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FLUORIDE UPTAKE IN DENTAL ENAMEL AFTER USING FLUORIDATED DENTIFRICE, PRECEDED OR NOT BY A $CaCl_2$ SOLUTION RINSE

INCORPORAÇÃO DE FLÚOR AO ESMALTE DENTÁRIO APÓS O USO DE DENTIFRÍCIO FLUORETADO, PRECEDIDO OU NÃO POR BOCHECHO COM SOLUÇÃO DE $CaCl_2$

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ABSTRACT

Introduction: The use of a calcium solution previously to brushing could favor the remineralization and the fluoride (F) uptake in dental enamel. Objective: This study evaluated the F in dental enamel after using a F dentifrice, preceded or not by a rinse with a $CaCl_2$ solution. Methods: Ten subjects (18-30 years) participated in a double-blind, cross-over protocol. Enamel biopsy and 3 min saliva samples were obtained at baseline and after brushing (1.5 g Crest®, 1,000 ppm F as NaF), preceded by a rinse with 10 mL of 20 mM $CaCl_2$ 50 mM acetate pH 7.2 or deionized water, for 1 min, followed by a 15 mL water rinse for 5 sec. Biopsies were made before the rinses and after 8-15 and 120 min. Saliva samples were collected before the rinses and after 4, 15, 30, 60 and 120 min. F concentration in enamel biopsies was analyzed with the F electrode (Orion 9409) and a miniature calomel reference electrode, while phosphorus concentration was analyzed by spectrophotometry. Saliva samples were analyzed for F with the electrode (Orion 9609) by the direct method. The data were analyzed by ANOVA and Tukey test ($p<0.05$). Results: Mean F concentration ± SD (µg/mL) at baseline up to 120 min for saliva samples were: 0.02±0.01; 9.06±4.26; 2.01±1.76; 0.47±0.33; 0.24±0.17; 0.08±0.08; 0.07±0.04 for the deionized water rinse, and 0.02±0.02; 10.96±14.21; 2.76±3.04; 0.87±1.29; 0.40±0.66; 0.16±0.23; 0.09±0.09 for the $CaCl_2$ rinse. The data for enamel biopsies were (µg/g): 1861.7±1011.0; 1790.5±953.2; 1117.6±585.2 for the deionized water rinse, and 1586.4±776.8; 1536.3±546.4; 1520.5±1340.7 for the $CaCl_2$ rinse. Conclusion: According to this protocol, there was not a significant variation in enamel F uptake after using a F dentifrice preceded or not by a rinse with a $CaCl_2$ solution.
Uniterms: Dentifrices; Fluorine; Calcium; Dental enamel; Saliva.

RESUMO

Introdução: O uso de uma solução de cálcio previamente à escovação com dentifrício fluoretado, poderia favorecer a remineralização e a incorporação de flúor ao esmalte dentário. Objetivos: Este estudo avaliou a concentração de flúor no esmalte dentário após o uso de dentifrício fluoretado, precedido ou não por bochecho com solução de $CaCl_2$. Métodos: Dez voluntários (18-30 anos) participaram deste estudo duplo cego e cruzado. Biópsias de esmalte e coletas de saliva de 3 min foram feitas no baseline e após a escovação com dentifrício (1,5g Crest®, 1000 ppm F, NaF), precedida por bochecho com 10mL de $CaCl_2$ 20mM, acetato 50mM, pH 7,2 ou água deionizada, durante 1 min, seguido de bochecho com 15mL de água deionizada por 5s. As biópsias foram feitas antes do bochecho e após 8-15 e 120 min. Amostras de saliva foram coletadas antes do bochecho e após 4, 15, 30, 60 e 120 min. A concentração de flúor nas biópsias de esmalte foi analisada com eletrodo para flúor (Orion 9409) e um microeletrodo calomelano de referência, enquanto a concentração de fósforo foi analisada por espectrometria. As amostras de saliva foram analisadas para flúor com o eletrodo (Orion 9609) pelo método direto. Os dados foram analisados por ANOVA e teste de Tukey ($p<0,05$). Resultados: A concentração média de flúor ± DP (µg/mL) no baseline e até 120 min para as amostras de saliva foram: 0,02±0,01; 9,06±4,26; 2,01±1,76; 0,47±0,33; 0,24±0,17; 0,08±0,08; 0,07±0,04, para os bochechos com água deionizada, e 0,02±0,02; 10,96±14,21; 2,76±3,04; 0,87±1,29; 0,40±0,66; 0,16±0,23; 0,09±0,09 para os bochechos de $CaCl_2$. Os dados para as biópsias (µg/g) foram: 1861,7±1011,0; 1790,5±953,2; 1117,6±585,2, para água deionizada, e 1586,4±776,8; 1536,3±546,4; 1520,5±1340,7, para o bochecho com $CaCl_2$. Conclusão: De acordo com este protocolo não houve uma variação significativa na incorporação de flúor ao esmalte após o uso de dentifrício fluoretado precedido por bochecho com solução de $CaCl_2$.
Unitermos: Dentifrícios; Flúor; Cálcio; Esmalte dentário; Saliva.

