Review Article

The Applications and Modifications of Casein Phosphopeptide-Amorphous Calcium Phosphate: An Update

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Abstract

Demineralization begins as a reversible process and current trends have surpassed the conventional options of invasive treatment to a more targeted non-invasive early management of reversible lesions using various new non-fluoride agents. Casein phosphopeptide-amorphous calcium phosphate is one such non-fluoride agent introduced with the potential of early remineralization in dental caries and dental erosion and to overcome conditions of dentine hypersensitivity. Various modifications of the agent have further expanded its applications in the dental field. This review was therefore, compiled from a final of 70 full-text articles searched on PubMed and Google Scholar that had the specified MeSH terms, published in English from the year 2000-2020, to present an update on the innumerable applications and modifications of this agent in pediatric dental practice.

Keywords: Casein phosphopeptide-amorphous calcium phosphate, dental erosion, remineralization, white spot lesions

Introduction

Tooth demineralization is a chemical reaction that entails an acidic attack on the enamel, which can be caused by dental erosion or dental caries, leading to calcium and phosphate ions dissolution. Demineralization begins first as a reversible process and thus, the partially demineralized hydroxyapatite crystals could return to their normal form if they are exposed to oral environments with the potential of remineralization.[1]

Currently new non-fluoride agents have been introduced in the market which have surpassed the conventional invasive treatments to a more targeted non-invasive management of reversible lesions using various remineralization agents. Casein Phosphopeptides (CPPs) were first introduced by Reynolds in 1993 as an anti-cariogenic and anti-calculus agent, and later in 1998 as a remineralizing agent at the University of Melbourne in Australia.[2]

This narrative review is compiled to provide insight on the applications and modifications of Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in pediatric dental practice.

Materials and Methods

A literature search using electronic databases such as PubMed and Google Scholar yielded 240 articles. The

articles were searched using MeSH terms: (casein phosphopeptide-amorphous calcium phosphate OR CPP-ACP) AND (enamel remineralization OR white spot lesions OR dental erosion OR dentine hypersensitivity), (casein phosphopeptide-amorphous calcium phosphate OR CPP-ACP) AND (modifications). A manual search was done which resulted in 10 additional articles to give a final of 250 articles. The titles, abstracts and full text of the identified articles were independently reviewed, and 75 duplicate studies were excluded. About 70 full-text articles that had the specified MeSH terms, published in English from the year 2000-2020 were included in the current review.

Mechanism of action

FitzGerald[3] discovered Casein phosphopeptides (CPPs), which are casein-derived phosphorylated peptides characterized with the ability to bind and solubilize minerals like calcium, magnesium and iron. The active sequence in CPP is –Ser(P)-Ser(P)-Ser(P)-GluGlu- referred to as the 'acidic motif'. The Seryl-phosphate group is the principle binding site for calcium.[4]

Calcium phosphate reservoir

CPP–ACP fosters the bond between ACP to the dental plaque causing a rise in the calcium phosphate content of plaque. Thus, plaque forms a calcium phosphate reservoir which maintains the supersaturation of tooth enamel.[5]

Buffering action

Under acidic pH, the CPP bound ACP dissociates to calcium and phosphate ions and induces a buffering action. The acid generated during the formation of hydroxyapatite as a result of enamel remineralization is also consumed by CPP-ACP by generating more CaHPO4, to maintain its concentration gradient.[6]

Antibacterial activity – delayed biofilm formation

Calcium phosphate sequestering phosphopeptides are tryptic peptides of casein which were discovered to be responsible for its anti-cariogenicity.[7]

CPP-ACP has the potential to substitute albumin in the pellicle which impedes the adherence of Streptococcus mutans and Streptococcus sobrinus. Casein also acts as an inert barrier to the diffusion of protons by providing basic amino acids and buffers the plaque acid.[8]

Rose et al[9] found a strong affinity of CPP-ACP to *S. mutans*, which binds to plaque and provides a pool of calcium ions besides slowing down the diffusion of free calcium ions. CPP-ACP also competes with calcium for plaque binding, resulting in a decrease in the amount of calcium-bridge produced between the pellicle and bacterial cells, as well as between the bacterial cells.

