these techniques are of immense use in determining the efficacy of the antimicrobial agent.

Microbial Resistance

Resistance mechanisms of biofilms and planktonic cells are dissimilar. In a study by Gilbert et al. (34), biofilms were found to be 10 to 1000 times less susceptible than planktonic cells to antimicrobial agents. This striking difference is due the different resistance mechanisms of biofilms. Resistance is defined as the ability of a microorganism to grow in the presence of high levels of an antimicrobial agent or to survive treatment with an antimicrobial agent. Microbial resistance is mainly of two types, intrinsic and acquired resistance. Intrinsic, or innate, resistance is the natural chromosomally determined resistance and physiological adaptation which is specific for a particular microorganism. Acquired resistance refers to the resistance resulting from mutations and the selection of resistant mutants from a population exposed to antimicrobial agents, or due to the incorporation of plasmids or transposons, which results in resistance to antimicrobials (78, 82). It is most likely that increased cell densities in biofilms result in the selection of spontaneously resistant mutants when exposed to sublethal concentrations of antimicrobials, while increased cell numbers cause a horizontal transfer of genes expressing resistance to antimicrobials (23). Various other mechanisms which explain microbial resistance include slow rate of growth of biofilm cells due to restricted availability of nutrients, the emergence of a biofilm-specific phenotype, stimulation of general stress response genes, the occurrence of persistent cells and physical and chemical diffusion-reaction barriers which limit occurrence the penetration of antimicrobials into the biofilm (23).

Sodium hypochlorite

The antimicrobial solution that has had extensive use in endodontics as a root canal antimicrobial is sodium hypochlorite (NaOCl), in concentrations ranging from 0.5 % to 5.25 %. This is due to its antimicrobial and dissolving effects on necrotic tissues (111). There is no consensus within the endodontic community regarding the most effective concentration of sodium hypochlorite to be used. However, a concentration of 2.6 % to 5.2 % has been found to have adequate tissue solvent activity. Sodium hypochlorite is a reducing agent with 5 % of available chlorine. It acts as

a lubricant, antiseptic agent, bleach and also dissolves tissue (39). However, the exact bactericidal mechanism remains obscure (25). It is suggested that the antibactericidal ability of NaOCl results from the formation of hypochlorous acid (HOCl) when in contact with organic debris. HOCl exerts its effect by the oxidation of sulphydryl groups within bacterial enzyme systems, thereby disrupting the metabolism of the microorganism (111). Cvek M et al. in his study reported that flushing with sterile saline had poor antibacterial action (9 %) when compared to sodium hypochlorite (25 %). In addition, 0.5 % or 5.0 % sodium hypochlorite solutions showed similar antibacterial effects. Inadequate mechanical cleansing of root canals in teeth with immature roots cannot be overcome by increasing the concentration of the solution, as it can cause tissue damage (20). The antibacterial action of NaOCl is time dependent. In an in vivo study, Ringel et al. noted that in root canals of permanent teeth 2.5 % NaOCl had a more powerful antibacterial effect than 2 % chlorhexidine gluconate, as NaOCl was a powerful solvent for necrotic and organic material (95). Naenni et al reported that only sodium hypochlorite showed effective necrotic tissue dissolution among 10 % chlorhexidine, 3 % and 30 % hydrogen peroxide, 10 % peracetic acid, 5 % dichloroisocyanurate (NaDCC), and 10 % citric acid. This finding assumes significance when other substitutes are used in place of NaOCl for endodontic irrigation (84).

An in vivo study was done by Ercan et al on 2 % chlorhexidine gluconate and 5.25 % sodium hypochlorite in infected root canals and he concluded that both chlorhexidine gluconate and sodium hypochlorite prevent microbial activity in non vital teeth with or without periapical pathologies (27). The main drawback of NaOCl is the toxicity to the periapical tissues(26, 50, 125), bad odour, discolouration of dental equipment, and

destruction of permanent tooth follicles and oral mucosa. It can also cause pharyngeal oedema and oesophageal burns when unintentionally swallowed (104), so its replacement by chlorhexidine gluconate is being carefully studied by various investigators (88).