INTRODUCTION

The effects of fluoride as an agent on dental caries control are highly known. The cariostatic effects of fluoride are associated to its ionic or ionizable presence in the apatite crystals aqueous phase surface[7,15,19], inhibiting demineralization process and activating the remineralization[1].

Many authors have suggested that fluoride, even in low concentrations, is necessary in oral fluids to obtain the maximum caries inhibition, and that its continuous concentration enhancement would be valuable[4,16]. Fluoridated dentifrices consist in a simple and rational form of fluoride use, and in many non-established market economy countries a decline in the caries prevalence was associated with the regular use of these products [6,9,17].

Fluoride uptake in the oral cavity following fluoridated products application, such as dentifrice and rinses, is associated with enamel and dental plaque calcium fluoride (CaF_2) formation. This compound acts as a fluoride reservoir, released by decreasing pH.[20] Thus, enamel and dental plaque CaF_2 formation following fluoridated products application is an important cariostatic mechanism of fluoride, because it keeps fluoride in the oral cavity for more time.

Whitford, et al.[24] (2001) made a cross-over, double-blind study on 13 adults volunteers from a community with fluoridated drinking water. The fluoride concentrations in saliva and total plaque after use of a fluoride dentifrice (1000 ppm fluoride, NaF) or placebo for 7 days were compared. Regression analyses showed a strong correlation between the Ca and F plaque concentration ($r=0.79$; $p<0.0001$), as well as a weak correlation between the Mg and F concentrations ($r=0.33$; $p=0.017$) and no correlation between the Zn and F plaque concentrations ($p=0.56$). These data support the hypothesis that the plaque fluoride concentration during the day depends more of the calcium concentration on the plaque, which would bind to fluoride, than on the fluoride concentration in the vehicle.

In an effort to reconcile these observations, it was necessary to evaluate the effect of a calcium rinse previously to brushing with a fluoridated dentifrice. It could be supposed that the use of calcium previously to brushing would cause an increase in plaque and dental enamel CaF_2. This would in turn be reflected as a lower fluoride salivary concentration. Thus, the aim of this work was to evaluate both the fluoride uptake in dental enamel and the fluoride concentration present in saliva after different time periods following brushing with a fluoride dentifrice, preceded or not by a calcium chloride ($CaCl_2$) solution rinse.

MATERIALS AND METHODS

Experimental design

Ten young adult volunteers (18–35 years old) participated in this cross-over, double-blind design protocol, approved by the Ethics Committee of Bauru Dental School – University of São Paulo. The volunteers were in good oral conditions. The volunteers lived in Bauru, a fluoridated community (0.7 ppm fluoride). The volunteers used a placebo dentifrice (no added fluoride) during two weeks and were asked to refrain from using fluoridated products, and to abstain from eating and drinking foods and beverages that are high in fluoride for these two weeks prior to the tests.

