

Salivary fluoride concentration after the use of a fluoridated dentifrice, preceded or not by a calcium rinse

Concentração de flúor na saliva após o uso de dentifricio fluoretado, precedido ou não por um bochecho com cálcio

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ABSTRACT

The use of the calcium previously to brushing could increase the dental plaque and enamel CaF_2 uptake, what would be reflected as a lower fluoride salivary concentration. The aim of this cross-over, double-blind design study was to evaluate the fluoride concentration present in the saliva of seven young-adults (18-28 years-old) at baseline and following several times (0, 4, 15, 30, 60 and 120 min) after a fluoridated dentifrice brushing (Crest., 1000 ppm F as NaF), preceded or not by a 20 mM CaCl_2 , 50 mM acetate, pH 7.2 rinse. Salivary samples were analyzed after the addition of TISAB III. There was no difference in the salivary flow after the treatments. There was no statistically significant difference in fluoride concentration in saliva between the groups at all times studied. Fluoride concentration in saliva was higher immediately after brushing with the fluoride containing dentifrice (Tukey's test, $p < 0.001$), whereas there were no differences in saliva fluoride concentration between any of the other time periods analyzed in this study ($p < 0.0001$). The results suggest that there was no relationship between a previous calcium rinse and a lower fluoride salivary concentration.

UNITERMS

Fluorine; calcium; saliva; dentifrices; dental polymers, fluorine

INTRODUCTION

Systemic or topic fluoridated supplements, and the calcium and phosphate ions present in saliva have been defined as potential tooth remineralizing agents⁹ 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 valuable³. Fluoridated dentifrices consist in a simple and rational form of fluoride use, and in many countries a decline in the caries prevalence was associated with the regular use of them^{4,7,13}. Fluoride uptake in the oral cavity following fluoridated products application is associated with enamel and dental plaque calcium fluoride (CaF_2) formation. This compound acts as a fluoride reservoir, released with decreasing pH¹⁵, whose cariostatic

effects are associated to its ionic or ionizable presence in the apatite crystals aqueous phase surface^{5,11,14} inhibiting demineralization process and activating the remineralization one¹. 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.

However, the remineralizing capacity of fluoride is limited by concentration of calcium ions available in the saliva. Calcium concentration can be increased through its supplementation on dentifrices, gum and rinse solutions⁹. The addition of this salt to dentifrices and rinses usually causes the inactivation of these compounds, due to insoluble CaF_2 precipitation^{12, 16}, essentially in the presence of fluoride, which impairs its clinic application. Thus, the solution would consist of an equilibrated

calcium-fluoride supplementary system, enhancing remineralization, inhibiting demineralization and increasing enamel fluoride uptake.

In an effort to reconcile these observations, it is necessary to evaluate the effect of a calcium rinse prior to the brushing with a fluoridated dentifrice. It can be supposed that the use of the calcium previously to brushing would enhance the plaque and dental enamel CaF_2 uptake, what would result in a lower salivary concentration of fluoride. Thus, the aim of this work was to evaluate the fluoride concentration present in saliva after several times following a fluoride dentifrice brushing, preceded or not by a calcium chloride (CaCl_2) rinse.

MATERIALS AND METHODS

Experimental design

Seven young adults volunteers (18–28 years old) participated in this cross-over, double-blind design, approved by the Ethical Committee of Bauru Dental School – University of São Paulo. The volunteers were of five females and two males, which had a full complement of teeth with a low-to-moderate past caries experience, no faulty dental restorations, no current caries activity, no active periodontal disease, and neither dental prosthesis nor orthodontics therapy in use. This was considered necessary to eliminate any possible effects on fluoride retention. The volunteers lived in a fluoridated community (0.7ppm fluoride). They 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, 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. The test regime (see below) was applied, and saliva was again collected immediately after treatment (0) and then at 4, 15, 30, 60 and 120 minutes after⁵. All subjects abstained from eating and drinking during the two hours experimental period and 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 not 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 10mL 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 subject¹⁹. Finally the subjects rinsed with 15 mL of deionized water for one minute.

