Published in final edited form as:

J Dent Res. 2008 May ; 87(5): 466–469.

Ca Pre-Rinse Greatly Increases Plaque and Plaque Fluid F after a F Rinse*

G.L. Vogel, G.E. Schumacher, L.C. Chow, S. Takagi, and C.M. Carey

American Dental Association Foundation, Paffenbarger Research Center, Gaithersburg, MD, USA

Abstract

This study examines if a Ca pre-rinse/F rinse increased plaque and plaque fluid F relative to the F rinse alone. Subjects ($n = 13$) accumulated plaque two days before rinsing with: (1) 12 mmol/L F (NaF), (2) 150 mmol/L Ca rinse, (3) the Ca rinse followed by the F rinse. A “no rinse” served as a control. One h later two plaque samples were collected, the plaque fluid recovered, and the plaque residues extracted 5 times with pH 6.8 or pH 4.8 buffers and then by strong acid. The F in each extract after Ca rinse/F rinse greatly exceeded the corresponding F from the NaF rinse. Consequently, Ca rinse/F rinse increased the total plaque F and the plaque fluid F by 12x and 5x compared to the F rinse alone. These and previous salivary results suggest that a Ca pre rinse may increase the cariostatic effect of F topical agents.

Keywords

fluoride; rinse; plaque; calcium; pre-rinse

INTRODUCTION

The concentration of fluoride (F) in oral fluids has a profound effect on the de/remineralization process (ten Cate, 1990; Margolis and Moreno, 1990; Featherstone, 2000). This observation has focused attention on the F reservoirs that maintain F concentrations in oral fluids, in particular, given the fundamental role of plaque in the caries process, “bioavailable” plaque F reservoirs. 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 (Rølla and Saxegard 1990, Arends and Christoffersen, 1990, Vogel *et al.*, 2006a, 2006b) or as or bacterial calcium-fluoride complexes (Rølla and Bowen 1977, Rose *et al.*, 1996). Unfortunately, the low concentration of plaque fluid or salivary Ca ($\approx 1 - 3$ mMol/L) relative to the amount of applied F after use of F dentifrice or rinse ($\approx 12 - 48$ mMol/L) implies that formation of these Ca-F reservoirs 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. Previous studies have shown that if this limitations is ameliorated by first pre-rinsing with a concentrated calcium lactate solution, a very large (up to 37x) increase in salivary fluoride can be obtained with from a commercial dentifrice or, optimally, a F rinse (Vogel *et al.*, 2006a, 2006b, Whitford *et al.*,

*Preliminary reports of some this data have been presented in abstract form: J Dent Res 85: (Spec Iss A); CD-ROM Abstract No. 0155 (IADR-2006).

All correspondence should be sent to G. L. Vogel. The contact information is: Gerald Vogel, American Dental Association Foundation, Paffenbarger Research Center, 100 Bureau Drive Stop 8546, National Institute of Standards and Technology, Gaithersburg MD 20899-8546, Tel: 301-975-6821, Fax: 301-963-9143, jvogel@nist.gov.

2007). The purpose of this study is to examine if these increases are also found in plaque fluid F. Since the source of the plaque fluid F is the bioavailable Ca-F reservoirs that can be easily released from the plaque mass, a second goal of this study is to examine the amount of buffer (pH 6.8 and pH 4.8) extractable F or Ca after the Ca pre-rinse procedure.

MATERIALS AND METHODS

Subject protocol

All procedures were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. The subjects were screened before participation to ensure good oral health and salivary gland function. The same thirteen subjects were used in all experiments, however in some experiments adequate plaque samples could not be recovered from all the subjects. All subjects lived in an area with fluoridated water and used their normal oral hygiene procedures between periods of plaque acumination. The rinses were given in a random order and the subjects 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 subjects accumulated plaque for 48 h and fasted overnight. In the morning subjects received the experimental treatment. Plaque samples were collected 1 h later.

Rinse administration

The subjects followed one of four regimens: (1) A No Rinse (“baseline” or “control”), (2) rinsing 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 ppm) F (NaF, Fisher Scientific, Pittsburg, PA, USA), (4) rinsing with the two rinses used in (2) and (3) with the F rinse being applied immediately after the calcium rinse. One week separated each regimen.

