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Effect of a Low-Fluoride-Content, Two-Component Rinse on Fluoride Uptake and on De- and Remineralization of Enamel Lesions: An in vitro Study

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Key Words

Fluoride rinse · Fluoride · Enamel · Mineralization · Calcium fluoride · Sodiumhexafluorosilicate · In vitro pH cyclic model

Abstract

The effect of calcium (Ca) concentrations and added ethanol on fluoride (F) depositions by experimental two-component rinses, each consisting of a Ca-containing and an F-containing component, was evaluated in an in vitro system. Among the tested rinses, a 3 mmol/l F two-component rinse with 200 mmol/l Ca and 10% v/v ethanol was found to produce the greatest F deposition relative to the F concentration in the rinse. Specifically, this rinse produced an F deposition that was about 7 times greater than a conventional 13.2-mmol/l sodium fluoride (NaF) rinse. In a second experiment, an in vitro pH cycling model was used to evaluate the potential anti-caries effects of 4 rinses: (1) placebo rinse (no F), (2) 13.2-mmol/l NaF rinse, (3) 52.6-mmol/l NaF rinse, and (4) the 3-mmol/l F two-component rinse in a 7-day in vitro pH cycling model. The changes in lesion mineral contents, delta(ΔZ), as assessed by quantitative microradiographic measurements, were as follows [mean ± standard deviation, n = 10]: (1) 72.5±10.2 μm, (2) 43.4±5.6 μm, (3) 17.3±10.2 μm and (4) 45.3±5.2 μm. These results showed that the 3-mmol/l F two-component rinse produced the same ($p>0.05$) protection against demineralization as did the 13.2-mmol/l NaF rinse which had 4 times the fluoride content. The results suggest that it is possible to formulate an effective low-F two-component rinse.

Introduction

The release of fluoride (F) from labile stores of loosely bound F increases the mineral saturation of oral fluid, and can promote the repair of lesions and reduce demineralization during periods of cariogenic attack [ten Cate and Duijsters, 1983; Wefel and Harless, 1984; Hoppenbrouwers et al., 1987; Feagin and Graves, 1988; Margolis and Moreno, 1990; White et al., 1994]. The precipitation of calcium fluoride (CaF_2; or 'calcium fluoride-like' deposits) has been suggested as a major source of this loosely bound F after a NaF rinse [Rølla, 1988; Lagerlof et al., 1988; Rølla and Saxegaard, 1990]. A two-component F rinse consisting of an F source (part A) and a calcium (Ca) source (part B) has been shown in both in vitro and in vivo studies to deposit significantly more loosely bound F on enamel surface and in saliva, plaque and plaque fluid than a sodium fluoride (NaF) rinse with the same F concentration [Chow and Takagi, 1991; Vogel et al., 1992; Vogel et al., 1997]. The 12-mmol/l F two-component rinse was also found to be significantly more effective than the NaF rinse in remineralizing enamel and root lesions in both in vitro and in situ studies

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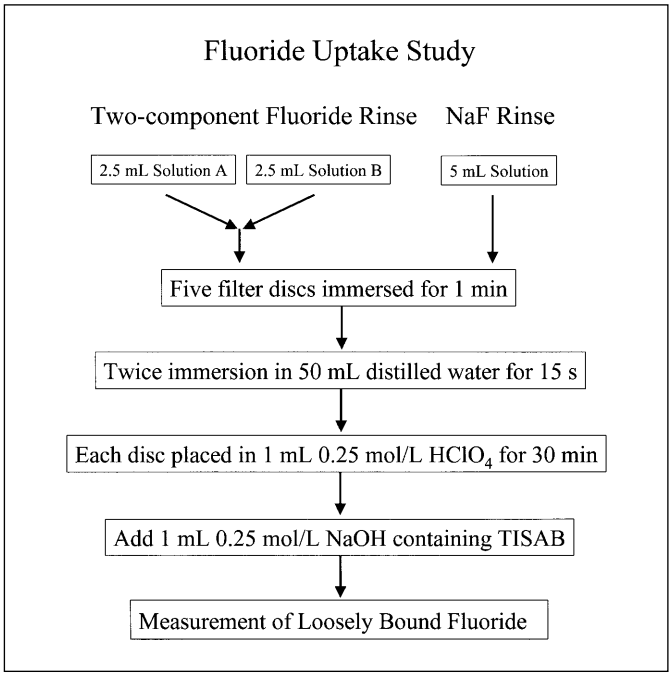

Fig. 1. Experimental design for an F uptake study.

