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Role of Biguanides in Endodontics: A Review

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Abstract: *Root canal irrigants play a significant role in the elimination of microorganisms, tissue dissolution, and the removal of debris and smear layer. Sodium hypochloride is the most commonly used endodontic irrigant, despite limitations. None of the presently available root canal irrigants satisfy the requirements of ideal root canal irrigant. Newer root canal irrigants are studied for potential replacement of sodium hypochloride like biguanides. Based on the actions and interactions of biguanides, a clinical irrigating regimen is proposed. Furthermore, some technical aspects of irrigating the root canal system are discussed, and recent trends are critically inspected.*

Keywords: *Alexidine, chlorhexidine, interactions, irrigants, antimicrobial activity, review.*

I. Introduction

A favorable outcome of root canal treatment is defined as the reduction of a radiographic lesion and absence of clinical symptoms of the affected tooth after a minimal observation period of 1 yr.[1] Alternatively, so-called surrogate outcome (dependent) variables yielding quicker results, such as the microbial load remaining in the root canal system after different treatment protocols, can be defined. However, these do not necessarily correlate with the "true" treatment outcome.[2] Endodontic success is dependent on multiple factors[3], and a faulty treatment step can thus be compensated. For instance if cultivable microbiota remain after improper canal disinfection, they can theoretically be entombed in the canal system by a perfect root canal filling[4], and clinical success may still be achieved.[5] On the other hand, in a methodologically sound clinical trial, single treatment steps have to be randomized and related to outcome. Otherwise, the results do not allow any conclusions and no causative relationships may be revealed.[6]

Microorganisms are the major causative factor associated with endodontic treatment failure.[7, 8] The success of endodontic treatment depends on the reduction or elimination of bacteria present in the root canal system. Residual pulpal tissue, bacteria, and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation.[9] Therefore, irrigant solutions should be used in combination with canal preparation.

Ideal Requirements Of Root Canal Irrigants[10]

1. Broad antimicrobial spectrum
2. High efficacy against anaerobic and facultative microorganisms organized in biofilms
3. Ability to dissolve necrotic pulp tissue remnants
4. Ability to inactivate endotoxin
5. Ability to prevent the formation of a smear layer during instrumentation or to dissolve the latter once it has formed.
6. Systemically nontoxic when they come in contact with vital tissues, noncaustic to periodontal tissues, and with little potential to cause an anaphylactic reaction.

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution because of its tissue-dissolving capability as well as its broad antimicrobial action and ability to neutralize toxic products.[10-14] On the other hand, NaOCl does not impart antimicrobial substantivity.[15] Similar to cholrohexidine(CHX), alexidine (ALX) is a bisbiguanide disinfectant that helps to inhibit the immune response of the major virulence factors (lipopolysaccharide and lipoteichoic acid) of bacteria[16] and contains 2 hydrophobic ethylhexyl groups in its structure.[17] ALX has been previously used as a mouthwash solution[18] and contact lens solution.[19]

The purpose of this article is to present an overview on biguanides in endodontics, their actions and interactions. Based on data derived from basic science studies, results obtained in clinical investigations are discussed and some general recommendations are given.

Anti Microbial Effectiveness of Biguanides

CHX is a cationic bis-biguanide with good efficacy against several gram-positive and gram-negative bacteria found in endodontic infections.[20,21] Its antibacterial effects are likely to be related to the induction of damage to the bacterial cytoplasmic membrane and precipitation of intracellular constituents.[22] Although some may claim for higher concentrations of CHX, in vitro antibacterial studies suggest that even lower concentrations may perform equally well[21]; 0.12% CHX is widely used as a mouthrinse and has good tissue compatibility.[23]

If antimicrobial activity were the only requirement of an endodontic irrigant, the results of this study would indicate that chlorhexidine gluconate is the irrigant of choice. It is as effective as sodium hypochlorite. A possible clinical advantage of chlorhexidine gluconate over sodium hypochlorite is that, even through both are effective as antimicrobial agents, chlorhexidine gluconate is relatively nontoxic.[24] Another advantage of using chlorhexidine gluconate is that it could be used in patients who are allergic to sodium hypochlorite.[25] The major disadvantage of chlorhexidine gluconate as a primary endodontic irrigant is that it lacks the ability to dissolve necrotic pulp tissue .[26] As mentioned, chlorhexidine gluconate is an effective antimicrobial against many Gram-negative and Gram-positive bacteria.

