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Protection of tooth structure by chlorhexidine and natural polyphenols: a review

Proteção da estrutura dental pela clorexidina e polifenóis naturais: revisão de literatura

Vinay PILLY
MSc candidate - Department of Biological and Diagnostic Sciences - Community Dentistry - Faculty of Dentistry - University of Toronto
Toronto - Ontario - Canada.

Carlos Henrique Lacerda BRITO JUNIOR
MSc candidate, Department of Biological and Diagnostic Sciences Preventive Dentistry - University of Toronto
Toronto - Ontario - Canada.

Getulio Rocha NOGUEIRA FILHO
Assistant Professor - Department of Biological and Diagnostic Sciences - Preventive Dentistry - University of Toronto
Toronto - Ontario - Canada.

Anuradha PRAKKI
Assistant Professor - Department of Clinical Sciences – Restorative Faculty of Dentistry - University of Toronto
Toronto – Ontario - Canada.

Abstract

Caries is a multifactorial and transmittable disease that results mainly from bacteria present in the oral cavity. Oral microorganisms can produce metabolic acids that diffuse through dental tissues and interfaces leading to dissolution of mineral. In dentine, after mineral is dissolved, organic matrix is also exposed to breakdown by host enzymes such as matrix metalloproteinases (MMPs) that are activated by low pH followed by neutralization. This review aims to discuss the activity of different antibacterial and antiproteolytic compounds such as chlorhexidine and natural polyphenols in the control of caries progression.

Keywords

Chlorhexidine; Catechin; Polyphenol; Caries; Dentine.

Resumo

A cárie é uma doença transmissível e multifatorial, resultante principalmente da presença de bactérias na cavidade oral. Os microorganismos orais podem produzir metabólitos ácidos que se difundem entre os tecidos e interfaces dentais levando a dissolução de minerais. Na dentina, após dissolução mineral, a matriz orgânica também é sujeita à degradação por enzimas, como as metaloproteinases (MMPs), que são ativadas pelo baixo pH seguido de neutralização. Esta revisão objetiva discutir a atividade de diferentes compostos antibacterianos e antiproteolíticos, como clorexidina e polifenóis, no controle da progressão de lesões cariosas.

Palavras-chave

Clorexidina; Catequina; Polifenol; Cárie; Dentina.

Background

Several reports have shown that caries progression and bond degradation within hybrid layer are likely to occur due to degradation of hydrophilic restorative monomers and/or self-degradation of dentine collagen fibrils [1,2]. In bond degradation, this mechanism is initiated from beneath the bonded interface with the breakdown of demineralized and denatured collagen matrices by host derived MMPs [3]. On the other hand, dental caries is a transmissible bacterial disease caused by acids (lactic, acetic, formic and propionic) from bacterial metabolism, diffusing into enamel and dentine and dissolving the mineral [4]. The caries process is a continuum resulting from successive cycles of demineralization and remineralization. Demineralization begins at the crystal surface inside the enamel and continues unless arrested with the end-point being cavitation. Remineralization is a natural repair process for disease confined into enamel. Here calcium, phosphate and fluoride ions rebuild a new surface on existing crystal remnants in subsurface lesions creating an acid resistant layer, being much less soluble than the original mineral [4]. During cavitation, the mineral part of dentine is dissolved, exposing the organic matrix to breakdown by bacterially-derived enzymes and MMPs present within dentine and also from saliva [5]. It has been reported that MMPs or matrixins hydrolyze components of the extracellular matrix [6] contributing to many biological and pathological processes. When activated by low pH followed by neutralization, MMPs have been suggested to play an important role in the digestion of dentine organic matrix, therefore in the progression of carious decay [5]. Studies have demonstrated that chlorhexidine (CHX) is capable of arresting caries when applied to dentine, and this has been attributed to its wide-spectrum antibacterial and antiproteolytic activity [7-9]. The MMP-inhibitory potential of several naturally derived compounds is also gaining attention especially in the field of cancer treatment. Natural polyphenols were found to have distinct inhibitory activity against different MMPs [10,11]. Thus, the focus of this review is to present and discuss the findings related to the activity of CHX and polyphenols extracted from green tea leaves or cranberry fruit in the inhibition caries progression.