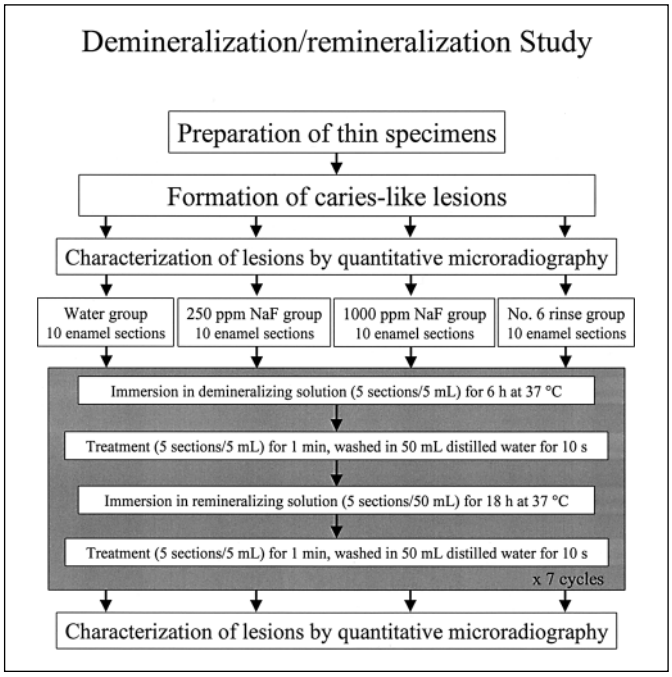

Fig. 2. Experimental design for the de/remineralization study.

[Chow et al., 1992; Takagi et al., 1997; Chow et al., 2000]. X-ray diffraction analyses, described later in the text, and plaque extraction studies [Vogel et al., 2000] strongly suggest that CaF_2 is indeed the in vitro and in vivo product of the two-component rinse. The greater deposition of CaF_2 by this rinse has been attributed to two factors. Firstly, a high concentration of Ca in part B of the rinse that compensates the need, in a NaF rinse, to scavenge this ion from the oral environment. Secondly, as confirmed by turbidity studies [unpubl. work], when the two components of the rinse are mixed and then placed in the mouth, CaF_2 precipitation is delayed for several seconds and once started, continues for several minutes. The slow formation of this mineral, which is governed by the rate of hydrolysis of Na_2SiF_6 in part A of the rinse, permits penetration of the rinse components into dental soft and hard tissue, and in dental plaque, before precipitating as CaF_2. With a conventional NaF rinse, the formation of CaF_2 appears to be primarily at or on the surface of these substrates where this mineral is rapidly lost.

Recent concerns about the prevalence of dental fluorosis [Pendrys and Stamm, 1990; Horowitz, 1992; Levy, 1993] suggest the need to develop low F rinses and dentifrices; however, it is important that such formulations maintain a high level of anticaries activity [Winter et al., 1989; Holt et al., 1994]. Low-F two-component rinses and perhaps dentifrices can be devised to address these concerns. Since CaF_2 precipitation is driven by both the F^- and Ca^{2+} ion activities, F deposition by the two-component rinse at an F concentration determined by the amount of Na_2SiF_6 can, in theory, be increased by increasing the calcium concentration. Furthermore, CaF_2 is very insoluble in ethanol solution, so an addition of ethanol to this rinse would be expected to further increase the rate of deposition of CaF_2. Thus, the first part of this study was to determine a combination of Ca, F and ethanol, which delivered a relatively high concentration of labile F at a low Na_2SiF_6 dosage, i.e. an 'efficient' two-component rinse made from a combination of these ingredients. The second aim of this study was to assess the remineralization potential of the two-component rinse found to be most 'efficient' in the first part of this study.