Plaque fluid and plaque recovery

Two pools plaque were recovered from the easily accessible buccal-interproximal 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 pointed mineral oil-filled plastic microcentrifuge tube (a heat sealed a 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 microcentrifuge tube and reweighed (plaque mass determination). (4) The micro-centrifuge tube was centrifuged (5 min, 2 °C, 14,000 rpm) and a plaque fluid samples were recovered with capillary micropipettes. (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 low plaque fluid ion concentration, fluid removal does not significantly reduce the total plaque Ca or F.

Extraction of plaque

Fifteen ul/(mg plaque) of either pH 50 mMol/L 6.8 pipes or 100 mMol/L pH 4.8 acetic buffer was added to the pooled plaque samples. The plaque was then dispersed into the buffer using a 250 ul plastic pipette tip attached to a pipettor in the manner of a mortar and pestle. After 1 h both the dispersed samples were centrifuged and the fluid recovered using capillary micropipettes. The pooled plaque samples were then re-extracted 4 more times

*DISCLAIMER

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.

with the same buffer however the extraction volume was increased to 45 μ l/(mg plaque). After the 5th extraction the remaining Ca and F in the plaque mass were extracted 1 h with a mixture of .9 M perchloric and .1 M formic acid and then neutralized by adding an identical volume of 1 M NaOH.

Analysis procedure

The fluoride in all samples was analyzed, after dilution 9:1 with TISAB III (Termo Electron Corp., Waltham MA) using 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 standard used for all analysis were made to contain, as much as possible, the same ion concentrations as the fluids use in these extractions.

Statistical methods

A significance level of $p < 0.05$ was used in all statistical tests, which were performed using SigmaStat statistical software (SPSS, Chicago IL). Much of the plaque data was not normally distributed and thus this data was normalized (Zero *et al.*, 1992, Vogel *et al.*, 2006a, 2006b, Whitford *et al.*, 2005) via a logarithmic transformation. In accordance with this transformation the geometric mean (Geo. Mean) and standard deviation factors (SDF) of this data are reported (Table 1 and 2) (Zero *et al.*, 1992). The total Ca data was found to be normally distributed and hence the conventional the arithmetic mean and standard error are reported (Fig. 1). The null hypothesis that there was no difference between 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). The Newman-Keuls multiple comparisons test was then used to examine the differences among the groups. A paired difference test was used to compare the plaque fluid F and total plaque F (or Ca) in the two samples recovered from each subject. The standard deviation factor and the standard deviation are used here as measures of the standard uncertainty.

RESULTS

The total F in the two (pH 6.8 and pH 4.8) recovered samples were not statistically different (Table 1, average agreement $\approx 8\%$). Except for the 5th pH 4.8 extraction and subsequent acid extraction, where the NaF rinsed samples were not different from the no-rinse samples, both experimental rinses increased the F in all extractions. The total 1 h post rinse plaque F recovered after the NaF rinse (total F, Table 1) was significantly increased ($\approx 5x$) over the No Rinse samples. With regard to the Ca rinse/F rinse, every buffer extraction, as well as the subsequent acid extraction, was significantly (and greatly) increased over the NaF rinsed samples. Reflecting these large increases, the total F of either Ca rinse/F rinse samples increased $\approx 64x$ over the baseline 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 samples values also did not differ significantly for any test regimen (average agreement $\approx 14\%$) and hence only the mean values are given in 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 ($\approx 3x$) increase with the NaF rinse, and much large increase ($\approx 16x$) with the Ca rinse/F rinse.

Fig. 2 shows the total Ca found in the extracts. Since, as with the plaque total F and plaque fluid F, the total Ca in the two samples were quite similar (average agreement 8%) the data was averaged in this table. Although there is a trend to high total Ca with the F rinse or the Ca rinse/F rinse, only the Ca rinse group was significantly increased. A correlation analysis

of the average total F vs average total Ca found that only the NaF rinse group had a significant correlation ($R^2 = .44$, $p < 0.02$, R = Pearson's correlation coefficient)

