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Original Article

Changes in the Concentration of Ions in Saliva and Dental Plaque after Application of CPP-ACP with and without Fluoride among 6-9 Year Old Children

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Abstract

Statement of Problem: The casein phospho peptide-amorphous calcium phosphate with or without fluoride (CPP-ACPF and CPP-ACP respectively) are of considerably new materials which are highly recommended for prevention of dental caries. However, there is a shortage in literature on how they affect the ion concentration of saliva or dental plaque.

Objectives: The aim of this study was to evaluate the concentration of calcium, phosphate and fluoride in the plaque and saliva of children with Early Childhood Caries (ECC) after applying the CPP-ACP paste in comparison with the use of CPP-ACPF paste.

Materials and Methods: One ml of un-stimulated saliva of 25 preschool children was collected and then 1 mg of the plaque sample was collected from the buccal surfaces of the two first primary molars on the upper jaw. CPP-ACP as well as CPP-ACPF pastes were applied on the tooth surfaces in two separate steps. In steps, plaque and saliva sampling was performed after 60 minutes. The amount of calcium ions was measured by Atomic Absorption Device and the amount of phosphate and fluoride ions was measured by Ion Chromatography instrument. Data were analyzed using Repeated Measurements ANOVA at a $p < 0.05$ level of significance.

Results: Application of both CPP-ACPF and CPP-ACP significantly increased the concentration of calcium, phosphate, and fluoride in both saliva and dental plaque. Moreover, significantly higher salivary fluoride concentration was seen after application of CPP-ACPF compared to CPP-ACP. No other significant difference was observed between these two materials.

Conclusions: CPP-ACPF can be more useful than CPP-ACP in protecting the primary teeth against caries process, especially when there is poor hygiene.

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Introduction

Dental caries is an infectious teeth disease defined as localized destruction of mineralized tooth structures by microorganism [1]. Although the prevalence of dental caries has decreased, it is still considered as a major health problem in the general population [2].

Children's oral cavity is a rather ideal environment for the growth of bacteria because it has a proper temperature, contains the necessary nutrients, and has sufficient moisture and proper surfaces for bacterial adhesion [3]. Currently, researchers have focused on non-invasive techniques to prevent dental caries [4]. In recent years, a calcium and phosphate remineralization technology has been introduced to decrease the incidence of dental caries that relies on Casein Phospho Peptide-Amorphous Calcium Phosphate (CPP-ACP) [5]. CPP-ACP is a bioactive material with a base of milk products, consisting of two parts: CCP and ACP [6]. This product binds to the plaque and servers as a reservoir for calcium and phosphate in the dental plaque [7]. In addition, it can prevent the adhesion of cariogenic Streptococci to tooth surfaces via participating in the pellicle structure [8].

CPP-ACP decreases the dental caries by increasing the calcium and phosphate levels in the plaque, decreasing the production of acid by the plaque, decreasing the concentration of cariogenic bacteria in the plaque, and by increasing the plaque PH [9].

CPP-ACP is used as an adjunct preventive agent in patients at a high risk for caries, those with poor plaque control, erosive lesions, white spot lesions, pregnant women, patients with tooth sensitivity resulting from bleaching of the teeth and in the treatment of dentin hypersensitivity [6].

The functional potential of CPP-ACP in inhibiting demineralization, increasing remineralization, and decreasing adhesion of Streptococci and its bacteriostatic/bactericidal activity is similar to those of fluoride; however, it does not exhibit the detrimental effects of the excessive use of fluoride [7,10].

A type of this product named Tooth Mousse (GC Co., Japan) is devoid of fluoride and is suitable for children under 2 years of age. It is safe to swallow any amount of this material. CPP-ACP has a favorable flavor and is tolerated well by children. It facilitates the maturation process of the teeth after their eruption due to the high levels of calcium and phosphate in the saliva since it replaces the minerals lost daily [11].

Although saliva is a natural source of calcium and phosphorus ions for remineralization, it is not a very effective remineralization environment in the absence of fluoride. An increase in the fluoride concentration of saliva is associated with an increase in remineralization and a decrease in caries rate [12].