Materials and Methods

This study consisted of both F uptake and de/remineralization experiments. The experimental procedures for the two experiments are schematically outlined in figures 1 and 2, respectively.

F Uptake Study

F Rinse Solutions. Reagent grade chemicals were used to prepare the F rinse solutions. The NaF rinse contained 13.2 mmol/l NaF,

Table 1. Mean amounts of F deposited on filter disc surfaces produced by 1-min application of various rinse formulations

F rinse type	Solution A F, mmol/l	Solution B[a] Ca, mmol/l	A + B[b] F, mmol/l	A + B Ca, mmol/l	F deposition μg/cm²	F deposition % of rinse 1	Efficiency (×10²)
One-component rinse							
NaF	13.2	–	13.2	–	0.21±0.08[d,*]	6	1.6
Two-component rinse							
1	24	20	12	10	3.48±0.5	100	29
2	12	20	6	10	2.03±0.13	58	33.8
3	12	100	6	50	2.55±0.18	73	42.5
4	6	20	3	10	0.36±0.12[*]	10	12
5	6	400	3	200	1.05±0.21	30	35
6[c]	6	400	3	200	1.48±0.13	43	49.3

[*] $p < 0.05$, statistically similar groups (Newman-Keuls)
[a] Solution B also contained 50 mmol/l sodium acetate.
[b] Mixed solution of equal parts of A and B.
[c] Solution A contained 20% v/v ethanol.
[d] ± denotes standard uncertainty (n = 5).

equivalent of 250 μg of F per ml of solution (approximately equal to 250 ppm F in conventional terms). Two-component F rinses 1 through 6 were prepared with various F and Ca concentrations (table 1). Solution A of rinse 6 contained a volume fraction of 20% ethanol, which by increasing CaF_2 saturation, should enhance the CaF_2 deposition.

F Rinse Procedure. A filter disc (Millipore, Bedford, Mass., USA), which is relatively inert with respect to fluoride, with a diameter of 8.2 mm (a total surface area of ≈1 cm²) was used as a substrate for F uptake. Five filter discs were immersed for 1 min in 5 ml of the NaF rinse or the mixed two-component rinse (2.5 ml of A and B mixed just before immersion). The filters were washed twice by immersion for 15 s in 50 ml of rapidly stirred distilled water to remove the residual F rinse solution, blotted dry with tissue paper, and placed in a plastic test tube containing 1 ml of 0.25 mol/l $HClO_4$ for 30 min. Then, 1 ml of TISAB containing 0.25 mol/l NaOH was added to this plastic test tube, and the solutions were thoroughly mixed. An F ion-selective electrode and a reference electrode (both from Orion Research, Cambridge, Mass., USA) that had been calibrated in F standards were placed in this solution, and the F concentration was measured [Vogel et al., 1983]. In order to identify the products formed by the various two-solution rinses, precipitates were collected by filtration of rinses 1, 3 and 6, and were characterized by powder X-ray diffraction (XRD: Rigaku DMAX 2200: Rigaku/USA, Mass., USA). Approximately 10 mg of the sample was placed in an aluminum sample holder for the XRD analysis.

De/Remineralization Study

F Rinse Solutions. Based on the results from the above F uptake experiment, rinse 6 containing 3 mmol/l F, 10% v/v ethanol, 200 mmol/l $CaCl_2$, and 25 mmol/l sodium acetate in the mixed A + B solution was selected for testing in the de/remineralization study. This rinse was found to have the highest 'F efficiency', defined in this study as amounts of F deposited (μg/cm²) divided by F concentration (mmol/l) in a rinse. In accordance with the recommendation by the Consensus Conference on Intra-Oral Models [Proskin et al., 1992], 3 other rinses, 0 mmol/l F, 13.2 mmol/l NaF and 52.6 mmol/l NaF, were also used in the pH cycling de/remineralization study as controls to validate the methods.