Matrix Metalloproteinases

The first collagenolytic enzyme (collagenase MMP-1) responsible for tadpole tail involution during amphibian morphogenesis was discovered in the 60s. In the present day, MMPs constitute a multigene family of over 24 structurally related but genetically distinct secreted or cell surface-associated proteolytic enzymes that can process or degrade numerous extracellular, pericellular and non-matrix substrates [12,13]. MMPs are divided in six groups based on their structural homology and substrate specificity (although there is some overlap): collagenases, gelatinases, stromelysins, matrylisins, membrane-type metalloproteinase, and others. All MMPs are regarded as derivatives of a 5-domain prototype structure formed by either the addition or deletion of regulatory domains [12]. The classical MMP structure must present two domains: 1) Prodomain (contains a highly conserved sequence of aminoacids with an unpaired cysteine sulfhydryl group whose interaction with the active site zinc maintains the enzyme in latent form - the cysteine switch) [14], and 2) Catalytic domain (with an active site Zn+2 that binds three conserved histidines in the sequence HEXXHXXGXXH(S/T)XXXXXXM and a conserved methionine to the carboxyl side of the zinc-binding site - metzincins) [15]. MMPs common features are: 1) requirement that zinc be bound at their catalytic site, 2) a family specific zinc-binding motif, and 3) propeptide domain located at the N-terminal end of the catalytic domain, maintaining the enzyme as inactive zymogen. MMP functional activity is regulated by positive or negative transcriptional controls of MMP genes, activation from latent state, differences in substrate specificity, and modulation by serum inhibitors or tissue inhibitors of MMPs (TIMPs – the most important inhibitors). MMPs are thus regulated in physiological processes, however when the mentioned regulatory controls are by-passed, excessive degradation and tissue destruction may occur. Before MMPs are activated, the prodomain and catalytic domains dissociate. This can be achieved by autocatalysis or action of proteolytic enzymes [16], by chemical agents such as reactive oxygens or thyol modifying agents, heat treatment, and changes in pH (low pH has been shown to activate gelatinases) [1].

Different MMPs (MMP-1, -2, -3, -9, -20) have been detected in dentine, either in odontoblasts or in predentine/dentine [17-19]. Collagenases and gelatinases (MMP-1, -2, -8, -9) were also detected in whole saliva [20-22]. MMP-9 was shown to be the main gelatinase in the whole saliva whereas MMP-2 was present in the form of higher molecular weight complex. Their origin is likely to be from salivary gland or the gingival crevicular fluid which is probably

the major source [23]. Collagenases have the ability to cleave interstitial collagen types I, II and III, and gelatinases degrade denatured collagen (gelatin).

Matrix Metalloproteinases and Dentine Caries

During caries activity, there is a drop of local pH caused by bacterial acid release. After mineral dissolution, dentine organic matrix (90% type I collagen and 10% phosphorylated proteins) becomes exposed to enzymatic degradation [24]. According to Tjaderhane et al., (1998) the activation of latent MMPs occur at low pH (due to alteration in propeptide conformation inducing cystein switch) followed by neutralization [1]. Thus, the conditions presented by caries lesions, with alternating periods of low pH demineralization and neutral pH due to the action of saliva buffers, are perfect to the enhancement of their activity. It has been reported that in a range of pH from 6 to 4.5, the greatest activation occurred at pH 4.5, which was the lowest pH evaluated. This in vivo study, using rat molars, also demonstrated a significant reduction in dentine lesion progression along with reduced salivary MMP activity, when MMP inhibitors were used [25]. Furthermore, Nordbo H et al. (2003) demonstrated that MMP collagenolytic activity in demineralized dentine affects its potential to further remineralization [26]. Bacterial collagenases were previously thought to degrade the organic matrix of dentine. However, experiments have shown that cariogenic bacteria can cause demineralization only, presenting weak protease activity [22]. Studies have shown that bacterial enzymes were able to cleave organic matrix proteins, but only at neutral pH and at the surface of dentine [27]. This is because bacterial proteases do not tolerate low pH ~4.3 [24]. Thus, while the role of bacterial acids in the dentine demineralization is undisrupted, uncertainty remains on the mechanism responsible for the proteolysis of dentine organic matrix. Moreover, studies have reported collagenolytic activity of sound mineralized dentine in the complete absence of bacterial colonization [28]. This implies that host-derived enzymes, localized either in dentine or saliva, may have a fundamental role in dentine organic matrix degradation and caries progression [29].

