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Ca Pre-rinse Greatly Increases Plaque and Plaque Fluid F

ABSTRACT

Previous studies demonstrated that a Ca pre-treatment greatly increases salivary F from a subsequent NaF rinse. This study examines if these increases are found in plaque and plaque fluid F. Thirteen individuals accumulated plaque before rinsing with: (1) 12 mmol/L NaF (228 μg/g F), (2) 150 mmol/L Ca rinse, or (3) the Ca rinse followed by the F rinse. One hr later, plaque samples were collected, the plaque fluid was recovered, and the plaque residues were extracted 5 times with pH 6.8 or pH 4.8 buffers, and then by acid. The F in each extract after the Ca rinse/F rinse greatly exceeded the corresponding F from the NaF rinse. Consequently, the Ca rinse/F rinse increased the total plaque F and the plaque fluid F by 12x and 5x, compared with the NaF rinse alone. These and the previous salivary results suggest that a Ca pre-treatment may increase the cariostatic effects of topical F agents.

KEY WORDS: fluoride, rinse, plaque, calcium, pre-rinse.

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INTRODUCTION

The concentration of fluoride (F) in oral fluids has a profound effect on the de-/remineralization process (Margolis and Moreno, 1990; ten Cate, 1990; Featherstone, 2000). Given the role of plaque in the caries process, this observation has focused attention on the "bioavailable" plaque F reservoirs that can persistently increase F concentrations in plaque fluid. Calcium-to-fluoride (Ca-F) binding appears to play a central role in the formation of these plaque F reservoirs, either in the form of phosphate-contaminated "calcium fluoride-like" plaque deposits (Arends and Christoffersen, 1990; Rølla and Saxegard, 1990; Vogel *et al.*, 2006a,b), or as bacterial calcium-fluoride complexes (Rølla and Bowen, 1977; Rose *et al.*, 1996). Unfortunately, the low concentration of plaque fluid or salivary Ca (≈ 1 mmol/L to 3 mmol/L) relative to the amount of applied F after use of F dentifrice or rinse (≈ 12 mmol/L) implies that Ca-F reservoir formation is limited by the rate at which additional Ca can be scavenged from Ca reservoirs in enamel, plaque, or saliva during the short period of F application. The relatively small amounts of these oral Ca-F reservoirs appear to produce only a transient increase in plaque fluid and salivary F (Vogel *et al.*, 1992; Whitford *et al.*, 2005). Previous studies have shown that if this limitation on the amount of easily available Ca is ameliorated by first pre-rinsing with a concentrated calcium lactate solution, a large increase in salivary fluoride can be obtained from a commercial dentifrice or, optimally, a F rinse (Vogel *et al.*, 2006a,b). The purpose of this study was to examine if these increases are also found in plaque fluid F. Since, except shortly after F administration, the source of the plaque fluid F is the bioavailable Ca-F reservoirs that can be easily released from the plaque mass (Vogel *et al.*, 1992), a second goal of this study was to examine the amount of buffer-extractable F or Ca (pH 6.8 and pH 4.8) in plaque samples after a F application with or without the Ca pre-rinse procedure.

MATERIALS & METHODS

Study Participant Protocol

All procedures were performed with the informed consent of the study participants following protocols approved by the institutional review board (IRB) of the American Dental Association and reviewed by the IRB of the National Institute of Standards and Technology. These participants were screened before inclusion, to ensure their good oral health and normal salivary gland function (assessed by the measurement of unstimulated salivary flow in each potential participant). The same 13 individuals participated in all experiments; however, in some experiments, adequate plaque samples could not be recovered from all the participants. All study participants lived in an area with fluoridated water (2003 average F = 1.01 μg/g; range, 0.69 μg/g to 1.13 μg/g) and used their normal oral hygiene procedures between periods of plaque accumulation (i.e., no special tooth-cleaning procedures were performed). The rinses (described below) were

Table 1. F in 5 Sequential Extractions of Plaque by a pH 6.8 or 4.8 Buffer Obtained 1 hr after One of the Treatment Regimens Shown (F remaining in the plaque, as measured by a subsequent strong acid extraction, is also shown.)