Other studies have observed a dose dependent manner of caries reduction by CPP-ACP. 1% CPP-ACP caused a 55% decrease in smooth surface caries and a 46% decrease in fissure caries, which was comparable to 500ppm fluoride.[10]

Anti-calculus action

ACP binds to CPP in a pH-responsive manner, i.e., the binding decreases with the fall in pH and vice-versa. As pH increases, free calcium and phosphate ions are stabilized, inhibiting spontaneous precipitation of calcium phosphate, and this inherently is the anti-calculus action of CPP-ACP.[11]

Interactions of CPP with saliva

Huq[12] demonstrated the interaction of CPP with saliva through hydrophobic or electrostatic bonds which lead to the concomitant retention of minerals and peptides derived from these complexes within the plaque. It also facilitated the remineralization process of early enamel lesions and subsurface enamel by the release of calcium phosphate on interaction with specific proteins.

Poureslami et al [13] deduced a significant increase in salivary and plaque calcium and phosphate levels among 6-9-year-old children after application of CPP-ACP. Plaque fluoride levels also increased but with no significant differences in the amount of fluoride in saliva.

Sub-surface remineralization potential

Prior remineralization of enamel lesions by CPP–ACP has demonstrated higher tolerance to subsequent acid challenge due to the remineralization of enamel subsurface lesions. CPP-ACP binds to dental plaque and slows calcium ion diffusion from enamel during acid challenge episodes, and acts as a calcium ion reservoir for eventual remineralization.[14]

Yengopal[15] described the successful dosage for short-term trials for preventing caries ranged from 10.0 to 18.8 mg CPP-ACP, and a dose of 54 mg CPP-ACP was suggested for long-term efficacy. The findings showed that lesions exposed to CPP-ACP had significantly better remineralization than lesions not exposed to CPP-ACP, supporting CPP- ACP's remineralization ability.

Applications of CPP-ACP

CPP-ACP has a significant remineralization potential which makes it a potential agent in various conditions where remineralization is necessary.

Early dental caries/white spot lesion reversal

White spot lesions (WSLs) clinically may be defined as a white, opaque area due to sub-surface mineral loss occurring below a relatively intact enamel surface.[16] High doses of fluoride have been suggested for preventing the progression of WSLs to caries. However, some authors have raised concerns regarding fluoride application due to the potential risk of dental fluorosis and hyper-mineralization of fluoride superficially which prevents sub-surface remineralization of the lesion.[17] Casein phosphopeptide is capable of reversing early enamel lesions by stabilizing calcium, fluoride, and phosphate ions on the tooth surface, leading to sub-surface remineralization of WSLs (Table 1).[18]

Ma et al[26] deduced excellent remineralization of WSLs by CPP-ACP and they recommend dentists to prioritize CPP-ACP when treating patients with WSLs, especially in children whose risk factors could be managed adequately, besides those with high aesthetic demands.

Dental erosion

Increased consumption of carbonated beverages, in addition to medical conditions like anorexia and bulimia, may lead to dental erosion in children.[27] A longer intra-oral exposure is thought to favor pH, pKa, and titratable acidity of the drink, while a shorter intra-oral exposure favors pH and pKa alone.[28] Citrate anions from the citric acid added in carbonated drinks, bind to calcium ions and form chelates, reducing the amount of free calcium ions available on the enamel and in saliva, resulting in demineralization.[29]

Studies have evaluated the possibility of reducing the erosivity of carbonated drinks by adding different compounds such as calcium, phosphate, and fluoride to it. A study by Xavier et al[30] added iron supplements to carbonated beverages which preserved the composition and microhardness of the enamel surface. Likewise, CPP-ACP added to carbonated drinks can reduce the erosive capacity of acidic soft drinks by raising the supply of calcium and phosphate ions and assisting nano-complexes in binding to the enamel surface, reducing the number of enamel dissolution sites (Table 2).[27]