Sodium hypochlorite is not carcinogenic in animals (52). However it is mutagenic in *Salmonella typhimurium* and not in *Bacillus subtilis* (52). Chromosome aberrations are seen in Chinese hamster lung cells but not in human fibroblasts (52). In vivo experiments on mice have shown that sodium hypochlorite does not elicit micronuclei, aneuploidy, and chromosome aberrations in bone marrow cells (57). Morphological transformation (135) and SCEs (80) are seen in SHE cells. However, UDS (42) is absent when sodium hypochlorite is used.

Chlorhexidine gluconate

Chlorhexidine (CHX) is widely used in periodontal and endodontic treatment as an irrigant. There are various mechanisms of antimicrobial action for chlorhexidine. It attaches electrostatically to negatively charged sites on bacteria and also to its cytoplasmic membrane. The leakage of intracellular material is due to the loss of osmotic balance by CHX. The binding of CHX to hydroxyapatite and soft tissues changes their electrical field to compete with the binding of bacteria (46).

Cetrexidin♦ (Vebas, San Giuliano, Milan, Italy) is another antiseptic agent that is being evaluated. It consists of 0.2 % chlorhexidine gluconate and 0.2 % cetrimide (22, 125). Cetrimide (cetiltrimethyl ammonium bromide), is a quarternery ammonium compound and a cationic detergent that is effective against many Gram positive and Gram negative bacteria (22).

A study on the antimicrobial effectiveness and cytotoxicity of 4 irrigant solutions, viz 5.25 % sodium hypochlorite (NaOCl), 0.2 % chlorhexidine gluconate plus 0.2 % cetrimide (Cetrexidin®), 2 % chlorhexidine gluconate and 0.9 % sterile saline solution demonstrated that NaOCl should remain in the canal for a substantial period so that it can act upon the bacterial cells located in the irregularities within the canal. In this study, 5 minutes following the irrigation process, chlorhexidine gluconate had a more rapid and stronger action on E. faecalis than NaOCl. Similar results were also obtained by D'Arcangelo et al. (22) and Türkün et al. (125). In the study it was seen that Cetrexidin® had a greater antibacterial effect than 5.25 % NaOCl. A plausible explanation for this seems to be that cetrimide acts as a detergent, thereby lowering the surface tension. Cetrimide, when combined with chlorhexidine, easily penetrates into the root canals and dentinal tubules. However, no significant difference between the antibacterial effects of Cetrexidin® and 2 % chlorhexidine gluconate was observed. Jeansonne & White (58) found that chlorhexidine gluconate had a residual antibacterial effect on the infected canals and the antibacterial effect was substantive after 48 h of chlorhexidine and Cetrexidin® application into the E. faecalis inoculated canals (58).

2 % chlorhexidine gluconate and Cetrexidin® had more antibacterial effect on anaerobic bacteria than 5.25 % NaOCl. This was due to their active cationic properties, which enable their adsorbtion by the dentine surface and their residual antimicrobial activity. In vivo studies have reported that chlorhexidine has antibacterial activity with residual effects in the root canal for 48 h. (72). Chlorhexidine appeared to be the most effective antibacterial substance in comparison to hydrogen peroxide, sodium hypochlorite and REDTA, while calcium hydroxide and saline solutions were least effective (99).

Calcium hydroxide

Calcium hydroxide is the most commonly used inter-appointment intracanal endodontic medicament (32, 91, 110, 120). The publication of research data on the antibacterial action of calcium hydroxide in root canal treatment by De Moor & De Witte led to increased use of calcium hydroxide

in endodontic treatment. Similar reports by several investigators resulted in widespread use of calcium hydroxide as an inter-appointment intracanal medicament (89, 112).