Extraction pH	Extraction #	Extract Vol., μL/mg	No Rinse			Ca Rinse			NaF Rinse			Ca Rinse/NaF Rinse		
6.8	1	15	3.5*	2.0	11	4.3[a]*	1.4	12	13[a]	1.4	12	212	2.6	13
	2	45	5.0*	2.0	11	6.4*	1.7	13	20	2.0	13	404	1.8	13
	3	45	4.8*	1.7	11	4.8*	1.7	13	15	1.9	13	264	1.9	13
	4	45	3.6*	1.8	11	4.5*	2.0	13	14	1.8	13	234	2.0	13
	5	45	3.5*	2.0	11	3.8*	2.1	13	11	2.4	13	193	1.9	13
	acid F[b]	45	13*	1.5	11	8.8*	2.6	13	61	5.2	13	916	2.4	13
	total F[c]		35*	1.6	11	39*	1.4	13	172	2.5	13	2455	1.8	13
4.8	1	15	6.6*	1.6	11	7.5*	2.1	13	52	2.1	12	405	1.6	12
	2	45	8.0*	3.0	11	16	1.8	13	65	3.5	12	662	1.4	12
	3	45	5.0*	3.0	11	7.7*	2.7	13	25	4.9	12	412	1.7	12
	4	45	2.0[a]*	1.9	10	3.5*	2.3	13	10	4.6	12	242	2.3	12
	5	45	2.3*	2.5	11	2.5*	1.3	13	4.6*	2.6	12	141	3.3	12
	acid F[b]	45	3.8*	2.0	11	2.0*	2.1	13	3.8*	2.8	12	108	5.0	12
	total F[c]		35*	2.0	11	45*	1.7	13	213	2.3	12	2149	1.6	12
	average total F[d]		36*	1.7	11	43*	1.5	13	199	2.2	13	2342	1.6	13

Data are given as the geometric mean of the values (F μmol/g x 100), the standard deviation factor (SDF), and the number of samples. Across the rows, each group is statistically different except as indicated by the same symbol ($p < 0.05$, repeated-measures ANOVA with the log-transformed values, Newman-Keuls multiple-comparisons test).

[a] Three extreme values from the sequential extractions were outliers according to the criteria of Grubbs and Beck (1972) and were omitted. These are: pH 6.8 extraction 1, Ca rinse = 19 and NaF rinse 40; and pH 4.8 extraction 4, No rinse = 59.

[b] The acid value is the amount of F remaining in the sample after the sequential extractions.

[c] The total F is the total of all the F extracted from the plaque sample. The total F from the pH 6.8 and pH 4.8 extractions did not differ statistically (paired difference test).

[d] The average total F is the geometric mean of the average of the individual pH 6.8 and pH 4.8 values (see text). The use of the geometric means is the reason that the total F is not equal to the sum of the extractions and the acid F values, and that the average total F is different from the average of the total 6.8 and total 4.8 values.

given in a random order, and the participants were not aware of the identity of the test material. The analysts were not aware of the identity of the recovered samples. Before each experiment, the participants accumulated plaque for 48 hrs and fasted overnight. In the morning, these individuals received the experimental treatment. Plaque samples were collected 1 hr later.

Rinse Administration

The study participants followed 4 regimens: (1) no rinse ("baseline" or "control"); (2) rinsing for 1 min with 20 mL of 150 mmol/L calcium lactate (calcium lactate pentahydrate; Sigma-Aldrich, St. Louis, MO, USA); (3) rinsing, similarly, with 12 mmol/L (228 μg/g) F (NaF; Fisher Scientific, Pittsburgh, PA, USA); or (4) rinsing with the 2 rinses used in (2) and (3), with the F rinse being applied immediately after the calcium rinse. The participants waited at least 1 wk between each regimen.

Plaque Fluid and Plaque Recovery

Two samples of plaque were recovered from the easily accessible buccal surfaces of all 1st and 2nd molar/premolar teeth. The plaque collection procedures have been extensively described (Vogel *et al.*, 1990; Tenuta *et al.*, 2006) and are only summarized here: (1) A plastic strip and a conical mineral-oil-filled plastic microcentrifuge tube (a heat-sealed 10-μL pipette tip) were weighed together. (2) The strip, grasped in a hemostat, was used to collect the plaque sample. (3) The strip and sample were transferred to a microcentrifuge tube and re-weighed (plaque mass determination). (4) The micro-centrifuge tube and contents were centrifuged (5 min, 2°C, 1466 rad/sec, or 14,000 rpm), and a sample of the plaque fluid was recovered with a capillary micropipette. (5) The tip of the centrifuge tube was cut and the residue expelled by centrifugation into a 600-μL plastic centrifuge tube. Due to the relatively low plaque fluid ion concentration, fluid removal does not significantly reduce the total plaque Ca or F.

Extraction of F and Ca from Plaque

A 15-μL/(mg plaque) quantity of either pH 6.8 (50 mmol/L PIPES) or pH 4.8 (100 mmol/L acetate) buffer was added to the 2 plaque samples. The plaque was then dispersed into the buffer with a 250-μL plastic pipette tip attached to a pipettor in the manner of a mortar and pestle. After 1 hr, both of the dispersed samples were centrifuged, and the fluid was recovered by means of capillary micropipettes. The plaque samples were then re-extracted 4 more times with fresh aliquots of the same buffer; however, the extraction volume was increased to 45 μL/(mg plaque). After the 5th buffer extraction, any remaining Ca and F in the plaque was recovered by extraction of the plaque mass for 1 hr with a mixture of 0.9 mol/L perchloric acid and 0.1 mol/L formic acid (45 μL/mg plaque). This mixture was then neutralized by the addition of an identical volume of 1 mol/L NaOH.