Chlorhexidine (CHX)

CHX is a cationic molecule with a wide-spectrum antibacterial activity [30]. It consists of two symmetric 4-chlorophenyl rings and bisguanide groups connected by a central hexamethylene chain. Studies have demonstrated that caries arrestment occur when carious dentine is treated with CHX varnish [31]. This has been initially attributed to its antimicrobial activity. Recently, it has however been demonstrated that CHX inhibits MMP-2, -8 and -9 activities [9] in sound dentine, and that the therapeutic mechanism in dentinal caries arrestment is very likely related to its antiproteolytic properties [32]. Both preparations of CHX (0.12%), either digluconate or diacetate seem to exhibit similar inhibitory effect on the activity of dentinal proteolytic enzymes. In adhesive Dentistry, in vivo and in vitro studies have also demonstrated that application of pure CHX solution on dentine, before restorative procedure, arrest self-destruction of acid etched dentine organic matrices probably due to MMPs inhibition [33,34]. The inhibitory effect of CHX on MMPs is still unclear but attributed to a chelating mechanism [9], since the inhibition of MMP-2 and -9 could be prevented by the addition of calcium chloride binding CHX. It has been also discussed that CHX may affect essential sulfhydryl groups and/or cysteine present in the active site of MMPs. At high concentrations above 0.2%, the inhibitory action of CHX might be related to protein denaturation rather than by chelation of cations [35]. CHX effectiveness is often related to its substantivity. This is due to its ability to bind anionic substrates like the oral mucosa, pellicle found on tooth surfaces, salivary proteins and mucous membranes [36]. Dental staining is a well-known and likely the most problematic side-effect of using CHX containing oral products. The probable cause of staining is the precipitation of anionic dietary chromogens onto adsorbed CHX surfaces [37]. It has been reported that adsorption of black tea components to the salivary pellicle is greatly enhanced by the immediate pretreatment with CHX. This is probably a result of the increased electrostatic attractions between the cationic CHX and negatively charged tea components. Clinically, it is expected that CHX will interact with a range of dietary chromogens (i.e., juices, red wine, coffee, curry, and soy sauces) and therefore produce colored precipitates [38]. Despite risks of staining, incorporation of CHX into resin composites and its antibacterial properties, mechanical properties, and release patterns are being evaluated [39,40].

Green Tea Polyphenols

Many health benefits related to the flavonoids (polyphenol extracts from black, green or oolong

teas) are being scientifically established. Green tea (GT) plant (Camellia sinesis) has flavonoids called catechins. Many studies suggest that consumption of GT may help prevent cancer with evidence that it has anti-mutagenic and anti-carcinogenic properties. Some beneficial effects of catechins have been attributed to their anti-oxidative effects. However, as it has been demonstrated that MMPs are very likely involved in human tumors [10], studies have been evaluating the alternative mechanisms of action, such as MMP inhibition, of the following catechin polyphenol analogs: (+)-catechin (C); (-)-epicatechin (EC); (-)-epigalattocatechin (EGC); epicatechin gallate (ECG); (-)-epigalattocatechin gallate (EGCG) [41].

Polyphenols usual composition in GT is: EGCG – 10-15%; EGC – 6-10%; ECG – 2-3%; and EC - 2% [41]. As reported by Demeule et al., (2000) among many natural products tested (polyphenols from red wine, soy extract genistein, GT polyphenols and compounds from garlic) for MMP inhibition, the GT polyphenols caused the strongest inhibition of MMP-2 and -9 in a dose dependent manner [42]. EGCG (the major constituent of GT polyphenols) was the most potent inhibitor followed by ECG. Gelatin zymograms also confirmed inhibition of MMP-2 and proMMP-9 by the other catechins (EGC, EC and C). MMP-9 was more sensitive than MMP-2 to these compounds. In addition, Suganuma M. et al., [41] indicated that GT extracts together (i.e., from the tea itself) have synergistic effects which resulted in a higher cancer-preventive activity than EGCG alone. In Dentistry, it has been recently shown that EGCG was able to reduce erosion and abrasion of bovine dentine in situ [43]. Although MMPs inhibition or collagen degradation had not been directly evaluated, the effects of GT polyphenol on dentine wear were attributed to possible MMPs inhibitory effect [44]. The mentioned study evaluated EGCG only. Different mechanisms were discussed for the inhibitory effects of GT polyphenols on the activity of MMPs. EGCG likely binds with the catalytic site (or close) of MMPs. There is also a possibility that the conformation of MMP-2, which is essential for its activity, could be altered by EGCG binding to any place of the enzyme. Interestingly, EGCG complexed with MMP-2 had no effect on MMP-2 binding to extracellular matrix proteins but significantly enhances both pro- and active-MMP-2 binding to TIMP-2 [45]. The presence of steric structure 3-galloyl radical in some tea catechins can play an important role in MMPs gelatinolytic activity [46,47].