It is used as an intracanal medicament due to the healing of periradicular tissues. However, a few reports of adverse reactions have been found. $Ca(OH)_2$ powder is made into a paste with water or saline and it is used as an intracanal dressing for a few days or weeks. The antibacterial activity is a result of free hydroxyl radical liberation (110) and diffusion of hydroxyl radicals resulting in a highly alkaline environment (pH 12.5). These hydroxyl ions penetrate the dentinal tubules and exert their effect. These hydroxyl radicals cause bacterial cell death by three possible mechanisms. The first mechanism is by splitting DNA strands and thereby preventing DNA replication and disrupting cellular activity (55, 97). Another method is by lipid peroxidation, which leads to the destruction of both phospholipid and cell membrane, finally resulting in loss of unsaturated fatty acids and massive destruction of membrane (68). The third mechanism is by protein denaturation and damage of cell metabolism. Calcium hydroxide also shows increased activity against anaerobes in comparison to paramonochlorophenol (32) and formocresol (120).

Resistance to $Ca(OH)_2$ by certain microorganisms have been reported (41, 85, 127). Nerwich et al. have shown differential diffusion rates of hydroxyl ions in cervical and apical root dentine (86). The pH of inner cervical root dentine peaked at 10.8 within hours after calcium hydroxide insertion, whilst apically a plateau pH of approximately 9.5 was reached only 2 weeks after the dressing was in place, and the outer root dentine pH reached a peak level of about 9.0 after 2–3 weeks.

In an extensive review of available clinical data on the efficacy of calcium hydroxide by C. Sathorn et al. was reported that calcium hydroxide has limited value as an antibacterial agent as evaluated by culture techniques (99). Ex vivo studies by Haapasalo et al. and Portenier et al. (40, 92) report that dentine may inactivate the antibacterial action of calcium hydroxide. Another study by Peters et al. (91) reported that the number of root canals positive for bacteria increased after intra canal medication with calcium hydroxide. Similar reports by other researchers also stated the inability of calcium hydroxide to effectively eradicate bacteria and the presence of positive cultures after using calcium hydroxide in the root canal (91, 94,128). Proton donors like H_2PO_4, HCO_3 and HCO found within the dentine neutralize hydroxyl ions, thereby preventing the attainment of optimal microbicidal pH and compromising the antibacterial potential of calcium hydroxide (86,129). In addition to this, necrotic tissue debris and/or cells may possibly interfere with the action of hydroxyl ions within the root canal and dentinal tubules (110).

Hydrogen peroxide

Another antimicrobial agent that has had extensive use in endodontics is hydrogen peroxide (H_2O_2). The mechanism of action is by the reaction of superoxide ions, resulting in formation of hydroxyl radicals. Hydroxyl radicals are strong oxidants and they destroy membrane lipids, DNA and other essential cell components. The oxidation of sulphydryl groups and double bonds in proteins, lipids, and surface membranes is responsible for the antimicrobial action. In addition, the chloride in the bacteria may be oxidized to hypochlorite when myeloperoxidase enzyme is present (11).

Hydrogen peroxide is an oxidizing solution and is usually used in combination with sodium hypochlorite for root canal irrigation. This results in two kinds of reactive oxygen species, the superoxide anion radical (O_2^-) and the hydroxyl radical (OH^-). Shiozawa A. studied the pH changes and dissolved oxygen values in the NaClO- H_2O_2 reaction mixture and found that the pH influenced the O_2^- and OH^- formation, and that H_2O_2 resulted in O_2 formation. Root canal irrigation with NaClO and H_2O_2 induces both biological and mechanical effects. The biological effect of NaClO and H_2O_2 owes to tissue irritation due to the chemical reactions of O_2^- and OH^-, while the mechanical effect results from O_2 bubbling (109). The effervescent action resulting in the release of nascent oxygen results in the agitation of the root canal contents and the debris is flushed out. The tissue dissolution and antimicrobial effect are the main mode of action of the combined solutions (18). The final irrigation of the canal should be done with sodium hypochlorite, as hydrogen peroxide can form gas in the presence of necrotic debris and blood leading to pain (39).

