Salivary Fluoride from Fluoride Dentifrices or Rinses after Use of a Calcium Pre-Rinse or Calcium Dentifrice

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Calcium · Dentifrice · Fluoride · Rinse · Saliva

Abstract
The low concentration of available calcium (Ca) in oral fluids limits the formation of Ca-mediated fluoride deposits that maintain oral fluoride (F) after a topical F treatment. The purpose of this study was to examine if a high concentration of Ca would increase salivary F when used before a F rinse or dentifrice. We found that a Ca pre-rinse (150 mmol/l Ca lactate) or Ca dentifrice (0.084 g Ca glycerolphosphate per gram dentifrice) used immediately before a 60 s 228-ppm F rinse (12 mmol/l NaF) produced a 4.6× or 3.6× increase (p < 0.05) respectively in the 1 h salivary F concentrations over the F rinse alone. Reducing the post-Ca F rinse to 10 s still produced a significant 2.2× increase in salivary F compared to the 60 s F rinse alone. Used with a conventional 1,100 ppm F (i.e. 1,100 µg F per gram) NaF dentifrice (Crest), the above Ca pre-rinse increased 1 h salivary F levels by 2.3× over the F dentifrice alone. However, a F rinse given before a Ca rinse produced no increase in 1 h salivary F concentrations. Although the persistence of these increases requires further study, these results suggest that a moderately high concentration of Ca given shortly before a F rinse or F dentifrice may increase the cariostatic effect of the F product.

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12–48 mmol/l, is many times greater than free oral calcium (Ca), which is about 1 mmol/l in saliva and plaque fluid [Vogel et al., 1998, 2000a]. The low concentration of Ca relative to the amount of applied F implies that Ca-F deposition is limited by the rate at which Ca can be scavenged from Ca reservoirs in enamel, plaque or tissue during the short period of F application. Thus the low level of oral free Ca (and other factors) precludes more than a fraction of the applied F from being deposited as a Ca-F reservoir in oral tissue. Recently a preliminary study with a small number of non-fasted subjects demonstrated that when a 150- or a 300-mmol/l Ca rinse was allowed to first penetrate oral tissue before the use of a sodium fluoride (NaF) rinse, a very large increase in the 1 h salivary F concentration was found compared to the NaF rinse alone.

The purpose of the study reported here was to further examine the effect of Ca pre-treatment on salivary F concentration. Thus we: (1) repeated the previous study with a larger group of subjects using the 150-mmol/l Ca lactate rinse that was found to produce the greatest increase in salivary F, (2) tested this Ca pre-rinse with a conventional NaF dentifrice, and (3) investigated the delivery of Ca in the form of a Ca-containing F-free dentifrice before a F rinse. Finally, we tested a ‘reverse application’ in which the NaF rinse was administered before the Ca rinse.

### Materials and Methods

#### Subject Protocol

Rinse administration and the collection of samples were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. The same 12 subjects were used in all experiments; however, as noted below, in two studies, 10 and 11 subjects participated. The subjects (3 females and 9 males) were recruited from the same pool as in the preliminary test of unstimulated saliva flow as described before participation to ensure good oral health, normal oral architecture, oral physiology and salivary gland function (demonstrated in a preliminary test of unstimulated saliva flow as described below). Subjects who had diabetes or who were using antihistamines were excluded from the study. All subjects lived in an area with fluoridated water and were instructed to use their normal oral hygiene procedures. The experiments were performed in the morning at least 2 h after food ingestion and 12 h since use of any dentifrice (or F rinse). For each subject, at least 1 week elapsed between any two rinse or dentifrice administrations. It should be noted that these administrations were not completely randomized since all four rinse administrations were performed before the four dentifrice administrations. However, within the rinse or dentifrice administrations the order of which the test materials were given was random. Because the same subject group participated in all experiments and the entire experimental period was only 10 weeks, we believe that the lack of complete randomization among all the rinse and dentifrice administrations would produce minimal statistical effects and that this factor did not materially affect the conclusions. Similarly, the analyst and subjects were only partially blinded: they were aware of which groups were being tested (rinses or dentifrices) but within these groups they were not informed of the identity of the test material or the recovered saliva sample.