Fluoride analysis

Fluoride content on saliva samples was analyzed with a specific electrode (Orion 9609) after the addition of TISAB III, in a volume corresponding of the 1/10 of the volume sample. A set of standards (ranging between 0.100-6.400 $\mu\text{g/mL}$ fluoride) was prepared, using a serial dilution from a 100 $\mu\text{g/mL}$ NaF stock solution (Orion#940907), in the same way as the samples. The millivoltage potentials were converted to mg fluoride using a standard curve with a coefficient correlation of $r \geq 0.996$.

Statistical Analysis

The differences in salivary fluoride concentration as a function of the time of collection were tested by repeated measures ANOVA and Tukey's post hoc test.

The differences between the different regimens were tested using the Wilcoxon matched-pairs signed-ranks test.

RESULTS

Mean salivary flow rate. Mean salivary flow rates (mL/min) \pm SD over the 2-hour time period are presented in Table 1 for all subjects. There were no differences in the salivary flow rates between the treatments. There was, however, a strong evidence of subject-to-subject variability in salivary flow rates (not show).

Mean saliva fluoride concentrations: mean saliva fluoride concentrations (mg/mL) \pm SD over the 2-hour time period are presented in Table 2 for both

groups. ANOVA revealed a significant difference among the different times in which saliva was collected ($p < 0.0001$). Tukey's test revealed that the saliva collected immediately after brushing (time 0) had fluoride concentrations significantly higher than samples collected at the other periods

($p < 0.001$). For the other periods, no statistical significant differences were observed ($p > 0.05$). Also, the differences between the two treatment groups were not statistically significant ($p > 0.05$), which suggests that the calcium rinse prior to brushing did not affect salivary fluoride concentrations.

Table 1 - Mean salivary flow rates (mL/min) \pm SD after brushing with a fluoridated dentifrice, preceded or not by a calcium rinse

Times of collection	Placebo	Calcium rinse
Baseline	0.67 \pm 0.34 ^{A,a}	0.57 \pm 0.39 ^{A,a}
0 min	0.61 \pm 0.13 ^{A,a}	0.69 \pm 0.34 ^{A,a}
4 min	0.50 \pm 0.15 ^{A,a}	0.50 \pm 0.33 ^{A,a}
15 min	0.64 \pm 0.32 ^{A,a}	0.50 \pm 0.21 ^{A,a}
30 min	0.57 \pm 0.39 ^{A,a}	0.53 \pm 0.29 ^{A,a}
60 min	0.59 \pm 0.39 ^{A,a}	0.56 \pm 0.31 ^{A,a}
120 min	0.59 \pm 0.38 ^{A,a}	0.50 \pm 0.22 ^{A,a}

Capital letters show the statistical differences among salivary flow rates as a function of the time of saliva collection. Lower case shows the statistical differences among salivary flow rates with the treatments. There was no statistical difference between the groups ($p > 0.05$).

Table 2 - Mean saliva fluoride concentrations (mg/mL) \pm SD after brushing with a fluoridated dentifrice, preceded or not by a calcium rinse

Times of collection	Placebo	Calcium rinse
Baseline	0.02 \pm 0.01 ^{A,a}	0.03 \pm 0.02 ^{A,a}
0 min	6.60 \pm 4.40 ^{B,b}	8.80 \pm 6.30 ^{B,b}
4 min	2.20 \pm 1.80 ^{A,a}	1.90 \pm 1.60 ^{A,a}
15 min	0.70 \pm 0.60 ^{A,a}	0.80 \pm 0.50 ^{A,a}
30 min	0.30 \pm 0.20 ^{A,a}	0.40 \pm 0.20 ^{A,a}
60 min	0.08 \pm 0.03 ^{A,a}	0.10 \pm 0.06 ^{A,a}
120 min	0.07 \pm 0.04 ^{A,a}	0.10 \pm 0.04 ^{A,a}

Capital letters show the statistical differences among saliva fluoride concentrations as a function of the time of saliva collection. Lower case shows the statistical differences among saliva fluoride concentration with the treatments. Times followed by different letters are statistically different ($p < 0.001$). There was no statistical difference between the 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 dentifrices. The level of fluoride in oral fluids following topical fluoride applications decreases exponentially in a biphasic manner to very low concentrations within a few hours¹⁵, as we can see in Table 2. Many factors are responsible for that. These factors aid in the retention of fluoride in the mouth and thus influence the clearance of fluoride from the mouth. Tooth structure, dental plaque, spaces between teeth and soft oral tissues are all possible sites of fluoride retention in the mouth, which may initially contribute to the clearance of fluoride from saliva^{10,18}. An additional source of oral fluoride is the redistribution of systemic fluoride via ductal salivary secretions⁸. The volunteers of this study have 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. Mean non-stimulated salivary flow rates were similar for both groups, showing that the statistical differences in salivary fluoride concentration found during the 2nd time following the treatments (0 min) (Table 2) cannot be attributed to differences in clearance due to salivary flow (Table 1).

