CPP-ACP: Effect on Dental Plaque Acidity after Water Rinsing Following Topical Fluoride Therapy

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Objectives: *There is some evidence that water rinsing immediately after topical fluoride therapy has the potential to reduce the effectiveness of fluoride. The aim was to determine if covering fluoridated teeth with a layer of mousse containing CPP-ACP could prevent the adverse effect of rinsing on fluoride and consequently its buffering effect on dental plaque pH during cariogenic challenge.* ***Study design:*** *This randomized, controlled, crossover, in situ study was conducted on 25 participants. The participants were subjected to acidulated phosphate fluoride (APF) application followed by five treatment protocols: (1) water rinsing after 30 minutes (APF-30) or (2) immediate water rinsing (APF-0); (3) using CPP-ACP immediately before water rinsing (F-CPP-ACP); and two control groups: (4) no fluoride therapy (No-F) and (5) using CPP-ACP and immediate water rinsing (CPP-ACP-0). After 48 hours, teeth were rinsed with 10% sucrose solution and plaque pH was measured before and after 5, 10, 15, 20 and 30 minutes.* ***Results:*** *The least pH changes, the lowest pH drop, and the quickest pH recovery were found in the APF-30 and F-CPP-ACP groups. APF-0 ranked in the middle and the highest values were in the control groups.* ***Conclusions:*** *The results show that in the case using CPP-ACP on fluoridated teeth, water rinsing immediately after topical fluoride therapy did not seem to influence the inhibitory effect of fluoride on plaque acidity.*

Key words*: acidulated phosphate fluoride; CPP-ACP; dental plaque.*

INTRODUCTION

Following the application of high concentrations of fluoride, calcium fluoride (CaF2) or calcium fluoride-like materials begin to deposit on the enamel surface. The CaF2 layer that has formed on the enamel surface or in plaque may serve as a pH-controlled reservoir of fluoride that can release fluoride durin g caries challenges[1]. Calcium fluoride formation is considered an essential cariostatic mechanism of topical fluoride, because it can provide fluoride ions in the oral cavity for a longer time[2]. However, calcium fluoride is soluble in normal saliva that will become a problem unless this compound transforms into fluorapatite. This transformation could restrict further dissolution of fluoride[3].

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To ensure that patients receive the complete benefit of their treatment, dentists have been recommended to ask patients to refrain from drinking or eating for 30 minutes following professional fluoride application, although there is conflicting evidence regarding this no-rinse period after fluoride therapy. Two studies have showed that rinsing immediately after fluoride therapy has no influence on the enhancement of remineralization or the inhibition of the demineralization effects of fluoride [4, 5]. Two other studies have suggested 15 min [6, 7] and in another, 30 min for refraining from rinsing after fluoride therapy [8].

Sometimes children, especially younger children, insist on rinsing after fluoride therapy and preventing them from doing so may decrease their acceptance of any future professionally applied topical fluoride therapy procedure. Aside from this, sometimes parents ask dentists to do some more dental treatments such as restoration of caries teeth after fluoride therapy, but dentists refrain from doing so because rinsing immediately after fluoride therapy may have an adverse effect on the anticaries activity of fluoride. If we have to use some rinsing after fluoride therapy (for example for restoring teeth or because a young child insists on rinsing), methods to prevent the potential loss of CaF2 globules or the anticaries effect of fluoride should be found.

It is known that CaF2 dissolves much more slowly in the oral cavity than in aqueous solution because of the presence of a phosphate or protein-rich coating of globular deposits of CaF2 on the enamel surface [1, 9]. On the other hand, in recent years a new approach to prevent dental caries has been introduced into dentistry, named CPP-ACP (casein phosphopeptide-amorphous calcium phosphate). It is derived from milk protein casein, so it contains protein and phosphate ions–two agents that may help prevent CaF2 from dissolving. However, it is uncertain as to whether CPP-ACP application following topical fluoride treatment decreases probable adverse effects of water rinsing.

The aim of the present study was to determine if covering fluoridated teeth with a layer of CPP-ACP could prevent the adverse effect of rinsing on fluoride and its buffering effect on dental plaque pH during cariogenic challenge.