Chlorhexidine (CHX) consists of two symmetric 4-cholorophenyl rings and two biguanide groups connected by a central hexamethylene chain.[27] CHX is a hydrophobic and lipophilic molecule which dissociates in solutions to form positively charged ion that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism.[28] Its efficacy is due to the interaction of the positive charge of the molecule and the negatively charged phosphate groups on the microbial cell walls[28], thereby altering the cells osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacterial cell. CHX is a base and is stable as a salt. The most common oral preparation, CHX gluconate, is water-soluble and, at physiologic pH, it readily dissociates and releases the positively charged CHX component.[28] At low concentration (such as 0.2%), low molecular weight substances – specifically potassium and phosphorous – will leak out. On the other hand, at higher concentrations (e.g. 2%), CHX is bactericidal and precipitation of cytoplasmic contents occurs which results in cell death.[28] It has a wide antimicrobial spectrum and it is effective against both Gram-positive and Gram-negative bacteria as well as yeasts, but mycobacteria, bacterial spores and most viruses are resistant to CHX.[36] The beneficial effect of CHX is a result of its antibacterial, substantive properties and its ability to inhibit adherence of certain bacteria.[28] CHX has much greater activity against Gram-positive than Gram-negative organisms. The least susceptible of the Gram-negative micro-organisms include strains of Proteus, followed by Pseudomonas, Enterobacter, Actinobacter and Klebsiella.[28] Chlorhexidine possesses adequate antimicrobial properties to enable it to be used as an antimicrobial endodontic irrigant. Gomes et al.[29] have compared the in vitro antimicrobial activity against endodontic pathogens of three concentrations (0.2%, 1% and 2%) of two forms of CHX (gel and liquid) and five concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%). All irrigants were effective in killing Enterococcus faecalis, but at different times. CHX in the liquid form at all concentrations tested (0.2%, 1% and 2%) and NaOCI (5.25%) were the most effective irrigants. However, the time required by 0.2% CHX liquid and 2% CHX gel to promote negative cultures was only 30 s and 1 min respectively. Even though all tested irrigants possessed antibacterial activity, the time required to eliminate E. faecalis depended on the concentration and type of irrigant used. On the other hand, Siqueira et al.[30] found that 4% NaOCl was statistically significantly better than 0.2% and 2% CHX against four black-pigmented Gram-negative antimicrobial aerobes and four facultative anaerobes. For the first time, Ferraz et al.[31] introduced the 2% CHX gel as an endodontic irrigant. They investigated both the ability of CHX gel to disinfect root canals contaminated in vitro with E. faecalis as well as its cleaning ability compared with commonly used irrigants, such as NaOCl and CHX liquid. The results indicated that the CHX gel produced a cleaner root canal surface and had an antimicrobial ability comparable with that obtained with other solutions tested. It was concluded that CHX gel had the potential for use as an endodontic irrigant. Sena et al.[32] investigated the antimicrobial activity of 2.5% and 5.25% NaOCl and 2.0% CHX gel and liquid as endodontic-irrigating substances against selected single-species biofilms. Findings showed that mechanical agitation improved the antimicrobial properties of the chemical substances tested using a biofilm model, favouring the agents in liquid presentation, especially 5.25% NaOCl and 2% CHX. In an in vivo antimicrobial study, Zamany[33] examined whether adding a 2% CHX rinse to their conventional treatment protocol increased the effectiveness of disinfection of the RCS. They reported that cultivable bacteria were retrieved at the conclusion of the first appointment in only one of the CHX cases, whereas the control group had seven out of 12 cases showing growth of micro-organisms. This difference was statistically significant. Recently, Siqueira et al.[34] compared the effectiveness of 2.5% NaOCl and 0.12% CHX as irrigants in reducing the cultivable bacteria in infected root canals of teeth with apical periodontitis. They found that both solutions were comparable in removing bacteria from infected root canals and suggested that both solutions could be used as irrigants. In another study, Siqueira et al.[35] evaluated the effectiveness of four

intracanal medications in disinfecting the root dentine in bovine teeth that had been infected with Candida albicans. Infected dentine cylinders were exposed to four different medications – namely, calcium hydroxide/glycerin,calcium hydroxide/0.12% CHX, calcium hydroxide/camphorated onochlorophenol/glycerin and 0.12% CHX/zinc oxide. They reported that the specimens exposed to pastes containing either calcium hydroxide/camphorated paramonochlorophenol/glycerin or CHX/zinc oxide were completely disinfected after 1 h of exposure whereas the calcium hydroxide/glycerin paste required 7 days of exposure and calcium hydroxide mixed with CHX was ineffective in disinfecting dentine even after 1 week.