Dentin hypersensitivity

Dentin hypersensitivity (DH) has been defined as a short, sharp pain arising from exposed dentin in response to a stimulus.[36] Increased CPP adsorption and release of calcium, phosphate, and fluoride ions within these nano-complexes occur by the CPP-ACP complexes' ability to adhere to dentine and dissolve in aqueous solutions. The release of these ions occludes the exposed dentinal tubules, lowers dentinal tubule permeability, and overcomes dentin hypersensitivity through the precipitation of fluorapatite crystals, which form a nanofilament coating (Table 3).[37]

Remineralization of molar incisor hypomineralization

Molar incisor hypomineralization (MIH) describes hypomineralized enamel of first permanent molars noted as demarcated opacities which easily chip off leading to exposed and sensitive dentine.[38-42] To increase mineralization and eliminate sensitivity, remineralization therapy is recommended and desensitization

Table 1. Role of CPP-ACP in treating white spot lesions

1. Munjal et al[19] Analytic and prospective study	Smooth surface WSLs occurred on 49.6% first permanent molars. CPP-ACP caused significant reduction in demineralization after 8-12 weeks on gingival-third and after 4-8weeks on middle-third.
2. Karabekiroglu et al[20] Randomized Controlled Trial	CPP-ACP with fluoridated toothpaste provided a synergistic impact on subsurface lesion regression.
3. Al Batayneh et al[21] Randomized Clinical Trial	CPP–ACP alone was similar to fluoride dentifrice, and their combination had no added benefits over either agent alone.
4. Sreekumar et al[22] *In-vitro* study	Clinpro showed superiority as a remineralization agent than CPP-ACP and Novamin.
5. Radha et al[23] Randomized Clinical Trial	Active WSL turned inactive after 24 weeks in all groups; statistically insignificant reduction in WSL by MI varnish
6. Das et al[24] Randomized clinical trial	Fluoride varnish and GC Tooth Mousse both reported only slight reduction in active WSLs.
7. Wang et al[25] Systematic review of 11 studies; 9 RCTs and 2 NRCTs.	CPP-ACP and its products may be effective, but are not significantly higher than fluoride alone

Table 2. Role of CPP-ACP in management of dental erosion

1. Manton et al[27] *In-vitro* study	The erosivity of drinks was eliminated as CPP-ACP was added
2. Carvalho et al[31] *In-vitro* study	Nano-phosphate group showed least mineral loss followed by CPP-ACP.
3. Agarwal et al[32] *In-vitro* study	Marked decrease in surface roughness after application of CPP-ACP in primary teeth compared to permanent teeth.
4. Rizvi et al[33] *In-vitro* study	Rapid remineralization of enamel increased the bond strength significantly at a shorter interval compared to saliva
5. Alencar et al[34] In-vivo study	Chewing gum containing CPP-ACP had no better defense against dental degradation or anti-erosive impact than regular chewing gum.
6. Velagala et al[35] *In-vitro* study	Decreased titratable acidity in all beverages with reduced loss of enamel.

Table 3. Role of CPP-ACP in management of dentine hypersensitivity

1. Prabhakar et al[38] *In-vitro*	MI paste plus showed weak tubule occlusion (29.51%) compared to NaF and Clinpro
2. Mahesuthi et al[39] Randomized Controlled Trial	Short-term efficacy of MI paste is due to the colloidal particles blocking the dentinal tubules.
3. Soares et al[40] SEM *in-vitro* study	CPP-ACPF effectively decreased DH after 1 month with increase in bioavailable calcium and phosphate concentration
4. Shah and Bajaj[41] SEM *in-vitro* study	CPP–ACP caused 65.33% tubule occlusion which was significantly higher compared to hydroxyapatite

should begin as soon as the defective surface is accessible to create a hypermineralized surface layer.[43]

MIH opacities have a lower mineral content and a higher organic content, which makes remineralization with CCP-ACP a difficult and time-consuming process. CPP binds to pellicle and plaque and retains elevated calcium and phosphate ion concentrations. Under the acid challenge, this ion reservoir retains a supersaturated mineral state, decreases demineralization, and improves enamel remineralization. This therapeutic approach, however, necessitates long-term care and a high level of patient cooperation.[44]

Tooth bleaching

Tooth bleaching causes demineralization with the prolonged exposure of teeth to bleaching agents as a result of its low pH and the by-products of protein denaturation.[45] Peroxide solutions in the oral environment trigger tooth sensitivity by contacting dentinal surfaces, retracting the odontoblastic processes, inducing rapid fluid movement within the dentinal tubules, and eventually stimulating mechanoreceptors at the pulp periphery.[46]