Concentration of H_2O_2 and irradiation time resulted in variation in the generation of hydroxyl radical from H_2O_2 exposed to light or laser radiation. Irradiation time also influenced the quantity of 5,5-dimethyl-1-pyrrolidone-(2)-oxyl-(1) (DMPO-X). The amounts of hydroxyl radicals generated from H_2O_2 after irradiation were highest with a plasma lamp and lowest with a Yellow He-Ne laser. The amounts of DMPO-X generated from NaClO after irradiation was greater with a plasma lamp and least with a He-Ne laser (64).

Formocresol

Formocresol consists of formalin and tricresol in a ratio of 1:1. Tricresol is a combination of o-, m-, and p-cresols. The application time and the concentration of formocresol influence the histologic reaction of vital pulp. Formocresol is a bactericidal agent and the mode of action is by fixation, which results in inhibition of bacteria. Formocresol causes zones of necrosis, fixation, and inflammation. It results in healing with inflammation and eventual replacement with granulation tissue, bone or osteodentin in some cases.

Smith et al. (113) and various other investigators have stated that clot formation replaced the pulp tissue when ferric sulfate was used. Inflammation and calcific changes in the coronal as well as in the radicular portions of the pulp were some of the other findings in their studies (113).

Cochrane Review (83) and evidence-based assessment of clinical trials of ferric sulphate and formocresol pulpotomies with meta-analysis (31) have reported similar clinical and radiographic success rates for both these agents.

Formaldehyde causes carcinogenesis in animals, mutation in bacteria, yeasts and Drosophila melanogaster and clastogenesis in mammalian cells and plants (51). Salmonella mutagenicity tests also reveal that formalin is mutagenic (13), while tricresol is non-mutagenic (44). Formaldehyde and m-cresol cause morphological transformation (135) chromosome aberrations (48), and UDS (42) in SHE cells. Formocresol also induces morphological transformation, UDS, and SCEs in SHE cells (122).

Formaldehyde is an ingredient in Buckley's Formocresol solution which is extensively used as a pulpotomy agent in grossly decayed deciduous teeth. In June 2004, the International Agency for Research on Cancer (IARC) classified formaldehyde as having carcinogenic potential in humans, as there is sufficient evidence which reveals that it causes nasopharyngeal cancer, limited evidence for cancer of the nasal cavity and paranasal sinuses, and 'strong but not sufficient evidence' for leukaemia (57).

Ferric sulphate

Ferric sulphate (15.5 %) is commonly used as a haemostatic agent in pulpotomy procedures. Landau and Johnsen in 1988 were the first who conducted animal experiments using ferric sulphate prior to the placement of calcium hydroxide over amputated pulps in monkey teeth. The persistence of an extrapulpal blood clot attributed to the decreased efficiency of calcium hydroxide. They studied the role of haemostasis by ferric sulphate and the resultant improvement in treatment with calcium hydroxide (71). The mode of action is by the formation of a ferric ion protein complex in the presence of blood resulting in the mechanical sealing of cut vessels by the membrane of this complex. This ultimately leads to haemostasis (102). The agglutinated protein complex forms plugs which seal the capillary orifices and inhibit clot formation (28, 70).

Several studies have been reported with the use of 15.5 % ferric sulphate. In an experimental study, ferric sulfate, diluted formocresol and IRM, when used on pulpotomized primary teeth of baboons, the degree of inflammation, periradicular or interradicular abscess or inflammatory root resorption and presence of dentinal bridge were similar.