GC Tooth Mousse Plus or CCP-ACPF contains CPP-ACP plus sodium fluoride 0.2% (900 ppm) that is very similar to the amount of fluoride in the toothpaste manufactured for adults. [13] CPP-ACPF binds to the tooth surfaces and plaque and provides biocompatible calcium, phosphate and fluoride in a localized manner. Thus, it provides all the ions that are necessary to build fluoro-apatite crystals which are more resistant to acid attack compared to hydroxyapatite. Studies have shown a synergistic effect of CPP-ACP and fluoride on remineralization, which might be attributed to the cumulative effect of CCP-ACP and fluoride in localizing calcium and phosphate ions in association with fluoride on the tooth surface [14].

The aim of the present study was to compare the concentrations of calcium, phosphate and fluoride after the application of CPP-ACP and CPP-ACPF, as two different pastes, in the plaque and saliva of children 6-9 years of age.

Materials and Methods

The study samples included one group consisting of twenty-five children aged 6-9 years in this clinical trial study. The subjects were selected from a charity foundation with proper oral hygiene and no systemic disease and active carious lesions. The sample size was determined according to similar studies [9,10]. The subjects participated in the study after signing written informed consent forms by their parents or guardians. Ethical approval for the present study was obtained from the Ethics Committee of Kerman University of Medical Sciences (IR.Kmu.REC.1394.594).

In the first stage of the study, a low-speed hand piece and a rubber cup, with no material, were used for cleaning the children's tooth surfaces. The subjects were asked to avoid brushing their teeth, and using toothpastes, dental floss and fluoride or other therapeutic agents for 48 hours. After 48 hours, the plaque and salivary samples were collected as follows:

Collection and preparation of salivary samples

Salivary samples were collected before collecting the plaque samples. Each subject was given a plastic container and asked to evacuate at least 1 ml of unstimulated saliva for more than five minutes into the container. Then, a sampler was used to transfer the salivary samples into coded microtubes. The samples were vortexed for 1 minute to homogenize them. To prepare the samples, 100 μL of 1-mol hypochlorite were mixed with 900 μL of each salivary sample. After 2 hours, 100 μL of 90% total ionic strength adjustment buffer (TISAB) solution was added and the samples were centrifuged at 1200 rpm at laboratory temperature for 3 minutes and then sent for laboratory evaluation.

Collection and preparation of plaque samples

The subjects were asked to swallow their saliva to eliminate any saliva from their teeth. Sterile Kerr applicators were used to collect the plaque samples from the buccal surfaces of teeth D and E in the upper jaw; 1 mg of the samples from each subject was transferred to microtube containing 1.5 mg of mineral oil. In order to increase the sensitivity of measurements, the plaque samples were weighed with the use of a digital weighing machine accurate to 1 mg. All the microtubes were centrifuged for 5 minutes.

Subsequently, the samples were mixed with 200 µL of Hypo-chlorite or subsequent analyses and diluted with 1800 µL of TISAB to stabilize and register the power of the ions. All the plaque and salivary samples were filtered through an 0.2-µm filter. The plaque and salivary samples were prepared using the technique introduced by Vogal *et al*. [15].

A chromatography unit (Metrom Co., Switzerland) was used to determine the fluoride and phosphate ion concentrations of the plaque and saliva with the use of specific columns. The chromatography unit was calibrated with the use of 11 standard samples. An ion meter (Metrom Co., Switzerland) was used to evaluate the concentration of calcium ions, using its specific calcium electrode.

After the first stage of the study, the subjects proceeded with their usual and routine diet and hygiene practices for 14 days. Then, similar to the first stage, rubber cups with no material were used for cleaning the children's tooth surfaces. Then, the subjects were asked to refrain from tooth brushing, toothpaste, dental floss, fluoride and other therapeutic agents for 48 hours. After that, on the day of sampling, CPP-ACP paste was placed on the tooth surfaces using a swab and plaque, and salivary samples were collected after 60 minutes similar to that in the first stage. The subject avoided eating and drinking between the applications of CPP-ACP paste and collecting the samples. The salivary and plaque samples were centrifuged and the concentrations of calcium, fluoride and phosphate ions were measured.