Demineralizing Solutions. The demineralizing solution (DS) 1 (pH 4) contained 100 mmol/l lactate, 3 mmol/l $CaCl_2$, 1.8 mmol/l KH_2PO_4, and 1% w/w carboxymethylcellulose [Teranaka and Koulourides, 1987]. DS1 was used to produce the initial caries-like lesions in enamel. DS2 was a pH 4.3 solution that contained 2.0 mmol/l $CaCl_2$, 2.0 mmol/l KH_2PO_4, and 75 mmol/l acetate [White and Featherstone, 1987]. DS2 was used in the cyclic de/remineralization model.

Remineralizing Solution. The remineralizing solution (RS) used in the cyclic de/remineralization regimen contained 1.2 mmol/l $CaCl_2$, 0.72 mmol/l KH_2PO_4, 30 mmol/l KCl, and 50 mmol/l HEPES buffer (Sigma Chemical Co., St. Louis, Mo., USA). The pH of RS was adjusted to 7.0 with KOH. The solution was supersaturated with respect to fluroapatite (FAp) and hydroxyapatite (OHAp), but undersaturated with respect to other calcium phosphate compounds and calcium fluoride (CaF_2). Thus, FAp and OHAp may be expected to precipitate on the tooth surface or in the lesion from the RS while any CaF_2 deposited by the rinse applications should dissolve.

Preparation of Tooth Specimens. Lingual or buccal surfaces of enamel slabs from defect-free human premolars and molars, extracted for orthodontic reasons, were sectioned longitudinally with a diamond blade (Isomet; Buehler Ltd., Lake Bluff, Ill., USA). A single-section method [Mellberg et al., 1992] that allowed the same lesion to be assessed before and after the treatment regimen, thus lowering the variances between samples, was used with two modifications: (1) a Nickel TEM grid (Ted Pella Inc., Redding, Calif., USA) was used as a marker (fig. 3), and (2) 120 μm-thick enamel-sections were cast in a polyester resin casting compound (Castin Graft casting resin #00175; E.T.I. Co., Fields Landing, Calif., USA). The cast enamel surface of the tooth specimen was exposed by grinding off the excess resin parallel to, and no closer than, 300 μm from the grid.

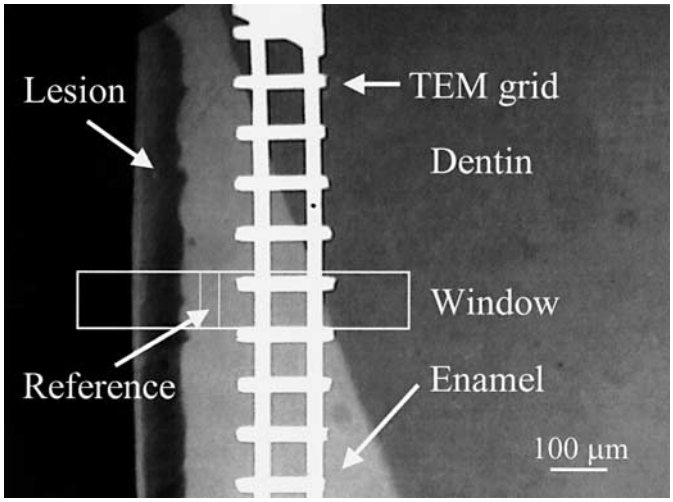

Fig. 3. Microradiograph of an enamel thin-section specimen showing the radio-opaque marker.

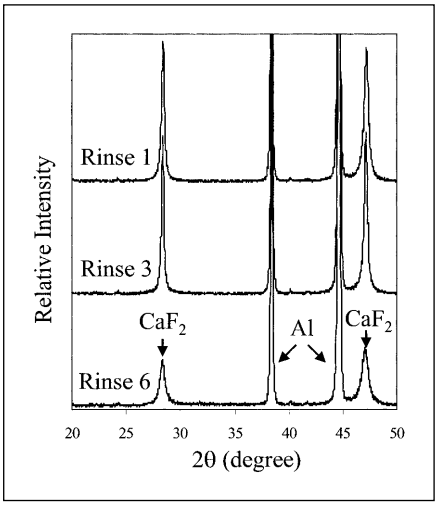

Fig. 4. XRD patterns of products formed from rinses 1, 3 and 6. The CaF_2 peaks and aluminum (Al) peaks from a sample holder are marked with CaF_2 and Al, respectively.

