J Res Dentomaxillofac Sci

http://www.jrdms.dentaiau.ac.ir

e(ISSN): 2383 -2754
p(ISSN):2588-4166

JRDMS Journal of Research in Dental and Maxillofacial Sciences

An in-vitro Comparison of Calcium Uptake by Intact Enamel after Using two Types of Casein Phosphopeptide-Amorphous Calcium Phosphate Paste

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Original Article

ARTICLE INFO

Article Type
Original Article

Article History
Received: Nov 2017
Accepted: Dec 2017
ePublished: Jan 2018

Keywords:
Casein phosphopeptide-amorphous calcium phosphate,
Tooth Demineralization,
Calcium,
Dental Enamel

ABSTRACT

Background and Aim: Dental caries is one of the most common childhood diseases worldwide. The implementation of strategies that facilitate the prevention of early dental caries and interrupt its progression has been recently advocated. It has been reported that casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) derived from casein reduces tooth demineralization and enhances the remineralization process. The aim of this in-vitro study was to assess the efficacy of two types of CPP-ACP paste in calcium uptake by enamel surfaces.

Materials and methods: Forty premolars were longitudinally dissected into experimental and control halves, and were coated with nail varnish, except for an enamel window of 4×4 mm. The samples were subjected to cycling in a demineralizing solution and were divided into two groups of GC Tooth Mousse CPP-ACP paste and Misswake CPP-ACP paste. The calcium contents of each half were examined using the acid etch enamel biopsy technique and were measured by atomic absorption. The values were statistically analyzed using Kolmogorov-Smirnov test and one-way analysis of variance (ANOVA).

Results: The enamel surfaces treated with the pastes exhibited higher calcium contents compared to the controls. There was a significant difference between GC Tooth Mousse CPP-ACP paste and Misswake CPP-ACP paste in terms of calcium uptake; a higher calcium uptake was witnessed with GC Tooth Mousse CPP-ACP paste than with Misswake CPP-ACP paste ($P<0.05$).

Conclusion: Misswake CPP-ACP paste presented some protective potentials; however, the samples treated with GC Tooth Mousse CPP-ACP paste were better able to uptake calcium. These pastes could be considered as effective means for the prevention of dental caries in susceptible patients.

Please cite this paper as:
Hekmatfar S, Jafari K, Mohammadpour S. An in-vitro Comparison of Calcium Uptake by Intact Enamel after Using two Types of Casein Phosphopeptide-Amorphous Calcium Phosphate Paste. J Res Dent Maxillofac Sci.2018;3(1):6-11.

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Introduction:

Dental caries is a common oral health problem affecting a large number of individuals.[1,2] This disease results from an imbalance between the demineralization and remineralization episodes. The reduction of demineralization and development of remineralization are considered as the first approaches for preventing dental caries.[3,4]

Fluoride is the most commonly used remineralizing agent. The effectiveness of topically-applied fluoride products has been well established in caries prevention studies.[5,6]

The inhibition of caries by fluorides is accomplished through the incorporation of fluoride ions into the hydroxyapatite structure of dental enamel in the form of fluorapatite or fluorhydroxyapatite.[7,8]

The only scientifically proven risk of fluoride application is the development of fluorosis in children younger than 8 years old, which may occur with fluoride ingestion during tooth and bone development.[9] Considering this risk in children, different modalities have been proposed for treating carious lesions.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes are novel dental caries preventive materials, and both laboratory and human in-situ studies have proven that these materials have anticariogenic properties.[10-12]

The CPP is derived from milk protein casein combined with calcium and phosphate. It contains a cluster of phosphoseryl residues stabilizing the nanoclusters of ACP in a metastable solution. The CPP binds to plaque, soft tissue, and dentin, creating a reservoir of bioavailable calcium and phosphate in the saliva and on dental surfaces. This eases the limitation of mineral loss during the cariogenic episode and reduces enamel demineralization while promoting the remineralization.[11,13-15] Several studies have demonstrated that the CPP-ACP shows greater remineralization in enamel, in comparison with fluoride, due to more bioavailable calcium and phosphate ions in CPP-ACP agents.[16,17] Moreover, CPP-ACP products are offered to be used for very young children since they can decrease the risk of caries without increasing the risk of fluorosis.[17]