MATERIALS AND METHOD

This study was carried out at the Pediatric Department of the Mashhad Dental School with the permission of the Research and Ethics Committee, School of Dentistry, Mashhad University of Medical Science (Mashhad, Razavi Khorasan province, Iran). The study was a randomized, controlled, crossover, single-blind, in situ clinical trial.

Initially, a pilot study was done using four patients. With a confidence level of 95% and a power of statistical test of 80%, 24 samples were calculated, but for more confidence, the sample size was expanded to 25.

Twenty-five healthy dental students (11 males and 14 females, average age: 26.4 years; range: 21-29 years) in good oral and general health took part in this study after signing an informed written consent form. Inclusion criteria were: healthy people with a normal unstimulated salivary rate, without active dental decay, not taking any medication that may interfere with saliva rate, no antibiotic (or antibacterial mouth rinses) use during the last four weeks, and no fluoride mouth rinse use during the previous two weeks.

Preparation of the enamel blocks and mandibular appliance:

Enamel blocks (3 x 3 x 1 mm) were prepared (using diamond burs) from the buccal surface of 125 impacted human third molars and sterilized by autoclave. Then a total of 125 enamel blocks were randomly distributed into the treatment groups. Two weeks before sampling, the alginate impression was taken from each volunteer and a mandibular removable appliance was prepared with one buccal flange (between the two premolars). In each session a new enamel block was placed in the buccal flange of the appliance and following the treatment (prophylaxis with a rubber-cup or prophylaxis and fluoride therapy and/or using CPP-ACP) a plastic mesh was placed by wax onto the acrylic surface. Dental plaque was allowed to form in a 1.0 mm space between the enamel blocks and plastic mesh affixed to the acrylic surface.

During the two week pre-experimental period and the washout periods, the volunteers brushed their teeth with non-fluoridated dentifrice. They also were instructed about not using any mouthwash or other fluoridated products during the study.

The volunteers received 1 to 5 treatments with the appliances in their mouth (two control and three experimental 48-hour periods). A two-week washout period was allowed between each experimental phase (Fig. 1). In each phase appliances containing a new human enamel block were used.

They received a complete professional teeth cleaning before each 48-hour phase and prior to performing the treatments the appliances were placed into the mouths of the volunteers. After that the plastic meshes were affixed with wax on the enamel blocks. The volunteers were instructed: to wear the appliances day and night during the next 48 hours, to remove them only during meals and to refrain from any oral hygiene for the next 48 hours.

The subjects were randomized into 5 treatment groups, through which they passed in a randomized sequence (using the Research Randomizer program available at www.randomizer.org).

Treatments included:

(1) (No-F) control, no fluoride therapy;

(2) (APF-0) immediate washing with water after APF (Nupro, Dentsply, Petrópolis, RJ, Brazil);

(3) (APF-30) APF treatment, followed by no rinsing and no consumption of liquids or solids for the next 30 minutes. After 30 minutes, they rinsed their teeth with water;

(4) (F-CPP-ACP) applying CPP-ACP (Tooth Mousse, GC Corp. Tokyo, Japan) after APF treatment and washing immediately with water.

(5) (CPP-ACP-0) control, applying only CPP-ACP without APF treatment and washing immediately with water.

Fluoride was applied to all of the experimental groups for 4 minutes with cotton tip applicators. Patients in group 3 were asked to spit 3 times immediately following the 4 minute fluoride application to remove the excess fluoride and rinsing with water was done 3 times after 30 minutes. After 48 hours of withholding all forms of oral hygiene, dental plaque sampling of the enamel blocks was done.

Assessment of dental plaque acidity

The "plaque sampling" method was used for dental plaque pH measurements. Two hours before the plaque samplings, which were scheduled for the morning, volunteers did not take any food or water. Refraining from oral hygiene for 48 hours and leaving a 1.0 mm space between the plastic mesh and enamel block caused adequate plaque accumulation for plaque pH assessment.

Forty-eight hours following the treatments, after removing the plastic meshes, plaque samples were taken by a sterile amalgam carver from the surface of the enamel blocks. A lip retractor was used to avoid contaminating samples with saliva.