Thus the pulpal reaction of ferric sulfate and formocresol did not differ from each other (31). Similar results were obtained for ferric sulphate and formocresol in rat teeth by Cotes and co-workers (19). However, less than 40 % of treated teeth presented with reparative dentine and fibrosis with ferric sulphate. A one-year prospective human trial (30) by Fei et al. revealed a success rate of 96 % for ferric sulphate and 78 % for formocresol on the basis of combined clinical and roentgenographic features. Investigations by Ibricevic and Al-Jame revealed similar success rates to that of Fuks et al. with ferric sulphate and formocresol treated teeth at an interval of 20 months (54) and subsequently at 48 months (53). However, the radiographic success rates decreased during this period from 97.2 % to 92 % for ferric sulphate. These results were significantly greater than that in the retrospective studies by Burnett and Walker (14) and Smith et al. (113). Casas et al. (16) used 16 % ferric sulphate equivalent in an aqueous vehicle and compared this with pulpectomy in primary molars. A higher success rate was reported after 2 years for ferric sulphate than with pulpectomy. However, the sample size after 3 years was inadequate to demonstrate statistically significant success rates for ferric sulphate.

Ferric sulphate is less toxic than formocresol and hence it may be considered as an alternative to formocresol for pulp therapy in primary molars (54). Dental caries involving greater than half the inter-cuspal distance demonstrated inflammation of the pulp horn. Thus extirpation of coronal pulp alone would be adequate, thereby maintaining vitality of the radicular tissue rather than tissue fixation, which is achieved by formocresol. As ferric sulphate causes only haemostasis, it is a more appropriate pulpotomy agent and may be considered a good replacement for formocresol in pulpotomy (24).

Application of ferric sulfate as a hemostatic agent for long duration leads to persistent inflammation and delays osseous wound healing. However, with adequate curettage and irrigation of the osseous wound before closure, there was no significant difference in the persistence of inflammation or delay in osseous wound healing when compared with controls (58).

Peracetic Acid

Peracetic acid has a wide spectrum of antimicrobial action at low concentration, and within short duration (33, 38, 66, 67, 114, 115, 116, 117, 123, I. J. Hutchings and H. Xezone, unpublished data). Aqueous solution of peracetic acid (PAA) has high microbicidal activity against a broad range of microorganisms (33, 37, 38, 59, 66). Peracetic acid is an effective germicide against bacteria, yeast, and viruses at 0.03 % or lower concentration (7, 90). Alasri et al. state that when peracetic acid and hydrogen peroxide are used together, they have a combined action on biofilms owing to the microbicidal activity of peracetic acid and dettachment of biofilm by hydrogen peroxide (2).

The sporicidal action decreased with storage due to hydrolysis of peracetic acid, whereas it increased with high pH concentration. The drawback of high pH concentration is the carcinogenic potential of 1 % peracetic acid, as it is a tumor promotor. The sporicidal action in a study by Jose-Luis and Aylin (98) was as follows: hypochlorite > peracetic acid > copper-ascorbate > glutaraldehyde > peroxide > phenol > formaldehyde. Ageing, pH, and temperature were found to greatly influence the order of the efficacy of these agents. Comet assay and Saccharomyces cerevisiae strain D7 stu-

dies have shown that the lowest effective dose which caused genotoxicity in human leukocytes was 0.2 ppm for chlorine dioxide, 0.5 ppm for sodium hypochlorite and peracetic acid. One of the limitations of PAA is that it is known to cause corrosion. However, it is used as a surface decontaminant for foods. This is mainly because there is no surface adsorption of PAA and its by products like acetic acid, water, and oxygen are nontoxic and can be washed off easily (38, 74). The armamentarium for gnotobiotic studies is also sterilized using PAA (9,124). According to Naenni N et al., among the commonly used endodontic irrigants like 10 % chlorhexidine, 3 % and 30 % hydrogen peroxide, 10 % peracetic acid, 5 % dichloroisocyanurate (NaDCC), and 10 % citric acid, all had lower tissue dissolution capacity in comparison to 1 % (wt/vol) sodium hypochlorite (NaOCl) (84).