Chlorhexidine has a unique feature in that dentine medicated with it acquires antimicrobial substantivity. The positively charged ions of CHX can absorb onto dentine and prevent microbial colonization on the dentine surface for some time beyond the actual period of medication.[28]

Antimicrobial Substantivity

Antimicrobial substantivity of CHX has been assessed in several periodontal and endodontic studies. In an in vivo periodontal study, Stabholz et al.[37] evaluated the substantivity of the human root surface after in situ subgingival irrigation with tetracycline HCL and CHX. They found that the substantivity of 50 mg mL-1 tetracycline was significantly greater than CHX over 12 days and greater than saline over 16 days. In an in vitro study, White et al.[38] evaluated the antimicrobial substantivity of a 2% CHX solution as an endodontic irrigant and they reported that substantivity lasted 72 h. In an in vivo study to evaluate the substantivity of 2% CHX solution, Leonardo et al.[39] found that CHX prevents microbial activity with residual effects in the RCS for up to 48 h. However, other studies have shown that the substantivity of CHX can last for longer periods of time. Khademi et al.[40] found that a 5-min application of a 2% CHX solution induced substantivity for up to 4 weeks while Rosenthal et al.[41] reported that a 10-min application of a 2%CHX solution resulted in CHX being retained in the root canal dentine in antimicrobially effective amounts for up to 12 weeks. Dametto et al.[42] found that 2% CHX gel and liquid were more effective than 5.25% NaOCl in keeping low colony-forming unit (CFU) of E. faecalis for 7 days after the biomechanicalpreparation.

Antimicrobial substantivity depends on the number of CHX molecules available to interact with the dentine. Therefore, medicating the canal with a more concentrated CHX preparation should result in increased resistance to microbial colonization. Recently, antibacterial substantivity of three concentrations of CHX solution(4%, 2% and 0.2%) after 5 min has been evaluated. Results revealed a direct relationship between the concentration of CHX and its substantivity.[43] In contrast, Komorowski et al.[44] reported that 5-min application of CHX did not induce substantivity at all and they recommended that the dentine should be treated with CHX for 7 days.

Intracanal Medicament

As mentioned above, CHX is a cationic biguanide. CHX's optimal antimicrobial activity is achieved within a pH range of 5.5–7.0.[28] Therefore, adding Ca(OH)2 to CHX (i.e. creating an alkaline pH) will precipitate CHX molecules and decrease its effectiveness.[28] When used as an intracanal medicament, CHX has been reported to be more effective than Ca(OH)2 in eliminating E. faecalis from inside dentinal tubules.[28] In a study by Almyroudi et al.[28], all of the CHX formulations used, including a CHX/Ca(OH)2 50:50 mix, were efficient in eliminating E. faecalis from the dentinal tubules with a 1%CHXgel working slightly better than the other preparations. These findings were corroborated by Gomes et al.[46] in bovine dentine and Schafer and Bossmann[47] in human dentine where 2% CHX gel had greater activity against E. faecalis, followed by the CHX/ Ca(OH)2 mixture and then Ca(OH)2 used alone. In an in vitro study using human teeth, Ercan et al.[48] showed 2% CHX gel was the most effective agent against E. faecalis inside dentinal tubules, followed by a Ca(OH)2/2% CHX mix, while Ca(OH)2 alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the Ca(OH)2/2% CHX mix against C. albicans after 7 days, although there was no significant difference after 15 and 30 days. Ca(OH)2 alone was completely ineffective against C. albicans. In another in vivo study using primary teeth, a 1% CHX gluconate gel, both with and without Ca(OH)2, was more effective against E. faecalis than Ca(OH)2 alone over a 48-h time period.[49] Schafer and Bossmann[47] reported that 2% CHX gluconate was significantly more effective against E. faecalis than Ca(OH)2 used alone, or amixture of the two. This was confirmed by Lin et al.[50] although in a study by Evans et al.[51] using bovine dentine, 2% CHX with Ca(OH)2 was shown to be more effective than Ca(OH)2 in water. In an animal study, Lindskog et al.[52] reported that teeth medicated with CHX for 4 weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption. In an in vitro study, Gomes et al.[53] investigated the time required for recontamination of the RCS of teeth with and without coronal restorations medicated with either calcium hydroxide,2% CHX gel or with a combination of both. The canals without a coronal restoration, but medicated with CHX, showed recontamination after an average time of 3.7 days; the group with Ca(OH)2 after 1.8 days and the group with CHX + Ca(OH)2 after 2.6 days. The canals medicated with CHX and restored with intermediate restorative material (IRM) showed recontamination within 13.5 days;

the group with Ca(OH)2 + IRM after 17.2 days and the group with CHX + Ca(OH)2 + IRM after 11.9 days. The group with no medication, but restored with IRM, showed recontamination after an average time of 8.7 days. There were statistically significant differences between the groups. All groups without a coronal restoration were recontaminated significantly more quickly than those restored with IRM, except those teeth that had a restoration but no medicament. The groups with intracanal medication and a coronal restoration were not significantly different from each other.

