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Anticariogenicity of Casein Phosphopeptide-amorphous Calcium Phosphate: A Review of the Literature

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Abstract

Aim: This review of the literature examines the role of the natural components of saliva in maintaining tooth mineralization and the role of different casein phosphopeptide amorphous calcium phosphate-based (CPP-ACP) compounds in controlling demineralization/ remineralization and their clinical applications.

Background: A group of peptides, known as CPP, have been shown to stabilize calcium and phosphate preserving them in an amorphous or soluble form known as amorphous calcium phosphate (ACP). Calcium and phosphate are essential components of enamel and dentine and form highly insoluble complexes, but in the presence of CPP they remain soluble and biologically available. This CPP-ACP complex applied to teeth by means of chewing-gum, toothpaste, lozenges, mouth rinses, or sprays is able to adhere to the dental biofilm and enamel hydroxyapatite providing bioavailable calcium and phosphate ions.

Review Results: Significantly high levels of calcium and phosphate have been found in both biofilm and subsurface incipient caries lesions and in lower level demineralization of enamel or dentine surfaces previously treated with CPP-ACP based compounds. When placed on the surface of a tooth with early carious lesions, pastes with CPP-ACP complexes can prevent tooth demineralization and improve enamel remineralization and enhance fluoride activity.

Conclusion: Remineralization of white spot lesions has been achieved clinically by applying pastes based on these compounds, and a similar effect to self-applied fluorides has been observed in reducing the appearance of new caries lesions in patients with xerostomia.

Clinical Significance: Use of CPP-ACP based compounds offers a potential for use in the prevention of dental caries.

Keywords: Casein phosphopeptide, CPP, amorphous calcium phosphate, ACP, demineralization, remineralization, caries, dental plaque

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Introduction

Better understanding of the etiology and pathogenicity of caries and the decline of the disease in recent years, especially in children and adolescents, have led to the use of more medical management and conservative measures in accordance with minimally invasive intervention criteria.[1] However, several risk factors for dental caries are still present and include:

- The frequent consumption of medicines effecting salivary flow, especially in older people, pose an added risk factor for the appearance of caries, especially root caries.[2]
- The public's dietary habits which include high consumption of soft drinks, especially among young people, has increased the risk of erosive lesions of teeth produced by chemical processes which reduce enamel hardness and can even lead to its complete disappearance.[3]
- Dietary disorders are also a high risk factor in dental erosion.[4]

Prevention of dental caries by milk-derived bioactive peptides is a complex physical and chemical sequence of cascading events. In general, bioactive peptides with anticariogenic activity have multiple functions to prevent dental lesions including bacterial inhibition, competitive exclusion to enamel binding sites, improved buffering capacity in the pellicle surrounding teeth, reduced enamel demineralization, and improved enamel remineralization. Anticariogenicity studies with dairy bioactive peptides have been accomplished with a number of *in vitro*, *in situ*, and *in vivo* model systems.[5] Through multiple phosphorylated residue,

CPP can free their own weight in calcium and phosphate to form colloidal complexes which prevent calcium phosphate crystals from growing to the size required for precipitation.[6]

In this present work we review the role of the natural components in saliva in maintaining tooth mineralization and the role of different CPP-ACP based compounds in controlling demineralization/ remineralization and the clinical applications.

Background

A literature review of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) was conducted. Papers were included for review from Medline since 1995 using the following descriptors:

- Remineralization and CPP-ACP
- Demineralization and CPP-ACP
- Dental plaque and CPP-ACP
- Caries and CPP-ACP

Out of a total of 50 publications identified, 31 were selected for this review because they were *in vitro* and *in vivo* studies on the role of CPP-ACP and subsequent revisions.

Review Results

Salivary Components in the Remineralization Process

Saliva contains a series of substances which are involved in the remineralization process: inorganic compounds, mainly calcium, phosphate, and fluoride, and organic elements which help to maintain calcium and phosphate supersaturation.

Ionic calcium is particularly important as it helps to maintain a balance between the calcium in the calcium phosphate in the tooth and the surrounding fluids. Ionic calcium concentration in saliva rises as salivary pH falls. Non ionic calcium is found attached to phosphate, bicarbonate, and other organic molecules. Ionic phosphate concentration is also determined by pH, between 10 and 25% of this compound is found in ionic form, and the rest is attached to calcium or proteins. The fluoride concentration in saliva is largely determined by external contribution through topical or systemic products. After topical fluoride application, fluoride rapidly spreads via saliva to the dental biofilm and concentrations increase over a short period of time forming calcium fluoride complexes and a smaller proportion of magnesium fluoride.[7]