PH measurement was done with a CupFET micro-combination electrode (ISFET, Sentron Inc. USA) and a portable, handheld Senron pH meter (Senron pH Meter, Type Argus X; Sentron Inc. USA). After allowing the pH meter to stabilize for 30 sec, the reading was done. Standard solutions of pH

4.0, 7.0 and 9.0 were used for system calibration. Between two measurements, the electrode was washed-out with distilled deionized water and placed in a standard solution of pH 7.0. Plaque pH measuring was done at baseline to evaluate the pH in resting plaque and at time intervals of 5, 10, 15, 20 and 30 min after rinsing with 10% sucrose solution (10 ml for 30 sec). The assessor who did the plaque sampling and determination of dental plaque pH was blind to the treatment done on the volunteers.

The following variables were measured: baseline-plaque pH, pH changes compared to baseline pH at all intervals, maximum pH drop, and maximum time required to reach baseline pH (recovery time).

Statistical analysis

Repeated measure ANOVA and Bonferoni tests were used for statistical analysis and the significance level was set at 5%.

RESULTS

Baseline plaque pH (before sucrose challenge) for 4 separate days in the same subjects showed no significant differences. PH changes between the groups in different intervals after sucrose rinsing had significant differences when compared to the baseline pH, but after 30 min the differences were not significant (Table 1).

The Bonferoni test showed no significant differences between the two groups APF-30 and F-CPP-ACP, which had the least pH changes. In addition, there were no significant differences between the two groups CPP-ACP-0 and No-F (control) except for in the 5 min interval, which had the highest pH changes. The APF-0 group had modest pH changes (Table 1). The least pH recovery times after exposure to sucrose were seen in the APF-30 and F-CPP-ACP groups (Table 2).

DISCUSSION

The results of the present study suggest that immediate water rinsing after topical fluoride therapy significantly results in a reduction in the preventive effect of fluoride on dental plaque acidity, but in the case of the application of a CPP-ACP-containing mousse on fluoridated teeth immediately before water rinsing, the results are similar to the APF-30 group (the group which refrained from rinsing or drinking after fluoride therapy for 30 minutes).

It has been shown that the key reaction material after a topical fluoride application to the tooth surface is calcium fluoride (CaF2) or a calcium fluoride-like material [7, 10, 11]. Calcium fluoride is highly soluble in aqueous solution. It was thought that due to being unsaturated saliva toward calcium fluoride, this salt dissolves in oral fluids whenever it is exposed to saliva [12]. However, several studies have shown that calcium fluoride has lower solubility in saliva. The

Table 1: **Comparison ofTable 1: Comparison of pH changes compared to baseline pH in different intervals after sucrose challenge.**

Intervals Treatments	After 5 (min)	After 10 (min)	After 15 (min)	After 20 (min)	After 30 (min)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
no fluoride (Control)	1.18 ± 0.21[c]	0.81 ± 0.23[c]	0.49 ± 0.29[c]	0.23 ± 0.23[b]	0.02 ± 0.09[a]
APF-0	0.48 ± 0.14[b]	0.35 ± 0.15[b]	0.21 ± 0.13[b]	0.11 ± 0.11[b]	0.01 ± 0.05[a]
APF-30	0.25 ± 0.12[a]	0.16 ± 0.09[a]	0.09 ± 0.08[a]	0.03 ± 0.05[a]	0.00 ± 0.00[a]
F-CPP-ACP	0.26± 0.13[a]	0.18± 0.13[a]	0.07± 0.08[a]	0.02± 0.04[a]	0.00 ± 0.00[a]
CPP-ACP (Control)	0.93± 0.29[d]	0.63± 0.28[c]	0.40± 0.25[c]	0.17± 0.18[b]	0.02 ± 0.07[a]
Repeated measure test	F= 536.93 P<0.01	F= 408.84 P<0.01	F= 188.32 P<0.01	F= 63.114 P< 0.01	F= 3.85 P= 0.06

Within each column, means with the same superscript letters are not statistically different from each other. pH changes compared to baseline pH in different intervals after sucrose challenge.