### Rinse/Dentifrice Administration

In the rinse experiments the subjects were requested to thoroughly rinse for the required time with the experimental or water rinses. No special brushing instructions were given for the dentifrice experiments (other than to specify the brushing time). Nine studies were done. The protocols of these experiments are summarized in Table 1. The numbering used in the experiments was chosen to produce the simplest presentation of the data in the results table (Table 2). In experiment (Exp.) 1, baseline (no rinse) saliva samples were collected. In Exp. 2 the subjects rinsed 1 min with 20 ml of 150 mmol/l Ca lactate (Ca lactate pentahydrate, Sigma-Aldrich, St. Louis, Mo., USA). The subjects were screened before participation to ensure good oral health, normal oral architecture, oral physiology and salivary gland function (demonstrated in a preliminary test of unstimulated saliva flow as described below). Subjects who had diabetes or who were using antihistamines were excluded from the study. All subjects lived in an area with fluoridated water and were instructed to use their normal oral hygiene procedures. The experiments were performed in the morning at least 2 h after food ingestion and ≥12 h since use of any dentifrice (or F rinse). For each subject, at least 1 week elapsed between any two rinse or dentifrice administrations. It should be noted that these administrations were not completely randomized since all four rinse administrations were performed before the four dentifrice administrations. However, within the rinse or dentifrice administrations the order of which the test materials were given was random. Because the same subject group participated in all experiments and the entire experimental period was only 10 weeks, we believe that the lack of complete randomization among all the rinse and dentifrice administrations would produce minimal statistical effects and that this factor did not materially affect the conclusions. Similarly, the analyst and subjects were only partially blinded: they were aware of which groups were being tested (rinses or dentifrices) but within these groups they were not informed of the identity of the test material or the recovered saliva sample.

### Table 1. Summary of experiments. Where multiple treatments were given, they were administered in the order shown (treatment 1/treatment 2/treatment 3)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Number</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>no treatment</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>60 s 20 ml Ca rinse</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>60 s 20 ml F rinse</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>60 s 20 ml F rinse/60 s 20 ml Ca rinse</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>60 s 1.5 g F dentifrice/10 s 10 ml water rinse</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>60 s 20 ml Ca rinse/60 s 1.5 g F dentifrice/10 s 10 ml water rinse</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>60 s 1.5 g Ca dentifrice/10 s 10 ml F rinse</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>60 s 1.5 g Ca dentifrice/60 s 20 ml F rinse</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>60 s 20 ml Ca rinse/60 s 20 ml F rinse</td>
</tr>
</tbody>
</table>
Table 2. Geometric means (Geo. mean) of the salivary F concentrations (µmol/l), standard deviation factor (SDF), and 95% confidence interval (CI) 1 h after one of the treatment regimens shown

<table>
<thead>
<tr>
<th>Exp. Description</th>
<th>1 no treatment</th>
<th>2 Ca rinse</th>
<th>3 F rinse</th>
<th>4 F rinse/Ca rinse</th>
<th>5 F dentifrice/water rinse</th>
<th>6 Ca rinse/F dentifrice/water rinse</th>
<th>7 Ca dentifrice/short F rinse</th>
<th>8 Ca dentifrice/F rinse</th>
<th>9 Ca rinse/F rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geo. mean</td>
<td>1.1</td>
<td>2.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>13.7&lt;sup&gt;B&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;B&lt;/sup&gt;</td>
<td>10.8&lt;sup&gt;B&lt;/sup&gt;</td>
<td>25.0&lt;sup&gt;C&lt;/sup&gt;</td>
<td>29.8&lt;sup&gt;C&lt;/sup&gt;</td>
<td>49.1&lt;sup&gt;D&lt;/sup&gt;</td>
<td>62.8&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>SDF</td>
<td>2.5</td>
<td>1.0</td>
<td>1.6</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
<td>2.1</td>
<td>2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>CI</td>
<td>0.6, 2.0</td>
<td>1.5, 2.6</td>
<td>10, 19</td>
<td>6, 6, 10</td>
<td>8, 15</td>
<td>18, 35</td>
<td>19, 48</td>
<td>29, 82</td>
<td>48, 83</td>
</tr>
</tbody>
</table>

Values with the same capital letter indicate groups that do not differ statistically.

* One value (284 µmol/l) was an outlier [Grubbs and Beck, 1972] and was omitted. The statistical results were not changed by this omission.