The ability of CPP to provide a constant supply of calcium and phosphate ions has led to the combination of CPP-ACP with bleaching peroxides to improve enamel remineralization. Studies have shown that it can be used in a 1:1 ratio without compromising the bleaching effect.[44]

The application of Tooth Mousse before bleaching can also improve enamel consistency and prevent reduction in surface microhardness induced by hydrogen peroxide from bleaching gels, with no effect on the color change caused by the bleaching procedure (Table 4).[47-50]

Avulsion: Re-implantation medium

Cehreli et al[51] investigated the potential of CPP-ACP as a re-implanting medium and discovered a 1–3 day increase in cell count. For a total of 7 days, a 'toxic threshold' was found at the cell surface where the maximum concentration of CPP-ACP was used due to the higher calcium content in Tooth Mousse. As a result of this research, it was discovered that low concentrations of CPP-ACP may be useful in maintaining the PDL's viability *in-vitro*.

Role in systemic conditions

Cerebral palsy (CP) is a term used to describe a group of syndromes that are characterized by motor dysfunction.

Table 4. Role of CPP-ACP in tooth bleaching

1. Borges et al[48]	Hydrogen peroxide office bleaching and CPP-ACP combined may reduce the tooth sensitivity and avoid morphological alterations on enamel after bleaching.
2. Heshmat et al[49]	Remineralization products used for 15 days decreased the surface roughness of enamel surfaces previously roughened by bleaching agents.
3. Kutuk et al[50]	CPP-ACP showed the highest microhardness 1 day after the bleaching treatment, which further increased 14 days after in-office bleaching.

[52] These children have a high incidence of gastro-esophageal reflux, thus a high risk of dental erosion. Ozdas et al[53] found that CPP-ACP increased the stimulation of saliva and its buffering capacity and had excellent anti-cariogenic activity as well. The added advantage of the use of CPP-ACP in children over fluoride-based products is that CPP-ACP can be swallowed without causing any internal harm to children.

Perić et al[54] found after 28 days of CPP-ACP use, there was a decrease in the number and size of enamel defects in patients with salivary gland hypofunction, and also the remineralization was faster and more pronounced. Despite the high caries risk, CPP-ACP can act as caries-preventive agents, as well as alleviate dry-mouth symptoms in Sjögren's syndrome patients.

Modifications of CPP-ACP
The ability to improve the remineralization potential and biological properties of CPP-ACP has led to various modifications of this product.

CPP-ACP with added fluoride (CPP-ACPF)
CCP-ACPF contains CPP-ACP plus 0.2% sodium fluoride i.e. 900 ppm fluoride (GC Tooth Mousse Plus). CCP-ACP and fluoride have shown a synergistic effect on remineralization by localizing calcium and phosphate ions in contact with fluoride on the tooth surface. As a result, it provides all of the ions needed to form fluoro-apatite crystals, which are more acid-resistant than hydroxyapatite.[55] Poureslami et al[56] found the salivary fluoride concentration was higher following the application of CPP-ACPF than with CPP-ACP and suggested it to be more effective in protecting primary teeth from caries. Imani et al[57] concluded a synergistic effect on enamel remineralization by CPP-ACP and fluoride combined due to the formation of stabilized ACPF.

CPP-ACP containing propolis
Biofilms with cariogenic bacteria such as Streptococcus mutans can lead to demineralization of the enamel surface as they break down sucrose and produce acid.[58] Propolis is a natural material of antibacterial properties,

besides being a strong antioxidant. As a result, by destroying the bacterial cell wall with its active ingredient flavonoid, it can effectively reduce gram-positive bacteria count and inhibit bacterial colonization.[59] In this regard, the use of CPP-ACP containing propolis for remineralization and as an antibacterial agent has good potential.

However, Darwita et al[60] found neither significant difference in the reduction of Streptococcus mutans count and plaque index nor in preventing demineralisation while using CPP-ACP paste with or without propolis.