Chloramine T

Chloramine T is N-chloro-p-toluensulphonamidesodium. It is used as an effective oral antiseptic agent. The mode of action is by the conversion of amino acids into aldehydes, carbon dioxide, ammonia and nitriles. Irrigation with a combination of hydrogen peroxide and chloramine, chloramine or glutaraldehyde were more effective irrigants than normal saline, 1% metronidazole or 3% hydrogen peroxide (138). A study by Wennberg A evaluated the cytotoxic effect in a cell culture system using HeLa cells and the initial tissue irritating effect of five antiseptics by applying the antiseptics onto an intact nonepithelialized tissue surface. 5% Chloramine-T produced the greatest cell and tissue reactions, while 0.04% Jodopax, 0.1% Biosept, 0.1 % Hibitane, or 0.5 % sodium hypochlorite showed no differences in cell and tissue reactions. HeLa cell recovery was best following use of Jodopax, Chloramine-T or sodium hypochlorite solutions, whereas tissue recovery for Biosept and Hibitane were the best (130).

Hexetidine

Hexetidine is 1,3-bis(2-ethylhexyl)-5-amino-5-methyl-hexahydropyrimidine. Hexetidine is a good antibacterial and antifungal agent with a wide spectrum of activity both in vivo and in vitro. Hexetidine rinse is widely used as an antiplaque and antigingivitis, as it decreases supragingival plaque and gingival inflammation. In vitro and in vivo action against Gram-positive and Gram-negative bacteria as well as yeasts (*Candida albicans*) is well known (5, 96, 131). In addition, it is also used as an astringent, local anaesthetic and deodorant. It has not been widely used in endodontic treatment. Studies on in vitro oral biofilm models demonstrate that antimicrobials like chlorhexidine, hexetidine, delmopinol, amine fluoride/stannous fluoride, triclosan, and phenolic compounds interfere with bacterial metabolism and may inhibit biofilm development and maturation (6).

Biochemical properties of hexetidine include oxidation of intramitochondrial pyridine nucleotides and stimulation of the rate of oxygen uptake and inhibition of the rate of ATP synthesis. Thus hexetidine exhibits uncoupling of mitochondrial oxidative phosphorylation (21). In vitro and ex vivo experiments on the adherence of yeast cells to buccal epithelial cells (BEC) and in vitro morphogenesis showed that hexetidine caused decreased adherence of *C. albicans* to buccal epithelial cells and modified or inhibited the morphogenesis (60).

Although some microorganisms develop resistance to hexetidine, it is only temporary and does not last long (63). A concentration of 0.1 % w/v when used as an oral rinse decreases the number of microorganisms. As the concentration of hexetidine in the oral cavity decreases with time there is a corresponding decrease in the antimicrobial effect. In an in vivo study, HPLC assay detected the presence of hexetidine in saliva up to 25 min after an oral rinse. This study also detected hexetidine below MICs for certain microorganisms (77). One of the advantages is that the extrinsic tooth staining is lower for hexetidine (107). Investigations testing the in vitro antifungal and fungicidal activities of antimicrobials demonstrated that cetylpyridinium chloride acheived significantly lower minimum inhibitory concentrations and had the maximum fungicidal activity in comparison to chlorhexidine digluconate and hexetidine (35).

Aminefluoride

38 % diamine silver fluoride, or $Ag(NH3)_2F$, is used as a Nd:YAG laser initiator. Yokoyama K and co-workers reported that pulsed Nd:YAG laser or iontophoresis following $Ag(NH3)_2F$ increased the permeability of the root canal wall and occlusion of dentinal tubules. Root canals treated using irradiation with an Nd:YAG laser that has been coated with $Ag(NH3)_2F$ solution showed improved results compared to either iontophoresis after coating with $Ag(NH3)_2F$ solution, or coating alone (137). In vitro studies have revealed that CO_2 laser effectively removes or melts the smear layer of root canal walls after it is treated with 38 % diamine silver fluoride [$Ag(NH3)_2F$] solution (29). Pulsed Nd:YAG laser irradiation for 2 sec after coating tooth surfaces with 38 % $Ag(NH3)_2F$ solution prevents fracturing of endodontically treated teeth (136). 4 % titanium tetrafluoride solution on root canal walls modified the smear layer on root canal walls, forming a massive structure which could not be eliminated with EDTA and/or NaOCl irrigations (136). The stability of this structure could be useful in preventing infection of dentinal tubules and microleakage, as it permanently occludes these tubules and avoids dissolution and disintegration of the smear layer (105). Biofilm inhibitory concentrations for chlorhexidine (300 times) and amine fluoride (75 times) is higher when *Streptococcus sobrinus* exists as a biofilm in contrast to the minimum bactericidal concentration for planktonic cells (106).