DISCUSSION

The No Rinse and 1 h NaF total F and total Ca concentrations observed in this study (Table 1, Table 2, Fig. 1) are similar to previous studies (Tatovissian 1990, Vogel *et al.*, 2000; 2001, Whitford *et al.*, 2005). The agreement in these quantities for the two collected samples demonstrates that they are nominally identical. Surprisingly the repeated extraction of the plaque samples with fairly large volumes of pH 6.8 buffer failed to recover all the F (Table 1). In fact the concentrations in these extracts are relatively similar (except extract 1, where a smaller extraction volume was employed). 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 5th pH 6.8 extraction (i.e. the acid F value) for the Ca rinse/F rinsed plaque samples exceeded the total F in the NaF rinse samples. With the pH 4.8 extractions four extraction of plaque appear to have exhausted the F reservoir in NaF rinsed samples, while the F remaining in the plaque after 5th extraction (the acid F) of the Ca rinse/F rinse contained more F than the sum of the F in the first two pH 4.8 extracts of the NaF plaque samples. As a consequence of these increases, the total F of the Ca rinse/F rinse group increased nearly 12x relative to the NaF rinsed samples. However a smaller 4x increase has been observed (Whitford *et al.*, 2007) in studies using a somewhat different collection and analysis protocol.

There is a trend toward higher total plaque Ca level in the NaF and Ca rinse/F rinse samples (Fig. 1) but only 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 an significant increase in total plaque Ca, in subjects that 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 salivary Ca relative to the amount of applied F severely limits the accumulation of Ca-F moieties from saliva, while the low amount of plaque fluid Ca limits the formation of plaque Ca-F from this source. The large increase in plaque F from the two rinse procedures, as well as the large Ca plaque reservoir from the Ca only rinse (Fig. 1), suggests the Ca pre-rinse procedure can overcome these limitations. The relatively modest increase in plaque Ca with the Ca rinse is combined with the F rinse, relative the NaF rinse alone, and the decrease in Ca relative to the Ca rinse alone (Fig. 1), suggests the F in Ca rinse/F rinse group, or the water component of F rinse, is acting as a "sink" (Vogel *et al.*, 2006b) to remove any Ca not firmly fixed in plaque by the formation of Ca-F plaque moieties. These results also suggests 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 (Schaecken and van der Hoeven 1990, Schaecken and van der Hoeven 1993) suggest that the Ca lactate used in this study decreases calculus formation.

The increases 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 is similar to the increases we have previously observed in saliva but is considerably less than the 32x increase recently reported (Whitford *et al.*, 2007). However, studies with a Ca pre-rinse and an 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 found increases in plaque F that are similar to the increases observed here (M. Buzalaf, private communication). The very large increase in salivary F, and more

importantly in plaque fluid F, produced by the Ca rinse/F rinse, and the fact that the Ca rinse/F rinse induced plaque F reservoirs that appear to persist despite repeated low pH extractions, suggest a Ca pre-rinse, or other Ca pretreatment, can be used to increase the cariostatic effect of F therapeutic agents.

Acknowledgments

This study was supported by a grant from the American Dental Association Foundation, and by USPHS Research Grants DE05354 and DE14707 to the American Dental Association Foundation from the National Institutes of Health – National Institute of Dental and Craniofacial Research and is part of the dental research program conducted by the National Institute of Standards and Technology in cooperation with the American Dental Association Foundation.