On the test day, the volunteers received professional prophylaxis with a placebo dentifrice on the maxillary central incisors. Dental enamel biopsy was done at baseline, and again between 8 and 15 minutes and at 120 minutes after the test regimen (see below) was applied, at different locations of the same tooth. The maxillary central incisors to be biopsied at the first experimental day were randomly selected. For the second experimental day, the contralateral central incisor was biopsied. While the volunteer was lying in the supine position, the tooth was dried with a sterile gauze. A strip of nonwettable adhesive tape (3M®) with a hole (1.1 mm diameter) punched in its center was burnished onto the tooth. For the control acid-etch biopsy, the hole was located in the middle third of the longitudinal aspect of the tooth to the left of the tooth midline. For the post-treatment biopsy, the hole was located in the middle third of the longitudinal aspect of the same tooth to the mid and to the right of the midline, respectively. The enamel biopsy was done by placing 5 µL of 0.5 M HCl on the enamel demarcated by the hole in the tape. The acid was dispensed using a fixed volume pipettor (5 µL) and nonwettable plastic tips. As the tape and enamel surface were not wettable, the acid drop was a hemisphere. After 5 seconds, the acid was removed by drawing it back into the plastic tip and was placed in a plastic Petri dish containing 50 µL of TISAB II (Total Ionic Strength Adjustment Buffer). Two 5-second separate rinses of the biopsy site, each using 5 µL of 0.25 M NaOH, then were done to neutralize and permit collection of any remaining acid. The NaOH rinses were added to the same plastic dish [16].

In addition, baseline non-stimulated whole saliva was collected with the subjects sitting quietly, swallowing, and allowing the saliva to pool in their mouths for three minutes. Each subject then emptied the contents of his/her mouth into a pre-weighed re-sealable plastic vial. Assuming the specific density of saliva as 1 mg/mL, the volumes were calculated by subtracting the initial from the final weight. Then the salivary flow (mL/min) was calculated dividing the salivary volume obtained by the time of collection. The test regimen (see below) was applied, and saliva was again collected immediately after treatment (0) and then at 4, 15, 30, 60 and 120 minutes. All subjects abstained from eating and drinking during the two-hour experimental period, were in a good state of health and took no medications that might affect their salivary flow rate either immediately, before or during the experiments. Saliva samples were weighed, and stored at 4°C for no more than two weeks prior to analysis.

Two test treatments were compared: placebo rinse (deionized water) and a 20 mM $CaCl_2$, 50 mM acetate, pH 7.2 rinse. Testing was randomized, with all subjects completing

the two aspects of this study. A minimum of two weeks elapsed between the cross-over tests with the same subject.

The method of delivery of the test regimens was highly standardized. The subjects rinsed with 10 mL of deionized water or $CaCl_2$ solution, over a one-minute period. Immediately after rinsing, the subjects brushed with a pre-measured amount (1.5 g) of a fluoridated dentifrice (CREST® toothpaste, 1000 ppm fluoride as NaF) for 1 minute with a toothbrush that was provided for each subject[20]. Finally the subjects rinsed with 15 mL of deionized water for 5 seconds.

Fluoride analysis

Fluoride analysis on enamel biopsy solution was made immediately after each biopsy with the ion specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a miniature calomel reference electrode (Accumet, #13-620-79). The final volume of the samples was adjusted to 65 µL with deionized water. Fluoride standards (0.08, 0.16, 0.32, and 0.64 µg F) were prepared in triplicate. The millivoltage potentials were converted to mg/mL fluoride using a standard curve with a coefficient correlation of $r \geq 0.99$.

Fluoride in saliva samples was analyzed with a specific electrode (Orion Research, Cambridge, MA, USA, model 9609) after the addition of TISAB III, in a volume corresponding to 1/10 of the sample volume. A set of standards (ranging between 0.100-6.400 µg/mL fluoride) was prepared, using a serial dilution from a 100 µg/mL NaF stock solution (Orion#940907), in the same way as the samples. The millivoltage potentials were converted to µg/mL fluoride using a standard curve with a coefficient correlation of $r \geq 0.99$.