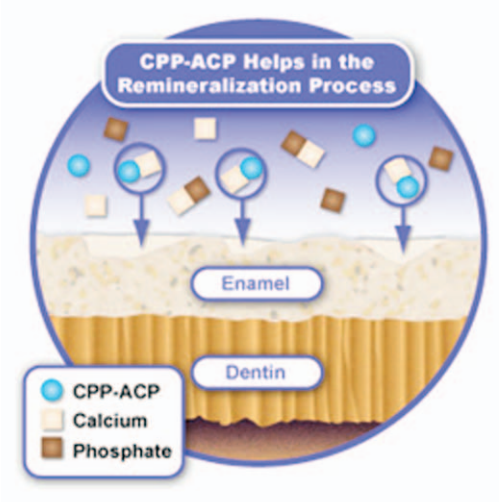
Statherin is a phosphoprotein with a strong affinity to calcium, enamel, and other apatite surfaces. Its functions include the capacity to inhibit the precipitation and growth of calcium phosphate crystals. Many statherin properties are shared by CPP.[8] Proline-rich proteins function in a similar manner to statherin and adhere to the surface of calcium phosphate crystals preventing their growth. Together with citrate, these proteins adhere to a significant amount of the total calcium in saliva and help to maintain correct proportions of ionic calcium and phosphate. These proline-rich proteins are key components of biofilms and adhere strongly through their amino-terminal portion to the enamel. The carboxy-terminal portion is the adhesion site for some bacteria in the early stages of plaque formation and is also the adhesion site for dietary tannins.[9]

Other salivary factors involved in remineralization are the salivary flow itself, which accelerates sugar clearance, buffer capacity, and saturation level of mineral elements in the tooth, especially calcium, phosphate, and fluoride.[7]

Role of Phosphopeptides in the Saliva and Dental Biofilm

Bovine milk is used to manufacture phosphopeptide based dental products for use in humans. There are three types of phosphopeptide based products: CPP, CPP-ACP, which contain 18% calcium ion and 30% phosphate ion in weight, and casein phosphopeptides with amorphous calcium fluoride phosphate (CPP-ACFP).[5] The latter provides all the elements necessary for dental remineralization (calcium, phosphate, fluoride, and water) on the tooth surface and in the dental biofilm. Plaque enzymes such as phosphatases and peptidases partially degrade CPP-based products, consequently increasing pH due to the production of ammonia. Adding fluoride to CPP limits phosphatase action by extending the action of molecular complexes.[8]

CPP-based products and particularly CPP-ACFP are the basis for the anticariogenicity of milk derivates. The anticaries action of CPP derivates has a topical effect based on the following: modulation of bioavailable calcium phosphate levels because they maintain ionic phosphate and calcium supersaturation; buffer effects on plaque; increased remineralization and reduction



of the hydroxyapatite solution; and difficulty for *Streptococcus mutans* and *Streptococcus sobrinus* to adhere and grow.[10,11]

CPP can adhere to 25 calcium ions, 15 phosphate ions, and 5 fluoride ions per molecule and can stabilize calcium phosphate in solution. ACP are formed when pH is neutral or alkaline. CPP, through its phosphorilated residue, can free its own weight in calcium and phosphate to form colloidal complexes. When CPP adheres to these ACP aggregates, their growth is controlled preventing them from reaching the critical size for precipitation.[12]

CPP-ACP complexes have shown an anti-caries effect in both experimental animals and human incipient caries models.[13] These complexes are found in ACP in the dental biofilm and increase calcium phosphate levels which serve as a reservoir for free calcium and phosphate ions. The final effect is saliva and plaque are kept in calcium and phosphate supersaturation with respect to the enamel which allows a reduction in demineralization and favors remineralization.[14] CPP-ACP complexes can be applied clinically to prevent caries and enamel erosion.

CPP and glycomacropeptides (GMP), which are also milk derivates and particularly present in cheese, reduce *S. mutans* and *S. sobrinus* adherence to dental biofilm; this selective inhibition can give rise to a less pathogenic plaque formation. Other proteins derived from milk such as lactoperoxydase, lysozyme, and lactoferrine also limit metabolism of *S. mutans* or interfere with biofilm adherence. GMP have been patented as antimicrobian for addition to toothpaste, mouth rinses, chewing-gums, and gels. Their effect can be strengthened when combined with fluoride or xilitol.[11]

CPP-ACP Action Against Dental Erosion

In vitro studies have shown lower enamel erosion due to citric acid when enamel is previously treated with a CPP-ACP paste (GC Tooth Mousse®, GC Corporation Itabashi-ku, Japan).[15] Likewise, enamel treated with cola derivatives which reduce its hardness and then subjected to the action of a CPP-ACP paste showed a significant increase in hardness independently of the presence of fluoride.[16] Adding CPP-ACP to energy drinks reduces their erosive capacity with no change in flavour when added in a proportion of over 0.09%.[17] The addition of CPP-ACP to citric acid and sugar free chewing-gums has shown greater remineralizing action than sugar-free chewing gums without citric acid or CPP-ACP. Remineralization after an acid attack was significantly greater when chewing-gums with CPP-ACP[18] were administered.

Introducing CPP-ACP nanocomplexes to soft drinks and other frequently consumed acid products, especially for the adolescent and young adult population, could help to reduce the erosive action of these products.