After 14 days, during which the subjects proceeded with their routine diet and oral hygiene practices, the third stage was carried out with the use of CPP-ACPF in a manner similar to that in the second stage. The salivary and plaque samples were centrifuged and the concentrations of calcium, fluoride and phosphate ions were determined. In all the three stages after collecting the samples, they were immediately sent to the laboratory and stored in a refrigerator until used for in the study.

Statistical Analysis

As data in this study were dependent, we analyzed them using the repeated measured Analysis of Variance (RM-ANOVA). A p-value less than 0.05 was considered as significant. Data were analyzed using SPSS, version 20.

Results

Table 1 shows the mean concentrations of calcium, phosphate and fluoride ions in the plaque and saliva in the control, CPP-ACP and CPP-ACPF paste groups. The results of the study showed that the use of CPP-ACP, compared to the control group, resulted in significant increases in calcium ($p < 0.001$), phosphate ($p < 0.001$) and fluoride ($p = 0.029$) ions in the plaque. In addition, the use of CPP-ACPF, compared to the control comparison, significantly increased the concentrations of calcium ($p < 0.001$), phosphate ($p < 0.001$), and fluoride ($p < 0.001$) in the plaque. However, there were no significant differences in the concentrations of calcium ($p = 0.638$), phosphate ($p = 0.46$), and fluoride ($p = 0.497$) ions between CPP-ACPF and CPP-ACP in the plaque.

In addition the results showed that the use of both

Table 1: Mean values and standard deviation (SD) of concentration of Phosphate, Calcium and Fluride Ions in the Saliva and Plaque samples in the groups (µg/ml).

Group		Phosphate Ions Mean (SD)	Calcium Ions Mean (SD)	Fluride Ions Mean (SD)
Saliva	Control	0.33 (0.06)[a]	19.04 (10.1)[a]	0.65 (0.15)[a]
	CPP-ACP	0.92 (0.26)[b]	43.87 (24.7)[b]	0.68 (0.15)[b]
	CPP-ACPF	0.95 (0.23)[b]	48.7 (19.6)[b]	0.77 (0.09)[c]
Plaque	Control	0.65 (0.08)[a]	22.2 (9.1)[a]	0.79 (0.15)[a]
	CPP-ACP	1.88 (0.56)[b]	48.8 (23.2)[b]	0.98 (0.3)[b]
	CPP-ACPF	1.78 (0.20)[b]	51.9 (22.5)[b]	1.03 (0.16)[b]

For each ion, different letters show a significant difference and the same letter shows a non-significant difference.

CPP-ACP and CPP-ACPF revealed in significant increase in the salivary concentration of calcium ($p < 0.001$) and phosphate ($p < 0.001$) ions, without significant difference between the two materials. However, in case of concentration of fluoride ion in saliva, although both materials increased its concentration, the effect of CPP-ACPF was significantly stronger then the CPP-ACP ($p = 0.043$).

Discussion

Studies have shown an inverse relationship between the concentrations of calcium and phosphate ions of the dental plaque and the prevalence of caries [5,16]. Furthermore, the balance between remineralization and demineralization depends on the environmental concentrations of calcium and phosphate ions [17]. Therefore, if a product such as CPP-ACP or CPP-ACPF can significantly increase the concentrations of calcium and phosphate ions of the dental plaque, it can play an anticariogenic role by decreasing demineralization, increasing remineralization, and possibly a combination of these two processes [7].

The results of the present study showed that the concentrations of calcium and phosphate in the saliva and plaque of children increased significantly after the application of CPC-ACP and CPP-ACPF pastes compared to the baseline; this is consistent with the results of previous studies [16,18,19].

Many studies evaluated calcium and phosphate concentrations after the use of CPP-ACP-containing chewing-gum, while the present study evaluated these concentrations after the use of CPP-ACP and CPP-ACPF pastes. It should be pointed out that no studies to date have evaluated calcium and phosphate concentrations of calcium and phosphate in the saliva or plaque after the use of CPP-ACPF paste and this study is the first study in this respect.

The results of the present study showed that after the use of both CPP-ACPF and CPP-ACP pastes, the concentrations of calcium in the plaque and saliva increased more than two folds and the concentrations of phosphate in the plaque and saliva increased three folds.