Interactions

Interaction of CHX/ALX and Naocl

The effect of a root canal disinfection regimen with a combination of CHX and NaOCl was investigated.[54-56] In particular, CHX, a dicationic acid, has the ability to donate protons, whereas NaOCl is alkaline and can accept protons from the dicationic acid. This proton exchange results in the formation of a neutral and insoluble substance, which is referred to as the precipitate, para-chloroaniline (PCA).[55,57] This precipitate acts as a chemical smear layer and can compromise the dentin permeability, the diffusion of intracanal medication, and sealing after obturation.[56,59,58] Regarding the formation of reaction precipitates, the association of ALX/NaOCl showed no precipitates covering the dentinal surface under SEM observations and also in the reaction solution after centrifuging. ALX has a slightly different structure, containing 2 hydrophobic ethylhexyl groups, whereas CHX contains p-chlorophenyl end groups . Consequently, ALX cannot produce a PCA precipitate when mixed with NaOCl.

Interaction of CHX and EDTA

When CHX and EDTA interact, a precipitate is formed that is over 90% CHX and EDTA, with less than 1% of the potential decomposition product, p-chloroaniline. The high recovery indicates that CHX is not degraded by EDTA under normal conditions. The precipitate is most likely a salt formed by electrostatic neutralization of cationic CHX by anionic EDTA. The clinical significance of this precipitate is largely unknown.[60]

Effect of CHX on dentin

CHX has the ability to bind anionic molecules such as phosphate present in the structure of hydroxyapatite. Phosphate exists in calcium carbonate complexes in dentin. CHX can bind phosphate, which leads to release of small amounts of calcium from the root canal dentin.[60]

CHX and bioflim

Spratt et al. have evaluated the effectiveness of 2.25% NaOCl, 0.2% CHX, 10% povidone iodine against monoculture biofilms of P intermedia, P miros, S intermedius, F nucleatum, and E faecalis. They reported that NaOCl was the most effective antimicrobial agent, followed by the iodine solution.[61] Clegg et al. evaluated the ex vivo effectiveness against apical dentine biofilms of three concentrations of NaOCl (6%, 3%, and 1%), 2% CHX, and Mixture of Tetracycline acid and detergents (MTAD). They reported that the 6% NaOCl and 3% NaOCl were capable of disrupting and removing the biofilm, the 1% NaOCl and the MTAD were capable of disrupting the biofilm but did not eliminate the bacteria, and the 2% CHX was not capable of disrupting the biofilm.[62]

CHX and dentin bonding (anticollagenolytic activity)

Human dentin contains at least collagenase (MMP-8), gelatinases MMP-2 and MMP-9, and enamelysin MMP-20.[63,64] Dentine collagenolytic[65] and gelatinolytic activities[65] can be suppressed by protease inhibitors, indicating that MMP inhibition could be beneficial in the preservation of hybrid layers. This was demonstrated in an In vivo study in which the application of CHX, known to have a broad-spectrum MMP inhibitory effect[66] ,significantly improved the integrity of the hybrid layer in a 6-month clinical trial.[67] Auto-degradation of collagen matrices can occur in resin-infiltrated dentine but may be prevented by the application of a synthetic protease inhibitor such as CHX.[68] On the whole, because of its broadspectrum MMP-inhibitory effect, CHX can significantly improve the resin–dentine bond stability.

Cytotoxicity of CHX

Cytotoxic effects of CHX on canine embryonic fibroblast and Staphylococcus aureus showed that bactericidal concentrations were lethal to canine embryonic fibroblasts while non-cytotoxic concentrations allowed survival of bacteria.[69] Ribeiro et al.[70] evaluated the genotoxicity (potential damage to DNA) of formocresol, paramonochlorophenol, calcium hydroxide, and CHX against Chinese hamster ovary cells. Results showed that none of the mentioned agents contributed to DNA damage. Thus, in the clinically used concentrations, the biocompatibility of CHX is acceptable.

Allergic reactions to CHX

Contant dermatitis is a common adverse reaction.[71] CHX may have a number of rare side effects, such as desquamative gingivitis, discoloration of the teeth and tongue, or dysgeusia.[72]

II. Conclusion

ALX and CHX are cationic molecules that exert their antibacterial effects by disrupting the integrity of the bacterial cytoplasmic membrane, causing the leakage of the intracellular contents.[73] Indeed, gram-positive bacteria are more sensitive to cations because they are more negatively charged.[74] ALX has greater affinity for the major virulence factors of bacteria than CHX. The difference in the hydrophobic moieties between these 2 compounds is believed to be responsible for the more rapid bactericidal action of ALX.[73] ALX had been tested in periodontologic and ophthalmologic fields. When used as mouth rinse, it reduced salivary bacterial counts and had residual antibacterial activity.[74] ALX has disinfection efficacy against all bacteria and fungi tested by contact lens disinfection standard.[75] A recent study has suggested the potential use of ALX as a novel anticancer compound.[76] Although ALX has less effect on plaque accumulation and shorter residual antibacterial activity than CHX[77], it might be valuable for endodontic irrigation purposes.

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