Fluoride on saliva samples at times 60 and 120 minutes after treatment was analyzed with the ion specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a miniature calomel reference electrode (Accumet, #13-620-79), after overnight HMDS (hexamethyldisiloxane) facilitated diffusion, according to Taves' method[18] (1968), as modified by Whitford[22] (1996). Prior to diffusion, 200 µL previously heated HMDS-H_2SO_4 was added to the samples, in order to remove CO_2. During the diffusion process, which was conducted at room temperature, the solutions in the non-wettable Petri dishes (Falcon, No. 1007) were gently swirled on a rotary shaker. Fluoride standards (0.00475, 0.0095, 0.019, 0.095 and 0.190 µg F) were prepared in triplicate and pre-diffused in the same manner as the samples by serial dilution of a stock standard containing 0.1 M fluoride (Orion 940906). Diffused standards, which differed from the pre-diffused standards only by the no addition of previously heated HMDS-H_2SO_4 were also prepared. The final volume of the samples and standards was 75 µL. Thus, considering the limit of detection of the ion-specific electrode as 0.02 µg F/mL, it was possible to analyze, with accuracy, saliva samples with a volume superior than 0.5 mL containing more than 0.0095 µg F/mL. In addition, nondiffused fluoride standards were prepared with the same solutions (0.05 M NaOH, 0.20 M acetic acid, plus NaF) that were used to prepare the diffused standards and samples. The nondiffused standards were made up to have exactly the same fluoride concentrations as the diffused standards. Comparison of the millivolt readings demonstrated that the fluoride in the diffused and pre-diffused standards had been completely trapped and analyzed (recovery >99%). The millivoltage potentials were converted to µg F using a standard curve with a coefficient correlation of $r \geq 0.99$.

Phosphorus analysis

Phosphorus analysis was made under Fiske and Subarrow[8] (1925) colorimetric method. The mass of enamel biopsied was calculated based on the assumptions that enamel is 17.4% phosphorus by weight. The depth of the biopsy was calculated based on the assumptions that the density of enamel is 2.95 g/cm^3 and that the geometry of the biopsied site was a cylinder.

Statistical Analysis

Data were analyzed by the ANOVA for repeated measurements and Tukey post hoc tests ($p<0.05$).

RESULTS

Saliva fluoride concentrations (µg/mL) over the 2-hour time period, for the groups treated with deionized water or $CaCl_2$ rinses are presented in Table 1. The differences between the two treatment groups were not statistically

TABLE 1- Mean salivary fluoride concentration (SD, unit µg/mL) as a function of the time (min) of saliva collection after rinsing with a placebo or a 20 mM $CaCl_2$ solution

Groups \ Time	baseline	0	4	15	30	60	120
Placebo	0.025	9.068	2.018	0.477	0.241	0.085	0.074
	(0.015)[a]	(4.269)[b]	(1.769)[c]	(0.331)[d]	(0.174)[e]	(0.084)[f]	(0.040)[f]
$CaCl_2$	0.029	10.968	2.763	0.871	0.405	0.162	0.096
	(0.025)[a]	(14.214)[b]	(3.099)[c]	(1.292)[d]	(0.663)[e]	(0.231)[f]	(0.093)[f]

Values followed by different letters in the same rows are statistically different ($p<0.05$). There were no significant differences between groups ($p>0.05$).

significant (p>0.05). Saliva collected immediately after brushing (time 0) had significantly higher mean fluoride concentrations than samples collected at the other periods. From then on, salivary fluoride levels decreased exponentially in a biphasic manner. At 60 and 120 min salivary fluoride levels were similar, but statistically higher than baseline levels.

Fluoride concentration (µg/g) in dental enamel biopsies for the groups treated with deionized water or $CaCl_2$ rinses are presented in Table 2. No significant differences were observed between the two treatment groups (p>0.05).

The mean depth (±SD) of the biopsy for the groups treated with deionized water or $CaCl_2$ rinses were 0.74 (± 0.13) and 0.67 (± 0.18) µm, respectively. No significant differences were observed between the two treatment groups (p>0.05).

DISCUSSION

Low concentrations of ionic fluoride have a beneficial effect on enamel and dentin de- and remineralization and are considered to play an important role in the effectiveness of fluoride treatments, such as topical applications, rinses or dentifrice. CaF_2 deposits formation appears responsible for the persistence of oral fluid fluoride after fluoride application. However, CaF_2 deposition is limited by the low concentration of labile oral calcium.