**Results**

The salivary flow rates in the 1 h post-treatment saliva samples were not statistically different: the average value of all the studies was (0.98 ± 0.53) g/min. The 1 h post-treatment salivary F concentrations in Exps. 1–9 are presented in table 2. The Ca rinse alone (in Exp. 2) produced a small but significant increase in F, compared to the baseline samples (Exp. 1). When Ca was given before a F rinse or dentifrice (Exps. 6–9), it produced significant increases in the 1 h post-salivary F concentration compared to the corresponding methods of delivering NaF alone (Exps. 3 and 5). The maximum increase, 4.6× and 3.8× respectively, was found when Ca was administered as a rinse or Ca-containing dentifrice with a 1-min F rinse (Exps. 8 and 9). The Ca rinse/F dentifrice (Exp. 6) and Ca dentifrice/10 s F rinse (Exp. 7) produced lower 1 h salivary F concentrations but they were still 2.3× and 2.2× greater than the corresponding method of delivering NaF without Ca (Exps. 3 and 5). The Ca content of the expectorate after use of the Ca dentifrice in Exp. 7 was 94 ± 14 mmol/l, which was somewhat lower than the target value of 150 mM for a Ca rinse. Administering Ca subsequent to a F rinse (Exp. 4) appeared to decrease the
1 h salivary F concentrations relative to the NaF rinse alone (Exp. 3). The decrease, however, was not significant.

Discussion

The baseline salivary F concentrations (table 2) were similar [Vogel et al., 2002] or slightly lower than reported in similar experiments [Vogel et al., 2000b, 1992, 2001]. The 1 h post-NaF rinse salivary F concentrations (Exp. 3) are also similar to the values found in these previous experiments. The 1 h post-NaF dentifrice values, which are not significantly different from the NaF rinse values, are somewhat higher than those found in other studies [Seppä et al., 1997; Zero et al., 1992a] in which a similar dentifrice and similar post-dentifrice water rinse protocol were used.

As noted in the Introduction, the low concentration of free Ca in oral fluid relative to the amount of applied F limits Ca-F deposition from a F dentifrice or F rinse. Several methods have been employed to supply additional Ca with F during these types of therapy. However, such procedures can be complicated by the rapid precipitation of CaF$_2$ particles that do not appear to adhere to tooth surfaces [Chow et al., 1992]. Chow and co-workers have pioneered a number of approaches to control CaF$_2$ formation during simultaneous application of Ca and F, most particularly an extensively tested method in which Na$_2$SiF$_6$ is mixed with Ca chloride immediately before use [Chow et al., 1992, 2000, 2002; Vogel et al., 1992, 2001]. In this study we have adopted a somewhat simpler approach: the penetration of the pores of oral tissue and plaque by high levels of Ca to create a high driving force for the formation of bioavailable Ca-F moieties when the F treatment is subsequently applied. Although the disadvantages of such a two-step procedure are apparent, the increase in 1 h salivary F concentration in the Ca pre-rinse/F rinse study in Exp. 9 (table 2) was nearly 4.6 x compared to a conventional NaF rinse. It should be noted, however, that this increase was only about half that found in a preliminary study [Vogel et al., 2004], perhaps due to the use of a subject panel who were allowed to eat up to 1 h before rinse administration in the previous study. Although the location of the oral Ca-F deposits produced by this procedure is not established, several authors have suggested that, due to its large surface area, oral soft tissue is the major source of F available for release into saliva [Zero et al., 1992b; Jacobson et al., 1992].

Evidence that the increase in 1 h salivary F is due to the reaction of F with elevated free Ca in the oral tissue, induced by the Ca pre-rinse, can be seen by noting that no increase in salivary F occurs when the order of administration is reversed in Exp. 4 (F before Ca). In fact in Exp. 4 there is a trend toward lower salivary F levels which might be due to the diffusion of the applied F from the tissue or plaque F into the fluid phase of the Ca rinse – an effect, as described below, which is well known with respect to dentifrices.

When the Ca was delivered as a dentifrice before the 1-min NaF rinse (table 2, Exp. 8), the 1 h salivary F appears to be somewhat reduced compared to that obtained with the 150-mmol/l Ca rinse/F rinse in Exp. 9. This trend may have been due to the 37% lower concentration of Ca found in the saliva/dentifrice expectorate compared to the 150-mmol/l Ca rinse. However, a second study (7 subjects) using a high concentration Ca glycerophosphate dentifrice (0.3 g/g) that produced a Ca concentration in the expectorate of 175 ± 40 mmol/l, increased F levels by only 20%. This suggests that, perhaps due to the formation of Ca compounds or complexes from the sodium lauryl sulfate (SLS) component of the Ca-containing dentifrice, the 1 h salivary F concentration is insensitive to moderate changes in the Ca content of the dentifrice.