Additionally EGCG has also been shown to have a broad spectrum antibacterial property. Several studies have reported its effectiveness in inhibiting acid production in dental plaque bacteria as well as antimicrobial activity against the cariogenic S. mutans [48,49]. In this sense, recent studies have been incorporating EGCG into restorative dental resins and testing these materials for drug delivery, mechanical and physical properties as alternatives to CHX [40]. Moreover, bonding agents incorporated with EGCG have also been tested for antibacterial and physicochemical properties, showing stability in resin-dentine bonding as well as inhibitory effect on the growth of S. mutans [50].

Cranberry Polyphenols

In recent studies, some benefits related to polyphenols in cranberry (Vaccinium macrocarpon) juice or extracted from cranberry fruit have been established. It has been demonstrated that cranberry polyphenols have the ability to decrease the cell surface hydrophobicity of streptococcal bacteria (S. sobrinus and S. mutans) [51], and adhesion between cariogenic bacteria and enamel-like structures (hydroxyapatite beads) [52] and enamel-like structures pretreated with glucans [53]. Other studies confirmed that cranberry extracts are not only able to inhibit the adhesion of S. sobrinus to enamel-like structures coated with saliva [54], but also led to desorption of the same bacterial species from an artificial dental biofilm [55]. More recently, the ability of cranberry polyphenols in reducing the formation of biofilm by S. mutans in vitro, and dental caries development in vivo (Sprague-Dawley rats) has also been reported [56]. Moreover, Bodet et al. [57] reported that low concentrations of cranberry extract inhibited the secretion of MMP-3 and MMP-9 by the gingival fibroblasts and macrophages following stimulation by the LPS of Aggregatibacter actinomycetemcomitans, a causative agent involved in periodontal disease. Their results also showed that the cranberry extract inhibited the catalytic activity of both enzymes and elastase. These seem to be promising results as inhibition of MMPs such as -9 might inhibit caries progression [1].

Most studies that evaluated the effects of cranberry polyphenols on cariogenic bacteria and MMP activity used a fraction of cranberries called the nondialyzable material (NDM), which is obtained by dialysis of concentrated cranberry juice [51,52,54,55,57]. The content of various cranberry polyphenols (phenolic acids, anthocyanins, flavonols, flavan-3-

ols, and proanthocyanidins) in NDM is subjected to variations depending on seasonal and varietal effects. Although, anthocyanins (monomer), flavonols and proanthocyanidins (polymer) are among the most abundant classes of polyphenols and have been associated with the health promoting benefits of cranberry [58], the biological action and potential usefulness of each individual compound are still unclear.

Conclusion

The broad-spectrum antibacterial CHX is a very commonly used agent for prevention or control of oral diseases. However, CHX has some disadvantages such as its synthetic origin, as concerns with toxicity, environment care, poisoning has been clearly rising by the population in general. Moreover bacterial resistance, tooth staining and mild toxicity to odontoblast-like cells [59] are motivating the investigation of alternatives. Natural products, especially food extracts, have been shown to be potential anticariogenic alternatives to synthetic chemicals due to their biological properties. Future research is however required to advance the knowledge in the effect of MMP inhibitors on caries prevention and progression.

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Corresponding Author

Anuradha Prakki
Restorative Department
University of Toronto - Faculty of Dentistry
Room 352C, 124 Edward St.
Toronto, ON, Canada
M5G 1G6
Phone: 416-979-4932 ext. 4389
E-mail: anuradha.prakki@dentistry.utoronto.ca