Analytical Procedure

The F in all samples was analyzed, after dilution of 9 parts sample with 1 part of TISAB III (Termo Electron Corp., Waltham, MA,

Table 2. Mean Plaque Fluid F for the Two Plaque Samples Obtained from Each Study Participant 1 hr after One of the Treatment Regimens Shown

Description	No Rinse F μmol/L	Ca Rinse[a] F μmol/L	NaF Rinse F μmol/L	Ca Rinse/NaF Rinse F μmol/L
Geo. mean	9.18*	8.8*	28.2	144.6
N	11	12	12	13
SDF	1.30	1.41	1.45	1.99
CI	7.6-10.8	7.1-10.9	22-36	96-219

The fluoride data are given as the geometric means (Geo. mean), standard deviation factor (SDF), and 95% confidence interval (CI). Across the rows, each group is statistically different except as indicated by the same symbol ($P < 0.05$, repeated-measures ANOVA with the log-transformed values, Newman-Keuls multiple-comparisons test).

[a] One value (39.7 μg/g) was an outlier (Grubbs and Beck, 1972) and was omitted. The statistical results were not changed by this omission.

USA), with an inverted F electrode apparatus (Vogel *et al.*, 1990). Calcium was spectrophotometrically determined as previously described (Vogel *et al.*, 1983; Tenuta *et al.*, 2006). The standards used for all analyses were made to contain, as far as possible, the same ion concentrations as the fluids used in these extractions.

Statistical Methods

A significance level of $p < 0.05$ was used in all statistical tests, which were performed with SigmaStat statistical software (SPSS, Chicago, IL, USA). Much of the plaque F data was not normally distributed, and thus these data were normalized (Zero *et al.*, 1992; Whitford *et al.*, 2005; Vogel *et al.*, 2006a,b) *via* a logarithmic transformation. In accordance with this transformation, the geometric mean (Geo. Mean) and standard deviation factors (SDF) of thse data are reported (Tables 1, 2) (Zero *et al.*, 1992). The total Ca data were found to be normally distributed, and hence the conventional arithmetic mean and standard error are reported (in the text below). The null hypothesis—that there was no difference among the treatments—was examined either by a one-way analysis of variance test (ANOVA) (plaque fluid F) or a one-way repeated-measures ANOVA (plaque extract Ca and F). We then used the Newman-Keuls multiple comparisons test to examine the differences among the groups. We used a paired-difference test to compare the plaque fluid F and total plaque F (or Ca) in the 2 samples recovered from each study participant. The standard deviation factor and the standard error are used here as measures of the standard uncertainty.

RESULTS

The total amounts of F in the 2 (pH 6.8 and pH 4.8) recovered samples (Table 1) were not statistically different (average difference ≈ 12%). Except for the 5th pH 4.8 extraction and subsequent acid extraction, where the NaF rinse samples were not different from the "no rinse" samples, both experimental rinses increased the F in all extractions. The total one-hour post-rinse plaque F recovered after the NaF rinse was significantly increased (≈ 5x) over the "no rinse" samples (Table 1). With regard to the Ca rinse/F rinse, every buffer extraction, as well as the subsequent acid extraction, was significantly ($p < 0.001$) increased over the NaF rinse samples. Reflecting these very large increases, the average total F of the Ca rinse/F rinse samples was significantly ($p < 0.001$) increased ≈ 64x (Table 1) over the "no rinse" samples. The Ca rinse alone did not significantly increase the F in any of the extracts.

The plaque fluid F from the pH 6.8 and pH 4.8 sample values also did not differ significantly for any test regimen (average difference ≈ 14%), and hence only the mean values are given (Table 2). The plaque fluid F results reflect the increases observed in the plaque extracts: No increase relative to the "no rinse" plaque samples was observed with the Ca-alone rinse, a significant ($p < 0.001$) increase (≈ 3x) was observed with the NaF rinse, and a much larger increase (≈ 16x) was observed with the Ca rinse/F rinse.

Since, as with the plaque total F and plaque fluid F, the total Ca extracted from the 2 (pH 6.8 and pH 4.8) samples was quite similar (average difference ≈ 8%), these data were also averaged. The average total plaque Ca values were (mean ± SE): no rinse = 33.2 ± 7.6 μmol/g, N = 10; Ca rinse = 89.9 ± 15.3 μmol/g, N = 12; NaF rinse = 46.8 ± 8.5 μmol/g, N = 12; and Ca rinse/NaF rinse = 56.5 ± 9.2 μmol/g, N = 12. Although there was a trend toward higher total Ca with the F rinse or the Ca rinse/NaF rinse, only the Ca rinse group was significantly increased ($p < 0.001$). A correlation analysis of the average total F *vs*. average total Ca found that only the NaF rinse group had a significant correlation ($R^2 = 0.44$, $p < 0.02$, R = Pearson's correlation coefficient).