As we can see in Table 1, the level of fluoride in saliva following topical fluoride applications decreased exponentially in a biphasic manner to very low concentrations within a few hours. This is in agreement with the findings of Ten Cate[20], 1997 and Blake-Haskins, et al.[3], 1992. According to Blake-Haskins, et al.[3] (1992) the first phase of the exponential decrease on the level of fluoride is abrupt, and happens between 40 and 80 minutes. The second phase remains unchangeable up to 3 hours. This is the result of a complex interaction of factors that influence the clearance of fluoride from the mouth and factors that aid in the retention of fluoride in the mouth. Tooth structure, dental plaque, spaces between teeth and soft oral tissues are all possible sites of fluoride retention in the mouth. An additional source of oral fluoride is the redistribution of systemic fluoride via ductal salivary secretions[10]. The volunteers of this study had a full complement of teeth with a low-to-moderate past caries experience. They had no faulty dental restorations, no current caries activity, no dental plaque, and neither dental prosthesis nor orthodontics therapy in use as possible sites of fluoride retention. One important consideration is that fluoride has high affinity for teeth and dental plaque, which may initially contribute to the clearance of fluoride from saliva[25,14].

TABLE 2- Enamel fluoride concentration (µg/g) as a function of the time before (baseline) and after rinsing with a placebo or a 20 mM $CaCl_2$ solution, and brushing

Time / Groups	baseline	8-15 min	120 min
Placebo	1861.7 (1011.0)[a]	1790.5 (953.2)[b]	1117.6 (585.2)[c]
$CaCl_2$	1586.4 (776.8)[a]	1536.3 (546.4)[b]	1520.5 (1340.7)[c]

Values followed by different letters in the same rows are statistically different (p<0.05).
There were no significant differences between groups (p>0.05).

Mellberg and Chomicki[13] (1985) considered that saliva and especially plaque fluid contain ionic calcium which can interchange with a sodium monofluorophosphate (SMFP) containing dentifrice. Thus, the rinse with calcium prior to toothbrush could potentiate this interaction.

The results shown in Table 1 suggest that there was no correlation between the CaF_2 rinse and higher salivary fluoride availability. A factor that could have influenced the similar results found for both groups was the low calcium concentration in the $CaCl_2$ rinse used. Vogel, et al.[21] 2004, determined if a concentrated calcium pre-rinse, which should form a tissue calcium reservoir, would increase saliva fluoride from a fluoride rinse. At experiment 1, subjects rinsed for 1 minute with 30, 150 or 300 mM calcium lactate (CaLac) and then 1 minute with 225 ppm NaF. At experiment 2, subjects rinsed with water 10 seconds after the 300 mM CaLac rinse to reduce calcium carry over to the fluoride rinse. The calcium pre-rinses increased saliva fluoride (mean ± SD) from 21 ± 18 µM (NaF - control) to 188 ± 138 µM (150 mM CaLac). The decrease in fluoride with the 300 mM calcium pre-rinse (F=130±35 µM), relative to the 150 mM calcium pre-rinse suggested that calcium carry over might be an important part of the mechanism by which the calcium pre-rinse increases saliva fluoride.

Blake-Haskins, et al.[3] (1992) evaluated calcium uptake by using a $CaCl_2$ solution with or without NaF. The authors found that a calcium rinse treatment followed by fluoride produced a 100% increase in calcium content on plaque model when compared with an artificial saliva treatment followed by calcium alone. This was attributed to CaF_2 formation. The effects of these increased plaque minerals on caries lesion formation were studied. Artificial plaque treated with a calcium rinse followed by a fluoride rinse reduced lesion size by 90%, compared with a 68% reduction by a fluoride rinse alone. The simulation of a pre-brush calcium rinse (180 ppm calcium) followed by a fluoride dentifrice suspension (110 ppm fluoride) reduced the lesion size in 46%, compared with a 32% reduction by the fluoride dentifrice suspension alone.