The results showed in Table 2 suggest that there was no relationship between calcium rinse and salivary fluoride concentration. Nevertheless, the results found in this study may be associated to the use of a final deionized water rinse for 1 minute. This may have removed all the additional calcium provided. It would be indicated an one final rinse with 15mL of deionized water for 10 seconds¹⁹. After considering the possible above-mentioned limitation in the design of this study, we hypothesized that if the fluoride delivered by brushing is not really taken up by the dental enamel surfaces as the desirable CaF_2 or CaF_2 -like deposits, it might diffuse throughout the soft oral tissues. Thus, the fluoride present in the salivary film coating the oral mucosa, which represents 80% of the surface

area of the mouth¹⁹, may act as an important reservoir of fluoride.

Blake-Haskins et al² (1992) evaluated calcium uptake by using a CaCl_2 solution with or without NaF and found that a treatment consisting of calcium rinse followed by fluoride produced a 100% increase in calcium content of model plaque, presumably because the formation of CaF_2 , compared with a treatment with artificial saliva followed by calcium alone. 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 (180ppm calcium) followed by a fluoride dentifrice suspension (110ppm fluoride) reduced the lesion size in 46%, compared with a 32% reduction by the fluoride dentifrice suspension alone.

Whitford et al¹⁷ (2001) compared the fluoride concentrations found in saliva and in total plaque after the use of a fluoridated dentifrice (1000ppm fluoride, NaF) or a placebo dentifrice. The Ca concentration was also analyzed. Salivary fluoride concentrations 1 and 12 hour after brushing with a fluoridated dentifrice (24.3 e 6.6 mmol/L) were significantly higher than the salivary fluoride concentration associated with the use of a placebo dentifrice (1.5 $\mu\text{mol/L}$). Fluoride concentration in plaque one hour after the use of a fluoridated dentifrice (22.9mmol/kg dried weigh) was higher than the 12 h one (12mmol/kg) and higher than the fluoride concentration found in plaque after 12 hours of use of the placebo one (10mmol/kg). Plaque fluoride concentration 12h after the use of a fluoridated dentifrice was not different from placebo. Regression analysis showed a strong correlation between the concentration of Ca and F in plaque ($r=0.79$; $p<0.0001$). These data support the hypothesis that the plaque fluoride concentration during the day depends more on the calcium concentration in the plaque, which would bind to fluoride, than on the fluoride concentration in the vehicle.

Additional studies are necessary to clarify the possible effects of a calcium rinse prior to brushing with a fluoridated dentifrice.

RESUMO

O uso de cálcio previamente à escovação poderia aumentar a incorporação de CaF_2 na placa e no esmalte dental, o que seria refletido como uma redução na concentração de flúor na saliva. O objetivo deste estudo cruzado e duplo-cego foi avaliar a concentração de flúor presente na saliva de 7 adultos jovens (18-28 anos) antes e em diversos períodos (0, 4, 15, 30, 60 and 120 min) após escovação com um dentífrico fluoretado (Crest., 1000 ppm F na forma de NaF), precedida ou não por um bochecho com CaCl_2 20 mM, 50 mM acetato, pH 7,2. Amostras de saliva foram analisadas após a adição de TISAB III. Não houve diferença no fluxo salivar após os tratamentos. Não houve diferenças estatisticamente significantes entre os grupos na concentração de flúor na saliva em todos os tempos avaliados. A concentração de flúor na saliva foi maior imediatamente após a aplicação do dentífrico fluoretado (teste de Tukey, $p < 0.001$), não havendo diferença nos períodos subsequentes analisados neste estudo, conforme testado pela ANOVA ($p < 0.0001$). Os resultados sugerem que não existe uma relação entre o bochecho prévio com cálcio e uma redução na concentração de flúor salivar.

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Flúor; cálcio; saliva; dentífricos; polidores dentários, flúor

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