DISCUSSION

The "no rinse" and one-hour NaF total F and total Ca concentrations observed in this study are similar to those reported in previous studies (Tatevossian, 1990; Vogel *et al.*, 2000a,b, 2001; Whitford *et al.*, 2005). The agreement in these quantities for the 2 collected samples demonstrates that they are nominally identical. The repeated extraction of the plaque samples with large volumes of pH 6.8 buffer failed to remove all the F. Indeed, the concentrations in these extracts were relatively similar (except extract 1, where a smaller extraction volume was used). However, each Ca rinse/F rinse plaque extract had much more F than the corresponding extract for the NaF rinse samples. In fact, the F remaining in the plaque after the 5th pH 6.8 extraction (*i.e.*, the acid F value) for the Ca rinse/F rinse plaque samples exceeded the total F in the NaF rinse samples by 5x. With the pH 4.8 extractions, 4 extractions of plaque appeared to have exhausted the F reservoir in the NaF rinse samples, while the F remaining in the plaque after the 5th extraction (the acid F) of the Ca rinse/F rinse contained more F than the sum of the F in the first 2 pH 4.8 extracts of the NaF plaque samples. As a consequence of these increases, the average total F of the Ca rinse/F rinse group increased nearly 12x relative to the NaF rinse samples.

There was a trend toward higher total plaque Ca levels in the NaF and Ca rinse/F rinse samples, but only the Ca rinse group showed a significant increase above the "no rinse" levels, and no correlation was seen between the plaque Ca and F except in the NaF rinse samples. Other studies (Whitford *et al.*, 2005; Pessan *et al.*, 2006) also found a significant correlation between plaque Ca and F, as well as a significant increase in total plaque Ca, in study participants who used a NaF dentifrice. Such results are compatible (Whitford *et al.*, 2005) with the fixing of salivary Ca into plaque Ca-F reservoirs by the applied F. Unfortunately, as noted in the INTRODUCTION, the low concentration of

salivary Ca relative to the amount of applied F severely limits the accumulation in plaque of such Ca-F moieties from saliva, while the low amount of plaque fluid Ca limits the *in situ* formation of plaque Ca-F from this source. The large increase in plaque F from the 2 rinse procedures, as well as the large Ca plaque reservoir formed by the Ca-only rinse, suggests that the Ca pre-rinse procedure can overcome these limitations. The relatively modest increase in plaque Ca when the Ca rinse was used immediately before the F rinse, relative to the NaF rinse alone, and the decrease in Ca relative to the Ca rinse alone, suggest that the water component of the subsequent F rinse was extracting Ca not yet firmly fixed in plaque (Vogel *et al.*, 2006b). These results also suggest that there is little increased potential for calculus formation, relative to the NaF rinse group, as a result of the Ca pre-rinse procedure. In fact, several studies (Schaeken and van der Hoeven, 1990, 1993) suggest that the Ca lactate used in this study decreases calculus formation.

The 12x increase in total plaque F from the Ca rinse/F rinse was reflected in a 5x increase in the average plaque fluid F relative to the NaF rinse. This increase in plaque fluid F was similar to the increases we have previously observed in saliva (Vogel *et al.*, 2006a,b). Recently, however, a 39x increase in whole saliva F, but only about a 4x increase in total plaque F, was reported at the 2007 IADR General Session (New Orleans, LA, USA) in studies done with this same Ca pre-rinse/NaF rinse system (Whitford, personal communication). It should also be noted that studies with a Ca pre-rinse and a F dentifrice found a much smaller increase in salivary F (Pessan *et al.*, 2006; Vogel *et al.* 2006), and no increase in plaque F (Pessan *et al.*, 2006). Although we have attributed these results to Ca binding by dentifrice surfactants (Vogel *et al.*, 2006b), two recent Ca rinse/F dentifrice studies involving an *in situ* model found large increases in plaque F (M. Buzalaf, private communication). In conclusion, although these very large increases in salivary F and, more importantly, in plaque fluid F suggest that a Ca pre-rinse, or other Ca pre-treatment, may increase the effectiveness of F-containing therapeutic agents, the persistence of these increases, which appears to be the key to obtaining an increased cariostatic effect, remains to be examined. It is noteworthy in this regard that the increase in plaque F induced by this treatment appears to persist, despite repeated neutral or low pH extractions.

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Certain commercial materials and equipment are identified in this paper to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or the American Dental Association Foundation, or that the material or the equipment identified is necessarily the best available for the purpose.

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