References

- Arends J, Christoffersen J. Nature and role of loosely bound fluoride in dental caries. *J Dent Res.* 1990; 69(Spec Iss):601–605. [PubMed: 2179320]
- Featherstone JD. The science and practice of caries prevention. *J Am Dent Assoc.* 2000; 131:887–899. [PubMed: 10916327]
- Grubbs FE, Beck G. Extension of sample sizes and percentage points for significance test of outlying observations. *Technometrics.* 1972; 14:847–854.
- Margolis HC, Moreno EC. Physicochemical perspectives on the cariostatic mechanisms of systemic and topical fluorides. *J Dent Res.* 1990; 69 (Spec Iss):606–613. [PubMed: 2179321]
- Pessan JP, Sicca CM, de Souza TS, da Silva SM, Whitford GM, Buzalaf MA. Fluoride concentrations in dental plaque and saliva after the use of a fluoride dentifrice preceded by a calcium lactate rinse. *Eur J Oral Sci.* 2006; 114:489–493. [PubMed: 17184230]
- Rølla G, Bowen WH. Concentration of fluoride in plaque - a possible mechanism. *Scand J Dent Res.* 1977; 85:149–151. [PubMed: 265087]
- Rølla G, Saxegaard E. Critical evaluation of the composition and use of topical fluorides with emphasis on the role of calcium fluoride in caries inhibition. *J Dent Res.* 1990; 69 (Spec Iss):780–785. [PubMed: 2179341]
- Rose RK, Shellis RP, Lee AR. The role of cation bridging in microbial fluoride binding. *Caries Res.* 1996; 30:458–464. [PubMed: 8946096]
- Schaeken MJM, van der Hoeven JS. Influence of calcium lactate rinses on calculus formation in adults. *Caries Res.* 1990; 24:376–378. [PubMed: 2261611]
- Schaeken MJM, van der Hoeven JS. Control of calculus formation by a dentifrice containing calcium lactate. *Caries Res.* 1993; 27:277–279. [PubMed: 8402801]
- Ten Cate JM. In vitro studies on the effects of fluoride on de- and remineralization. *J Dent Res.* 1990; 69(Spec Iss):614–619. [PubMed: 2179322]
- Vogel GL, Chow LC, Brown WE. A microanalytical procedure for the determination of calcium, phosphate and fluoride in enamel biopsies. *Caries Res.* 1983; 17:23–31. [PubMed: 6571804]
- Vogel GL, Carey CM, Chow LC, Ekstrand J. Fluoride Analysis in Nanoliter- and Microliter-size Fluid Samples. *J Dent Res.* 1990; 69(Spec Iss):522–528. [PubMed: 2312891]
- Vogel GL, Mao Y, Chow LC, Proskin HM. Fluoride in plaque fluid, plaque, and saliva measured for 2 hours after a sodium fluoride monofluorophosphate rinse. *Caries Res.* 2000; 34:404–411. [PubMed: 11014907]
- Vogel GL, Zhang Z, Carey CM, Ly A, Chow LC, Proskin HM. Composition of plaque and saliva following use of an α -tricalcium phosphate-containing chewing gum and a subsequent sucrose challenge. *J Dent Res.* 2000; 79:58–62. [PubMed: 10690661]
- Vogel GL, Zhang Z, Chow LC, Schumacher GE. Effect of a water rinse on 'labile' fluoride and other ions in plaque and saliva before and after conventional and experimental fluoride rinses. *Caries Res.* 2001; 35:116–124. [PubMed: 11275671]

- Vogel GL, Zhang Z, Chow LC, Schumacher GE. Changes in lactate and other ions in plaque and saliva after a fluoride rinse and subsequent sucrose administration. *Caries Res.* 2002; 36:44–52. [PubMed: 11961330]
- Vogel GL, Chow LC, Carey CM, Schumacher GE, Takagi S. Effect of a calcium prerinse on salivary fluoride after a 228-ppm fluoride rinse. *Caries Res.* 2006a; 40:178–180. [PubMed: 16508278]
- Vogel GL, Shim D, Schumacher GE, Carey CM, Chow LC, Takagi S. Salivary fluoride from fluoride dentifrices or rinses after use of a calcium pre-rinse or calcium dentifrice. *Caries Res.* 2006b; 40:449–454. [PubMed: 16946616]
- Tatevossian A. Fluoride in dental plaque and its effects. *J Dent Res.* 1990; 69(Spec iss):645–652. [PubMed: 2179325]
- Tenuta LM, Del Bel Cury AA, Bortolin MC, Vogel GL, Cury JA. Ca, Pi, and F in the fluid of biofilm formed under sucrose. *J Dent Res.* 2006; 85:834–838. [PubMed: 16931867]
- Whitford GM, Buzalaf MAR, Bijella MFB, Waller JL. Plaque fluoride concentrations in a community without water fluoridation: Effects of calcium and use of a fluoride or placebo dentifrice. *Caries Res.* 2005; 39:100–107. [PubMed: 15741721]
- Whitford GM, Roehl B, Adair SM. Intraoral [F] (2007): Effect of Calcium Rinse before a Fluoride Rinse. *J Dent Res (Spec Iss A)*. Abstract 1873.
- Zero DT, Raubertas RF, Fu J, Pedersen AM, Hayes AL, Featherstone JDB. Fluoride concentrations in plaque, whole saliva, and ductal saliva after application of home-use topical fluorides. *J Dent Res.* 1992; 71:1768–1775. [PubMed: 1401438]

Fig. 1.