The integration of fluoride into CPP-ACP leads to the co-localization of calcium and phosphate ions with fluoride ions on the tooth surface in the form of CPP-ACPF nanocomplexes. The enhanced concentration of calcium, phosphate, and fluoride ions on the tooth surface results in the diffusion of ions into the enamel and the underlying lesion, which in turn, allows for higher levels of remineralization and fluoride absorption in the mineral phase.[18-21] The present study was conducted to compare the calcium uptake by enamel surface after the application of two types of CPP-ACP paste.

Materials and Methods

This experimental study was conducted on 40 caries-free permanent premolars extracted for orthodontic reasons. The exclusion criteria included any visible or detectable caries, hypoplastic lesions, or white spot lesions (WSLs). The teeth were thoroughly cleaned from debris, calculi, and soft tissues. Each tooth was longitudinally sectioned into experimental and control halves which were coated with nail varnish, except for an enamel window of 4×4 mm on the buccal and lingual surfaces of the middle one-third of the crown.

A demineralizing solution was prepared using a combination of 2.2 mM calcium chloride ($CaCl2.2H2O$), 2.2 mM monosodium phosphate ($NaH2PO4.7H2O$), and 0.05 M lactic acid ($C3H6O3$). The final pH was adjusted to 4.5 using 50% potassium hydroxide (KOH). All the samples were then incubated with 50 ml of the demineralizing solution at 37°C for 48 hours.[21] Afterwards, the teeth were washed with deionized water and were then dried. The prepared teeth were randomly assigned into three groups:

1) GC Tooth Mousse CPP-ACP paste (GC Corp., Tokyo, Japan)
2) Misswake CPP-ACP paste (Misswake Co., Switzerland)
3) Control (no treatment)

The samples were rubbed with the respective pastes for 3 minutes,[19,22] were washed with deionized water and were placed in clean glass containers of artificial saliva for 24 hours at 37°C using an incubator (except for the control group). The samples were treated with the pastes every 24 hours for 7 days, were washed with deionized water and were placed in fresh artificial saliva.[21]

After 7 cycles of remineralization, the surfaces of the control and experimental groups were assessed using the acid etch enamel biopsy technique. Subsequently, the samples were immersed in 0.2 M of KOH solution in order to reach an acid-base condition at 2 ml. The level of calcium absorption in each group was evaluated by atomic absorption at the wavelength of 422.7 nm.

Statistical analysis:

Kolmogorov-Smirnov test was applied to check the normality of distribution. Subsequently, one-way analysis of variance (ANOVA) and Tamhane's T2 were performed to compare the data among the three groups.

Results

The mean level of calcium in the treated enamels was higher than that in the control group (P<0.05). The comparison of the two groups revealed that the GC Tooth Mousse samples showed a significantly higher calcium absorption than the Misswake group (P<0.05; Table 1).

Table 1. Mean and standard deviation (SD) of calcium absorption (ppm) in each group

	Maximum	Minimum	Mean	SD
GC Tooth Mousse CPP-ACP paste	589.00	347.00	454.70	65.21
Misswake CPP-ACP paste	553.00	225.00	405.45	92.676
Control	331.00	195.00	266.30	42.068

CPP-ACP= Casein phosphopeptide-amorphous calcium phosphate, ppm=part per million

Discussion:

Dental fillings or restorations are used as a therapeutic option for the management of childhood caries.[23] Considering the high prevalence of carious lesions among children in most countries, noninvasive interventions have been adopted as a new approach.[24-27] The minimally invasive approaches can arrest caries progression with the aid of therapeutic agents that promote remineralization.[27]

The present study compared the remineralization potential of two CPP-ACP-containing pastes on the enamel surface. MI paste, a water-based, sugar-free cream, is the first product for professional use that contains RECALDENT™ (CPP-ACP). The flavoring of the paste helps to stimulate the salivary flow and provides longevity in the mouth, enhancing the effectiveness of the cream. MI paste has considerable buffering capabilities, resulting in the continual release of calcium phosphate ions for over 3 hours.[27]

Misswake CPP-ACP paste is a new product for younger children, which is claimed to contain calcium and milk protein without any risks during routine applications. There is no published report regarding the remineralizing potential of this paste. Regarding the costs, Misswake is significantly less costly than other CPP-ACP products.