A sufficient amount of calcium and phosphate ions should be present for remineralization to occur and in the majority of cases the process is limited due to the limited availability of calcium and phosphate ions [17]. In addition, studies have shown that fluoride ions can induce remineralization of demineralized enamel if sufficient amounts of calcium and phosphate ions are present in the saliva when fluoride is applied because 2 fluoride ions, 10 calcium ions and 6 phosphate ions are necessary to form one fluoroapatite unit [2].

Srinivasan *et al*., too, showed that the saliva alone cannot induce favorable conditions for remineralization; therefore, the use of remineralizing agents is necessary to create an environment favorable for remineralization [20]. In addition, studies have shown that in the enamel remineralized with the use of CPP-ACP, the minerals deposited in hydroxyapatite have a higher ratio of calcium and phosphate compared to normal hydroxyapatite; therefore, they were more resistant to acid attack [6].

In addition, it is possible that CPP-ACP can exert an increased anti-plaque effect by maintaining a high concentration of calcium, highlighting the importance of the presence of calcium and phosphate ions [6,7,21-23].

The results of the present study showed that the fluoride concentration of the plaque was significantly higher in the CPP-ACP and CPP-ACPF groups compared to the control group. However, the difference between the CPP-ACP and CPP-ACPF groups was not significant. In this context, the salivary fluoride concentration was significantly higher in the CPP-ACPF group only, compared to the control group. In addition, comparison of the salivary fluoride concentration showed a significant difference between the CPP-ACP and CPP-ACPF groups. Researchers believe that deposition of fluoride in the plaque depends on the presence of calcium ions, which is attributed to the effect of calcium and bonding of fluoride to bacterial cell wall [24,25]. This explains a significant increase in fluoride concentration of the plaque when CPP-ACP is used and also the lack of a significant difference in the concentration of plaque fluoride after the use of CPP-ACP and CPP-ACPF. This can be justified by the availability of a high concentration of calcium ions, resulting in greater deposition of fluoride in the plaque, while the salivary fluoride increased significantly only after the use of CPP-ACPF.

Reynold *et al.* carried out a study to evaluate the potential of CPP-ACP to increase the penetration of fluoride into the plaque and improve remineralization. The results showed that incorporation of CPP-ACP into mouthwashes containing 450 ppm of fluoride resulted in a two-fold increase in the penetration of fluoride into the plaque, which was considered significant [5].

The results of the present study showed no significant differences in the plaque and salivary calcium and phosphate concentrations and also in the fluoride plaque concentrations between the CPP-ACP and CPP-ACPF groups; in this context, only the salivary fluoride concentration in the CPP-ACPF group was significantly higher than that in the CPP-ACP group.

Jayrajan compared the remineralizing effects of CPP-ACP and CPP-ACPF and reported that both pastes increased remineralization significantly. Although CPP-ACPF resulted in a higher rate of remineralization, the difference was not significant [13].

Srinivasan *et al*. compared remineralization of the human softened enamel after exposure to CPP-ACP and CPP-ACPF pastes and reported that remineralization induced by CPP-ACPF was significantly higher than that induced by CPP-ACP, indicating the significant effect of fluoride and CPP-ACP on remineralization [20].

The protective effect of fluoride is basically attributed to the formation of CaF_2 layer on the tooth surface, which can serve as a reservoir for fluoride. During the acid attack, the fluoride released from the CaF_2 layer can take part in the formation of fluoroapatite and fluorohydroxyapatite, decreasing susceptibility to dissolution in future. In addition, the CaF_2 layer might serve as a mechanical barrier to prevent the contact of acid with the underlying enamel or as a reservoir for minerals; it creates a buffering action during the acid attack on the underlying enamel or decreases the hydrogen ions of the acid. The formation of the CaF_2 layer depends on the pH and concentration of fluoride and the duration of application. At high concentrations of fluoride or a long duration of application, a thicker or more stable CaF_2 layer might be deposited [26]. It appears that combing CPP-ACP with fluoride results in accumulation of calcium and phosphate on the enamel surface in association with fluoride. Therefore, this paste provides all the ions required for the formation of fluoroaptite crystals that can resist the acid attack [14].

Conclusions

According to this study, CPP-ACPF can be more useful than CPP-ACP for protecting the primary teeth against caries process, especially when there is poor hygiene.

Conflict of Interest: None declared.

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