CPP-ACP Action to Prevent Demineralization and Improve Remineralization

Fewer morphological surface changes were observed with SEM after adding enamel and dentine to a CPP-ACP solution followed by immersion in a 0.1 M lactic acid solution, in

comparison to specimens not treated with CPP-ACP, which is evidence of lower demineralization in the former.[19] In addition, specimens which were treated with Tooth Mousse GC® after demineralization showed higher remineralization than the untreated specimens.[20] *In vitro* use of Tooth Mousse GC® reduced demineralization around orthodontic brackets, especially when the brackets were cemented with resin-reinforced glass-ionomer cement.[21]

Experimental studies have shown adding CPP-ACP to chewing gums containing xilitol or sorbitol as sweetners significantly increases subsurface remineralization of experimental caries lesions with a dose-related response.[22]

The addition of CPP-ACP (Recaldent™, Recaldent Pty Limited, Victoria, Australia) to chewing gums has demonstrated its capacity to promote subsurface remineralization of experimental caries lesions and that remineralization is dose-dependent.[23]

Microradiographs and densitometry have shown the use of lozenges with different CPP-ACP concentrations increases remineralization in subsurface caries lesions, and this remineralization is dose-dependent. Therefore, lozenges containing CPP-ACP may also be considered a suitable vehicle for remineralizing incipient caries lesions.[24]

Two percent CPP-ACP solutions have also shown their effectiveness in reducing subsurface caries lesions, obtaining higher remineralization with longer application times.[24] Studies on patients with demineralization white spots after removing orthodontic brackets showed daily application of a CPP-ACP cream for three months, followed by brushing with a fluoride toothpaste for three months achieved complete removal of the spots after monitoring for 12 months. CPP-ACP have also been successfully used to prevent the appearance of these demineralization white spots after attaching orthodontic brackets.[25]

Clinical cases of root caries lesions have been described which have been stabilized by fluorescence laser using a CPP-ACP paste (Tooth Mousse GC®).[26] Enamel microabrasion together with prolonged use of a CPP-ACP-based paste is useful for treating white spot enamel lesions.[27]

The presence of CPP-ACP compounds was detected using ELISA techniques in supragingival plaque, adhering to bacteria walls and the intercelullar substance when it was supplied in the form of mouth rinses or chewing-gum, providing higher concentrations of calcium and phosphate ions on the plaque surface than other calcium-based products supplied by the same channels.[28] Similar results were obtained using experimental models on *Stretococcus* plaques.[29]

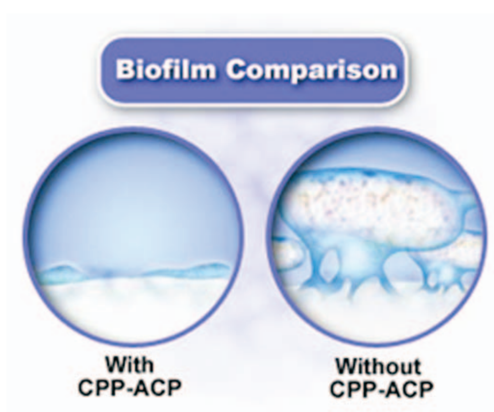
CPP-ACP Action in Patients with Xerostomia

In vivo studies in patients with xerostomia treated with CPP-ACP-based mouth rinses show a lower rate of new caries lesions than for patients treated with 0.05% fluoride mouth rinses, although there are no significant differences between the groups after monitoring for 12 months.[30]

Discussion

The addition of CPP-ACP to chewing gums, lozenges, mouth rinses, toothpaste, and even some foods is a promising tool for preventing dental caries and early treatment of incipient lesions, especially in children, young people, and patients with xerostomia. Numerous experimental studies support the beneficial effect of CPP-ACP in controlling the demineralization/remineralization of both enamel and dentine.

They have a multiple action mechanism on the one hand providing an oversaturation of calcium and phosphate ions in the dental biofilm and saliva, and on the other hand inhibiting adhesion of cariogenic bacteria to the hyroxiapatite making it possible to modulate the activity of plaque



bacteria and favour colonization by less cariogenic bacteria. This can help to reduce acid formation in the biofilm and reduce enamel demineralization.[5]

Prolonging contact time with tooth surfaces by including these bionanocomplexes in chewing gums and toothpastes has proven effective in experimental studies on the remineralization of subsurface lesions and reduction of demineralization where portions of enamel previously in contact with CPP-ACP-based substances were subjected to artificial demineralization.[24]

Furthermore, the combination of CPP-ACP nanocomplexes and fluoride in toothpaste provide a greater concentration of fluoride ions in the dental biolfilm and a greater increase in remineralization than if only fluoride toothpaste is applied.[31]

These experimental findings offer promising results which need to be confirmed with clinical and epidemiological studies, especially for application in patients with high caries risk, due to hyposalivation, irradiation, dental sensitivity, eating disorders (anorexia and bulimia), etc., where the demineralization/remineralization balance is always unfavourably inclined towards demineralization.

Conclusion

Remineralization of white spot lesions has been achieved clinically by applying pastes based on CPP-ACP compounds and a similar effect to self-applied fluorides has been observed in reducing the appearance of new caries lesions in patients with xerostomia.

Clinical Significance

Use of CPP-ACP based compounds offers a potential for use in the prevention of dental caries.

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