The results of the present study showed a significant difference among the three groups regarding the enrichment of the calcium content of enamel. In this regard, the GC Tooth Mousse group showed the highest calcium content among other groups. Our results were also in accordance with those of the studies performed by Chaudhary et al,[16] Chokshi et al,[28] and Sinfiteli et al.[29] The proposed mechanism of action of CPP-ACP products describes that these products act as a calcium-phosphate reservoir, buffering the activities of free calcium and phosphate ions in the plaque fluid, helping to preserve a state of supersaturation of enamel, inhibiting demineralization and promoting remineralization.[12,16]

Lata et al studied the remineralization potential of fluoride and CPP-ACP in enamel lesions and reported that fluoride was more effective in remineralization although CPP-ACP had the ability to reinforce the enamel.[30]

Rahiotis and Vougiouklakis conducted an in-vitro study on sound human dentin and observed that the presence of CPP-ACP on dentinal surfaces triggered less demineralization in comparison with untreated dentinal surfaces.[31] Tantbirojn et al conducted an in-vitro study in which cola-softened enamel surfaces were examined.[32] In the mentioned study, treatment with CPP-ACP reinforced the surfaces significantly compared to fluoride treatment.[32] The reason for this discrepancy can be due to the use of different methodologies; we evaluated the calcium absorption by enamel surfaces treated with two types of CPP-ACP paste.

Thakkar et al compared the extent of inhibition of demineralization and promotion of remineralization in sound permanent molar enamel with and without the application of CPP-ACP paste, CPP-ACP paste with 900 ppm (part per million) fluoride, and 5% sodium fluoride-containing varnish and observed that CPP-ACPF varnish has the greatest remineralization effect in preventing demineralization and promoting remineralization of enamel.[33] Also, they observed that CPP-ACP in combination with fluoride increases the remineralization potential when compared to CPP-ACP paste alone.[33]

MI paste contains xylitol, which is a non-cariogenic substance that can enhance the benefit of the paste in caries prevention. Xylitol has an antimicrobial effect on Streptococcus mutans (S. mutans), which is dose/frequency-dependent.[33,34] There are several studies investigating the caries-preventive effect of xylitol among children.[34,35]

Misswake bioactive glass-containing paste is a type of mineralizing agent. When bioactive glass comes in contact with saliva or other liquids, its active ingredients (e.g., calcium sodium phosphosilicate) bind to dental surfaces to activate the remineralization process. The bioactive glass reacts with saliva, inducing the dissolution of calcium, phosphate, and silicate ions at the glass surface. The following precipitation of a polycondensed silica-rich layer, which functions as a pattern for the formation of calcium phosphate, crystallizes into hydroxyapatite.[36-38]

Consequently, bioactive glass acts as a remineralizing agent in non-cavitated lesions and in high-risk patients.

Narayana et al investigated the efficacy of bioactive glass-containing toothpaste on the remineralization of artificial incipient enamel lesions using the pH cycling method to compare its efficacy with that of fluoride-containing toothpaste, CPP-ACP-containing tooth cream, and CPP-ACPF-containing tooth cream.[38] They concluded that bioactive glass can be considered as an effective remineralizing agent.[38]

One of the limitations of the present in-vitro study was the difficulty in simulating the oral environment; further studies are needed to confirm these results.

Conclusion

Although Misswake CPP-ACP paste presented some protective potentials, the samples treated with GC Tooth Mousse CPP-ACP paste were better able to uptake calcium. It can be concluded that these pastes are effective in the prevention of dental caries in susceptible patients.

Acknowledgements

This study was part of the doctoral thesis (#63). The authors would like to express their gratitude to the research deputy of ardabil university of medical sciences for fi nancially supporting this study.

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