The mean depth of the biopsies did not differ between the two treatment groups. The enamel fluoride concentrations also did not. Despite the correlation between these two variables, it would not be accurate to assure that the $CaCl_2$ solution cannot diffuse through the enamel. This is because there is the possibility that in the present study a low calcium concentration was used in the $CaCl_2$ solution rinse. A study conducted by Koo and Cury[11], in 1998, showed

that the association between a soluble calcium (as 0.45% $CaCl_2$) and a sodium monofluorophosphate (SMFP - 1450 ppm fluoride) containing dentifrice promoted the enhancement of fluoride uptake on deeper layers of enamel. Enamel microhardness was also enhanced when compared with the use of a SMFP (1450 ppm fluoride) containing dentifrice only or with the use of a placebo dentifrice. The results suggested that the SMFP$CaCl_2$ can diffuse through the enamel lesion, enhancing the fluoride uptake in the caries lesions. In addition, enamel biopsies showed a 50% and a 40% increase on enamel remineralization after the use of a fluoride dentifrice associated or not with the $CaCl_2$, respectively.

In our study, however, the pre-rinse with $CaCl_2$ solution was not able to increase salivary fluoride, nor enamel fluoride concentrations. These findings could be explained by a conjunction of factors: first, the calcium concentration in the rinse; second, the kind of acid used to perform the enamel biopsy; third, the biopsy protocol chosen; and fourth, the caries risk of the volunteers. The first hypothesis may have had some influence both in saliva and enamel. It is not probable that the second hypothesis had an influence on our negative findings. Despite most of the protocols for *in vivo* enamel biopsy use perchloric acid[23], which is stronger than hydrochloric acid, this one has been successfully used for enamel biopsies in many *in vitro* studies involving pH cycling. As for the biopsy protocol chosen, it acts removing total fluoride (CaF_2 and fluoroapatite). Thus, if the concentration of the newly formed CaF_2 was not high enough, it could be masked by the concentrations of fluoroapatite and previously formed CaF_2. In addition, the volunteers in our study had a low caries risk. It does not seem to exist a direct scientific evidence for the fact that the formation of CaF_2 after the clinical use of fluoride is an important fluoride reservoir in sound teeth.[26] A study performed by Belser, et al.[2], 1975, showed that biopsied intact, not treated enamel had an average fluoride content of the integral of 550 ppm. Seven days after one 3 minutes topical application of amine fluoride on intact enamel the integral average fluoride concentration increased from 550 to 1150 ppm. Moreover, 7 days after one amine fluoride application on enamel previously etched with pyruvic or orthophosphoric acid, the average fluoride content was the integral of 3400 ppm and the integral of 2800 ppm, respectively. These data clearly indicated that the uptake and retention of fluoride are difficult in the intact enamel. Another point of consideration is the treatment time. The treatment protocol was performed just on the experimental day. Thus, it could not have had enough time to the small CaF_2 particles to penetrate into the enamel pores allowing further growth and aggregation. Markovic, et al.[12], 2004, investigated the correlation between CaF_2 precipitation and deposition kinetics in a 12 mM NaF solution to which a 0.1 M calcium solution was added at a calcium-addition rate of 7.5 mM per min (37 °C, stirring at 400 rpm). The authors concluded that CaF_2 deposition during an earlier time interval is prerequisite for a higher deposition in the subsequent intervals. This suggests that only small particles that nucleated and/or penetrated into the pores in the early time interval were fixed inside the pores by their further growth and aggregation. Duckworth, et al.[5] (1992) observed an increase in salivary fluoride levels with increasing fluoride dentifrice concentration (0, 1000, 1500, and 2500 ppm F as SMFP) after a single dentifrice application or after a four-week daily use. In this sense, if the treatment time of the present study was increased to at least one week, it is possible that a positive correlation could have been found in enamel fluoride uptake and in the salivary fluoride after using a fluoridated dentifrice preceded by a rinse with a $CaCl_2$ solution. Thus, additional studies using solutions with higher calcium concentrations for a bigger treatment time and involving volunteers with a high caries risk are needed to clarify the role of a pre-rinse with calcium on the salivary and enamel fluoride concentrations following the use of fluoridated dentifrices.

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