Table 2: Comparison between groups for maximum time to return to the baseline pH (Recovery time)

Treatments	Recovery time (min)
	Mean ± S.D.
no fluoride (Control)	27.00 ± 5.00[b]
APF-0	26.20 ± 6.00[b]
APF-30	20.40 ± 6.91[a]
F-CPP-ACP	20.00 ± 6.12[a]
CPP-ACP (Control)	27.00 ± 5.00[b]
Repeated measure test	F=2.23 P=0.001

Means with the same superscript letters a or are not statistically different from each other.

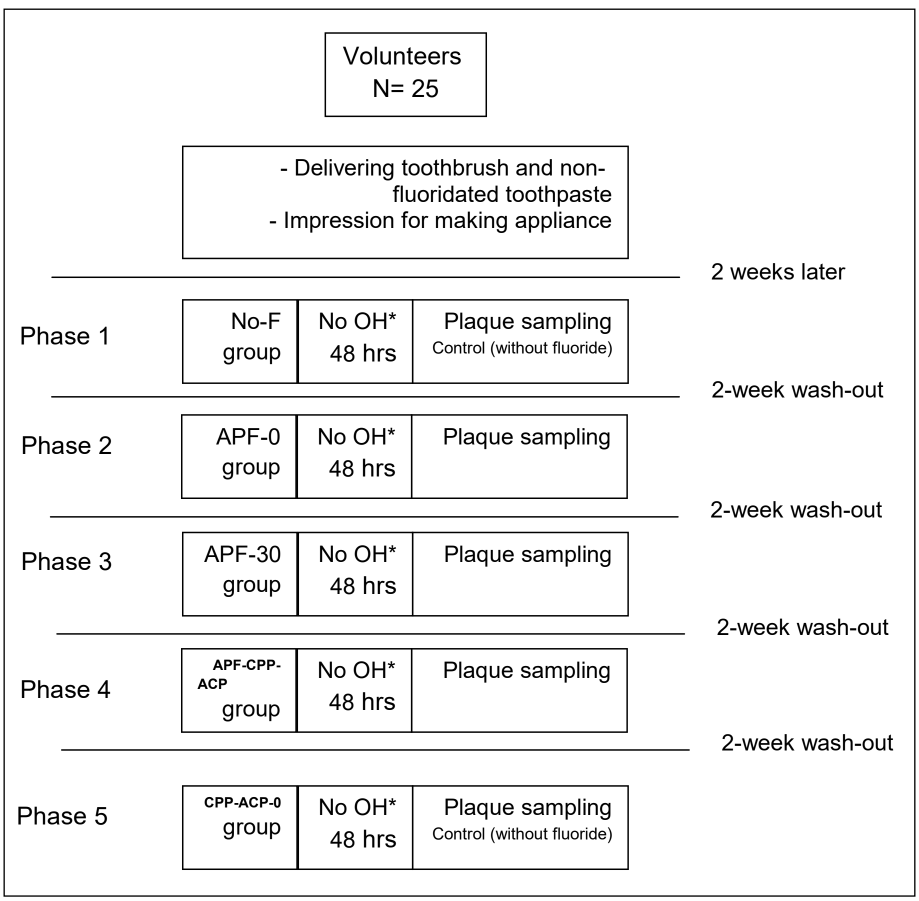
resistance of calcium fluoride is attributed to the presence of a phosphate or protein-rich coating of the globular deposits of calcium fluoride on the enamel surface [1, 2, 10, 13-16]. At low pHs during an acid attack, the phosphate groups in the protective layer bind protons and F-ions are released from the CaF2. Thus, the CaF2 layer functions as a pH controlled fluoride reservoir and is the most likely source of free ions during cariogenic challenges [1, 17, 18].

On the other hand, a new calcium phosphate remineralization technology has now been developed based on casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [RecaldentTM CASRN691364- 49-5], where it is claimed that the CPP stabilizes high concentrations of calcium and phosphate ions together with fluoride ions at the tooth surface by binding to pellicle and plaque. Considering that the CPP-ACP contains both protein and phosphate, which are essential for reducing the solubility of CaF2 in aqueous solutions, we hypothesized that the application of a CPP-ACP-containing mousse on fluoridated teeth immediately before water rinsing could protect CaF2 globules from dissolving. The results of the present study showed that there was no statistically significant difference between the F-CPP-ACP and the APF-30 groups. They had the least pH changes and pH recovery times after exposure to sucrose (caries challenges). But when water rinsing was done immediately after fluoride therapy without using the CPP-ACP in the APF-0 group, there was a higher statistically significant pH change and recovery time. This could imply that CPP-ACP can protect CaF2 globules from dissolving. It could be assumed that due to the application of APF with an acidic pH, H+ concentration in the fluid surrounding the tooth increases, which subsequently will protonate phosphate ions (PO43–) to HPO42–, the form of phosphate ion that protects the CaF2 globules.