pH-Cycling Experimental Design (fig. 2). Each resin-cast enamel section was placed in 1 ml of DS1 for 1 day at 37 °C to produce caries-like lesions. Forty sections were randomly assigned to the four study groups: the water rinse, the 13.2-mmol/l NaF rinse, 52.6-mmol/l NaF rinse, and the test rinse 6. The daily treatment regimen included immersion of the samples in each group in 10 mL of DS2 for 6 h, 1-min treatment with 10 mL of the test rinse, immersion in 100 mL of RS for 16 h, and 1-min treatment with 10 mL of the test rinse. After each F rinse treatment, the samples were washed for 10 s in 100 mL of rapidly stirred distilled water before returning to either DS2 or RS. The daily regimen was performed for 7 days. Contact microradiographs of all specimens were produced for measurement of mineral contents of the lesions before and after the treatment regimens. This experimental regimen may be regarded as a demineralization model, since preliminary experiments demonstrated this regimen produced a net demineralization of the sections in the no-F control group.

Quantitative Assessment of Mineral Content. The mineral contents of the partially demineralized enamel in the lesions were measured by quantitative microradiography as described previously [Chow et al., 1991; 1992]. Results from a previous study [Chow et al., 1991] showed that the standard mineral content errors determined by using this procedure were in the range of 2.9 to 4.6% relative to the mineral content of the sound portion of the sample.

Calculation of Delta(ΔZ). The mineral losses (ΔZ) in the unit of thickness (μm) for each specimen before and after the treatment regimen were calculated from the relative mineral content profiles as previously described [Chow et al., 1991; 1992; Takagi et al., 1997]. For each window, the difference in mineral loss before and after treatment (i.e. delta(ΔZ) was calculated as the difference between the ΔZ measured after the treatment regiment and the ΔZ measured before the treatment regiment. ANOVA tests and Newman-Keuls multiple comparisons were performed on the ΔZ and delta(ΔZ) values to determine whether there were significant differences among any groups before or after the treatment regimens.

Results

F Uptake Study

The amounts of F deposition on the filter disc surfaces by the various rinse solutions are given in table 1. The mean F depositions of the 13.2-mmol/l NaF rinse and rinse 1 were 0.21 and 3.48 μg/cm^2, respectively. Rinses 2 and 3 with an F content of 6 mmol/l deposited the mean F amounts of 2.03 and 2.55 μg/cm^2, respectively. Even when the F content was reduced to 3 mmol/l for rinses 4, 5 and 6, they produced mean F depositions of 0.36, 1.05 and 1.48 μg/cm^2, respectively. Statistical analysis of the results showed that F deposition by all the two-component rinses was significantly greater than by the 13.2 mmol/l NaF ($p < 0.05$) except for rinse 4. The 'F efficiency' calculated as amounts of F deposition (μg/cm^2) divided by F concentration (mmol/l) in rinse (table 1) showed that rinse 6 appeared to have the highest 'F efficiency': it produced an approximately 7 times greater F deposition than did the NaF (13.2-mmol/l) with a F concentration of only 3 mmol/l, resulting in a 30 times greater efficiency than that of the 13.2 mmol/l NaF rinse. It was because of this high efficiency that rinse 6 was chosen for use in the de/remineralization study. XRD results (fig. 4) showed that precipitates formed from rinses 1, 3 and 6 were CaF_2.