Cetylpyridinium chloride

Cetylpyridinium chloride (CPC) is a quaternary ammonium salt ($C_{21}H_{38}ClN$; molecular weight, 358.07) having a combination of hydrophilic and lipophilic affinities. CPC is commonly used as a broad-spectrum antimicrobial against oral bacteria and with properties and uses typical of cationic surfactants. The primary mechanism of action of CPC is by cell membrane penetration, which results in leakage of cell contents, disturbance of bacterial metabolism and inhibition of cell growth. These eventually cause cell death (11, 101, 134). It exhibits surface-active properties. Thus the long duration of action is by virtue of the binding of CPC to the glycoproteins covering the teeth and oral mucosa. It does not alter the composition of the normal oral microbiota, which is in accordance with the American Dental Association (Council on Dental Therapeutics).

Cetylpyridinium chloride (CPC) is recognised as an effective antiplaque agent and commonly found in oral hygiene aids. It is less commonly used in root canal treatment. It is available as an over-the-counter drug regulated by the Food and Drug Administration (FDA) (1, 17, 61, 62, 69, 81, 97, 101, 116, 126) products. Mouthwashes and throat lozenges containing 1 to 2 mg of CPC and the use of 1 lozenge every 2 hours for adults and children above 6 years of age have been recommended. From April 2, 2004, the FDA permitted the use of a fine mist of CPC for antimicrobial action during the processing of poultry (level not to exceed 0.3 g of CPC per pound of poultry). Several animal studies (4, 36, 93) on the cytotoxicity of CPC have shown it to be a highly safe and effective antimicrobial agent. Studies investigating incorporation of 2.5 % CPC in orthodontic adhesive have shown that antimicrobial properties are imparted by CPC, while the diametral tensile strength of the material remains the same. Slow and continuous release of CPC over a prolonged period has numerous clinical benefits. However, the maximum safe level for such slow release activity is unknown (3).

Investigations by various researchers have proved that cetylpyridiniumchloride (CPC) mouthrinses are effective anti-plaque agents, either when used alone or along with toothbrushing(5, 17, 43, 76, 119, 133, 134). This has led the US Food and Drug Administration Dental Plaque Subcommittee to state that "it is reasonable to assume that formulations containing (at least) 72–76 % available CPC are active in reducing plaque and gingivitis (Federal Register 2003)." However certain studies have shown that the antimicrobial action of CPC is inactivated by anionic surfactant substances like sodium lauryl sulphate (SLS) found in toothpaste (17, 108). The same applies to chlorhexidine rinses and hence a 30 min. time span between toothbrushing and chlorhexidine has been suggested (8).

CPC has the distinction of being recognised by the FDA Plaque Subcommittee after a six year review of over 40 active ingredients as being one of the only three (stannous fluoride and essential oils – the remaining two safe agents) antimicrobial agents which is safe and effective (concentration range of 0.05 and 0.10 %) for the treatment of plaque-induced gingivitis. (47, 134).

Conclusion

The field of endodontics is rapidly changing with technological advances based on sound scientific research. A sterile endodontic canal is the cornerstone of successful treatment of infected root canals. It will facilitate ease in obturation and result in less post endodontic failures and complications. Hence the search for an ideal root canal antimicrobial agent which will completely eliminate endodontic pathogens from the root canal is one of the primary goals of endodontics.

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