Chitosan added CPP-ACP
Chitosan is a linear copolymer of glucosamine and N-acetyl glucosamine with a β1-4 association obtained by N-deacetylation of chitin.[61] It contains an amino group-NH2, which exhibits high reactivity to cariogenic acids and can reduce acid dissolution of hydroxyapatite.[62] It further triggers the formation of organic layers, and the addition of mucins to the enamel surface, forming an attached multilayer that is more resistant to acids and which prevents the release of minerals in the enamel.[63]

Pchi is a chitosan derivative that has antibacterial, and biocompatible properties. Pchi chelates with calcium ions and stabilizes ACP by forming nano-complexes of Pchi–ACP which further remineralizes enamel sub-surface lesions.[64]

Batubara et al[65] found the combination of CPP-ACP-Chitosan nanoparticles to cause little morphological enamel changes and concluded that both CPP-ACP with and without chitosan have the same ability to increase tooth enamel remineralization.

Sodium tripolyphosphate (TPP) added CPP-ACP
TPP is considered the biomimetic copy of dentin matrix protein 1 (DMP1).[66] It helps with mineral accumulation on dentin, as well as their entry into the collagen fibrils for complete mineralization. Zhou et al[67] showed a high degree of remineralization, which was more even, and dentinal tubule occlusion after treatment with CPP-ACP combined with TPP on dentin.

Thus, treating demineralized dentin with TPP and CPP–ACP improved the remineralization effect and restored the mechanical properties of dentin.

CPP-ACP incorporated in glass ionomer and calcium-silicate cements

CPP–ACP as a bioactive additive to glass ionomer cement has shown to promote remineralization, as well as increase fluoride release under neutral and acidic conditions, without compromising the mechanical properties of GIC. Both micro tensile bond strength and compressive strength were increased significantly, as did the release of calcium, phosphate, and fluoride ions. [68] Zraikat et al[69] suggested the addition of 3% CPP–ACP to GIC. Zhao et al[70] concluded no difference in shear bond strength. In terms of remineralization, CPP-ACP-modified GIC outperformed traditional GIC and resin-modified GIC. Dawood et al[71] suggested adding no more than 1% CPP-ACP to BioDentine and 0.5% to MTA.

ACP incorporated in pit and fissure sealants

This pit and fissure sealant facilitates the development of hydroxyapatite crystals. It helps to release calcium and phosphate ions and improving the tooth's natural repair mechanism. When compared to other forms of fissure sealants, the ACP-containing pit and fissure sealant showed the greatest inhibition in enamel demineralization.[72]

Lasers combined with CPP-ACP

Laser irradiation is a new technology in the treatment of dental caries. Subramaniam[73] conducted an *in-vitro* study and concluded that laser irradiation of primary teeth followed by CPP-ACP application increased surface micro-hardness of enamel significantly. Yassaei et al[74] reported the highest release of calcium ions and increased efficiency of Er:YAG laser to decrease demineralization when combined with CPP ACP products. A study by Hajizadeh et al[75] also found that the combined effect of Er:YAG laser with CPP-ACP and fluoride significantly increased dentin hardness and Er:YAG laser alone did not lead to increased dentin resistance to caries.

Glycyrrhizic acid added CPP-ACP

With its extraordinary medicinal powers, licorice has been used in medicine for over 3,000 years. Glycyrrhizin (glycyrrhizic acid) and glabridin are the most bioactive components of licorice plants. They promote saliva production and defend against dental caries.[76]

Considering primary tooth enamel, Sahin and Oznurhan[77] showed that CPP-ACP and CPP-ACP containing glycyrrhizic acid improved remineralization, with no substantial difference between the two groups. When compared to the control group, they both reduced biofilm, but the difference was not statistically significant. Casein inhibits *S. mutans* adhesion to the tooth surface and selectively modifies the microbial content of plaque biofilm, while licorice inhibits *S. mutans* glycosyltransferase enzyme activity, thus inhibiting the synthesis of insoluble glucans required for biofilm formation.

Conclusion

The prodigious role of CPP-ACP in the non-invasive management of early caries lesion, dental erosion, and various other conditions with its different modifications can thus, be accepted as an effective preventive measure in pediatric dental care.

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