De/Remineralization Study

Results of the Newman-Keuls multiple comparison tests, given in table 2, showed that there were no significant ($p > 0.05$) differences in ΔZ_b values for all the groups be-

Table 2. Mean mineral loss ΔZ before (ΔZ_b) and after (ΔZ_a) treatment and difference in mineral loss (delta(ΔZ)) in the four study groups

Group	Before ΔZ_b, μm	After ΔZ_a, μm	Paired difference delta(ΔZ), μm
Control	77.7±14.6*	150.1±19.3	72.5±10.2
13.2-mmol/l F NaF rinse	68.0±24.9	111.4±25.0	43.4±5.6
3-mmol/l F two-component rinse 6	64.2±17.8	109.4±16.3	45.3±5.2
52.6-mmol/l F NaF rinse	77.6±12.4	95.0±9.8	17.3±10.2

The net mineral loss in delta (ΔZ) indicates overall demineralization.

* $p > 0.05$, the means of any two groups connected by a vertical line are not significantly different. All values are means ± SD (n = 10)

fore the pH cyclic treatment. The ΔZ_a value of the control group after de/remineralization regimen was significantly greater ($p < 0.05$) than those of the F rinse groups (higher numbers in table 2 denote greater demineralization and lower numbers less demineralization). Statistical analysis showed that the delta(ΔZ) values for the 13.2-mmol/l NaF rinse and rinse 6 were not significantly different ($p > 0.05$). In accordance with the net demineralization observed in this experimental model, they were significantly larger ($p < 0.05$) than that of the 52.6-mmol/l NaF rinse and smaller than that of the control group.

Discussion

Table 1 showed that when the F concentration of the two-component rinse was reduced from 12 to 6 mmol/l, F deposition by rinse 2 was decreased to 58% of that of the rinse 1. However, this F deposition was increased to 73% of that of the rinse 1 when the Ca concentration (A + B) was increased from 10 mmol/l in rinse 2 to 50 mmol/l in rinse 3. Further reduction of F concentration to 3 mmol/l in rinse 4 greatly reduced the F deposition to 10% of that of the rinse 1, but again, the F deposition was increased to 30% of that of the rinse 1 by increasing the Ca concentration (A + B) to 200 mmol/l in rinse 5. These observations are in good agreement with what may be expected from thermodynamic solubility considerations, i.e. when the F concentration is reduced by a factor of 2, the Ca concentration needs to be increased by 4 in order to maintain the same level of saturation with respect to CaF_2. Further improvement of F deposition by rinse 5 was achieved to 43% of that of rinse 1 by adding ethanol in rinse 6. This observation was expected because ethanol has been known to increase the association constants of ionic compounds and to decrease their solubilities in aqueous solution, resulting in an increase in precipitation rate in the ethanol-containing solution [Tung and O'Farrell, 1993]. Thus, the reduction in F deposition caused by a decrease in the F concentration can largely be offset by both increasing the Ca concentration and adding ethanol, the latter being a common ingredient in mouthrinses. XRD patterns (fig. 4) showed that CaF_2 formed from rinse 6 appeared to be less crystalline than those formed from rinses 1 and 3. This may be a result of more rapid precipitation of CaF_2 due to ethanol.

The results from the de/remineralization study showed that the 3-mmol/l rinse 6 produced equal protection to the 13.2-mmol/l NaF rinse that contained almost 4 times more F. Since dental plaque was not present in this model, the remineralization effect of rinse 6 should be a result of the greater amount of CaF_2 deposited directly on teeth and in the lesions.

There is increasing evidence that the F ingestion from rinses, dentifrices and other F agents may contribute to an increase in dental fluorosis [Pendrys and Stamm, 1990; Horowitz, 1992; Levy, 1993]. It is therefore desirable to have F agents that deliver a high concentration of labile F and therefore a high level of caries protection at a lower F dosage. This rinse provided a degree of demineralization protection equal to a 13.2-mmol/l NaF rinse; a level of F at which some clinical studies have indicated a high level of anticaries protection [Koch et al., 1982]. Furthermore, 13.2-mmol/l F is near the effective F concentration seen in saliva 30 s after use of a conventional 57.9-mmol/l F dentifrice [Bruun et al., 1984]. More importantly, the extrapolation to the oral environment may be complicated by the effects of plaque and saliva; however, the findings presented here suggest that two-component rinses and dentifrices that produce higher F deposition at lower F doses can be created.

Acknowledgment

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