Mean total Ca (ppm) and standard error (error bars) for the two plaque samples obtained from each subject 1h after one of the treatment regimens shown. Only the Ca rinse group (capital letter) had a significantly increased total Ca ($P < 0.05$, repeated measure ANOVA, Newman-Keuls multiple comparisons test). The total plaque calcium values from one subject were all extreme outliers (Grubbs and Beck, 1972) and were omitted. The values were no rinse = 8,387 ppm, Ca rinse = 6,255 ppm, NaF rinse = 10,143 ppm and Ca rinse/NaF rinse = 9,869 ppm. The numbers on the figure are the number of subjects.

F (ppm) in 5 sequential extraction of plaque by a 6.8 or 4.8 buffer obtained 1 h after one of the treatment regimens shown. The acid value is the amount of F remaining in the sample after the sequential extractions. The total F is the total of all the F extracted from the plaque sample. The average total F is the average of the pH 6.8 and pH 4.8 values (see text). Data is given as the geometric mean of the values, the standard deviation factor (SDF), and the number of samples. The same capital letters indicate groups that do not differ statistically ($P < 0.05$, repeated measure ANOVA using the log transformed values, Newman-Keuls multiple comparisons test). Note that use of the geometric means is the reason that the total F is not equal to the sum of the extractions + the acid F

Table 1

Exaction	Extract Vol. ul/mg	pH 6.8 extraction									
		No Rinse		Ca Rinse		NaF Rinse		Ca rinse/NaF rinse			
1	15	0.7*	2.0	11	0.8*	1.4	12	2.5B	1.4	12	40.3A
2	45	1.0	2.0	11	1.2	1.7	13	3.8B	2.0	13	76.7A
3	45	0.9	1.7	11	0.9	1.7	13	2.9B	1.9	13	50.A
4	45	0.7	1.8	11	0.9	2.0	13	2.7B	1.8	13	44.4A
5	45	0.7	2.0	11	0.7	2.1	13	2.1B	2.4	13	36.7A
acid F	45	2.5	1.5	11	1.7	2.6	13	11.7	5.2	13	173.4A
total F**		6.7	1.6	11	7.4	1.4	13	32.7B	2.5	13	466.A
pH 4.8 extraction											
1	15	1.2	1.6	11	1.4	2.1	13	9.8B	2.1	12	76.9A
2	45	1.5	3.0	11	3.0C	1.8	13	12.4B	3.5	12	125.0A
3	45	1.0	3.0	11	1.5	2.7	13	4.8B	4.9	12	78.3A
4	45	0.4*	1.9	10	0.7	2.3	13	1.9B	4.6	12	46.0A
5	45	0.4	2.5	11	0.5	1.3	13	0.9	2.6	12	26.7A
acid F	45	0.7	2.0	11	0.4	2.1	13	0.7	2.8	12	20.5A
total F**		6.9	1.7	11	8.0	1.5	13	37.8B	2.2	13	445.0A
average total F		6.6	2.0	11	8.5	1.7	13	40.B	2.3	12	408.3A

* Three extreme values from the sequential extractions were outliers by the criteria of Grubbs and Beck (Grubbs and Beck, 1972) and were omitted. These are: pH 6.8, extraction 1 No Rinse = 7.5, and extraction 1 Ca rinse 3.7 ppm. pH 4.8 extraction 4 No Rinse = 11.2 ppm.

** The total F from the pH 6.8 and pH 4.8 extractions did not differ statistically ($p < 0.05$ paired difference test)

Table 2

Mean Plaque fluid F ($\mu\text{mol/L}$) for the two plaque samples obtained from each subject 1 h after one of the treatment regimens shown. The fluoride data is given as the geometric means (Geo. Mean), standard deviation factor (SDF), and 95% confidence interval (CI). Values with the same capital letter indicate groups that do not differ statistically ($P < 0.05$, ANOVA using the log transformed values, Newman-Keuls multiple comparisons test).

description	No Rinse	Ca rinse *	NaF Rinse	Ca rinse/NaF rinse
Geo. Mean	9.1	8.8	28.2 ^B	144.6 ^C
N	11	12	12	13
SDF	1.30	1.41	1.45	1.99
CI	10.8, 7.6	10.9, 7.1	36, 22	219, 96

* one value (39.7 ppm) was an outlier (Grubbs and Beck, 1972) and was omitted. The statistical results were not changed by this omission.