Tenuta *et al* [19] showed that fluoride released into the “plaque fluid” significantly correlated to the CaF2 concentration built on enamel, which had a dose response effect between them. Thus, the more CaF2 reservoirs on enamel surface, the higher F in the fluid of subsequently formed plaque and hereby it could prevent glycolysis and consequently reduce acid production more efficiently. Bradshaw *et al* [20] showed that high fluoride concentration in plaque fluid contributed to the inhibition of acid production and subsequent enamel demineralization.

With regard to the results of other studies that have shown that CPP-ACP contains high calcium and phosphate ions [21] and can penetrate into dental plaque [22], so it could increase the calcium concentration of plaque and the CaF2 formed on the teeth surface. Therefore, it can be suspected that CPP-ACP has caused this effect due to the increasing calcium and phosphate ions that are available, not as a protective layer to prevent CaF2 from dissolving. Caruana *et al* [21] have showed that the application of a paste containing CPP-ACP can reduce the decrease in plaque pH following

Figure 1. **Flowchart of the study phases (sequences of the 5 phases were randomly determined for each volunteer).**



* No OH 48 hrs = No Oral Hygiene for 48 hours

immediate carbohydrate challenge. To reject this hypothesis, another control group was designed in which CPP-ACP was followed by immediate water rinsing. The results showed that there was no statistically significant difference between this group and the No-F group in all time intervals except the 5-min interval. This could imply that using CPP-ACP and rinsing immediately has no effect on increasing calcium and phosphate ions and the effect is probably due to reducing the solubility of calcium fluoride in water.

Based on the results, it seems that if inhibiting mouth rinsing in some young children causes them to not accept this important way of dental caries prevention in the future, an application of a layer of CPP-ACP on their fluoridated teeth before rinsing could protect the clearance of CaF2 globules created on the enamel surface. Also, in conditions in which splashing water immediately after topical fluoride therapy was necessary due to restoring the teeth using high speed handpieces, using this protective layer seems helpful. However, a specialized research should be done because washing the teeth using minimal water or splashing with a turbine could have different influences on removing the CaF2 globules.

As we can see in the APF-0 group, the effectiveness of fluoride treatment decreased because of immediately rinsing with water after APF application. An explanation for this finding is that water consumption after fluoride application influences the clearance of calcium fluoride globules in the mouth. So that pH recovery times and pH drop after exposure to sucrose in the APF-0 group was higher than other treatment groups (APF-30 and F-CPP-ACP groups), although it had better results in comparison with the two control groups (No-F and CPP-ACP-0 groups).

The result was similar to Stookey *et al* [8] and Lopes *et al.'s* [6] studies. However, the former evaluated the effect of immediately rinsing on fluoride uptake into artificially induced incipient lesions and the latter assessed that effect on residual fluoride concentration in the saliva. The result of the present study did not agree with the two studies performed by Delbem *et al* , which evaluated the effect on enamel demineralization [4] and remineralization [5] rates. This subject has been discussed in detail in a previous study [7], so for more information the readers have been invited to read the article.

Some limitations of the present study need to be addressed. First, the subjects were adults because of having better compliance with the requirements of the different phases of the study. Second, they were dental students with low caries risk. Further research that includes children with both low and high caries risk is recommended to investigate the effect of CPP-ACP after fluoride therapy on dental plaque pH.

CONCLUSION

The findings led to the following conclusions: (1) Immediately rinsing after APF application reduced the buffering effect of fluoride on plaque acidity. (2) The mousse containing CPP-ACP could be used as a protective layer after APF treatment in cases where immediate rinsing is necessary in order to maintain the effect of inhibitory fluoride on dental plaque pH drop during a sucrose challenge.

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