Surprisingly, decreasing the post-Ca dentifrice F rinse time to only 10 s (Exp. 7) only decreased the 1 h salivary F concentration by about 39%. The use of a smaller volume (10 vs. 20 ml) for the NaF rinse in this experiment probably had a minimal effect on the observed F concentration since only a fraction of the applied F is orally deposited from the rinse during application and 10 ml should be enough to ensure complete oral coverage.

In accordance with studies on commercial rinses and dentifrices, the 1 h salivary F concentrations were reduced for the NaF dentifrice (Exp. 3) [Zero et al., 1988, 1992a] relative to the NaF rinse (Exp. 5). However, in the current study the difference was not significant. Several factors decrease the delivery of F from a dentifrice relative to a rinse, most importantly, water rinsing, which is customarily done after dentifrice application, has been shown to drastically reduce salivary F [Duckworth et al., 1991; Seppä et al., 1997; Sjögren and Birkhed, 1994]. Although the 1 h salivary F concentrations from the NaF rinse and dentifrice were similar (table 2), the Ca pre-rinse produced a much higher salivary F concentration when used before the F rinse (Exp. 9) than when used before the F dentifrice (Exp. 6). Given the very high concentration of salivary F found when the Ca pre-rinse or
Ca dentifrice was used before a F rinse (Exps. 8 and 9), this suggests that the post-F dentifrice water rinse had a highly deleterious effect on high levels on Ca-F moieties. Such an effect is not unanticipated given the high driving force for Ca-F dissolution under these circumstances. The formation of Ca complexes or compounds by component of the F dentifrice, for example SLS, may also be a factor in removing some of the Ca held in the tissue after the Ca rinse.

The Ca pre-rinse alone (Exp. 2) produced a small but statistically significant increase in the 1 h salivary F concentrations (table 2) relative to baseline levels (Exp. 1). Such results, and the very high levels of F found in Exps. 8 and 9, suggest that high concentrations of Ca in a pre-treatment could induce the formation of large amounts of bioavailable F reservoirs from rinses or dentifrices with a reduced F concentration. It is noteworthy in this regard that a 114-ppm Na2SiF6 type F rinse with an enhanced level of added Ca produced more remineralization in an intraoral model than a conventional 228-ppm F rinse [Chow et al., 2002]. Although a sequential rinse procedure has not been tested in this regard, the concentrated Ca pre-rinse has the potential advantage of reducing oral fluid phosphate through precipitation of Ca-PO4 minerals. Phosphate, which is very high in oral fluid [Vogel et al., 1998, 2000a], appears to induce the formation of non-bioavailable (insoluble) Ca-F-PO4 phases when high levels of Ca are used to enhance F deposition from F rinses more dilute than 114 ppm [Chow et al., 2002].

There are a number of limitations on the use of the sequential Ca-before-F procedure described here. Firstly, the Ca application should be done immediately before the F rinse or dentifrice. Secondly, low levels of Ca appear to be relatively ineffective [Vogel et al., 2004]. Thus, Whitford et al. [2005] found that a 20-mmol/l Ca pre-rinse in a 50-mmol/l acetate buffer (equivalent to 15 mmol/l of free Ca) had no effect on salivary F concentrations in samples collected 1 h after brushing with a F dentifrice. Whitford’s results may also partly reflect the reduction in salivary F levels noted above when the F source is a commercial dentifrice rather than an aqueous rinse. Thirdly, there appears to be also a limitation on the maximum Ca that can be used since Ca carryover into the subsequently applied F agent can lead to loss of F by precipitation of CaF2 in the applied phase [Vogel et al., 2004]. Finally, there is the possibility of increased calculus formation when high concentrations of Ca are frequently applied. However, Ca lactate, which was used in the F rinse studies, has been shown to actually reduce calculus in clinical studies [Schaeken and van der Hoeven, 1990, 1993]. With regard to Ca glycerolphosphate, which was used in the Ca dentifrice studies, this compound has been used in high concentrations in sodium monofluorophosphate dentifrices for many years. There are no published reports of this dentifrice formulation producing an increase in calculus formation.

The clinical implications of this study are unclear, especially since the persistence of the high post-treatment 1 h salivary F levels shown in table 2 has not been investigated. Furthermore, the F concentrations in phases that are more relevant to caries, such as tooth mineral, plaque, and especially plaque fluid, remain to be fully examined. However, promising preliminary plaque fluid data has recently been presented using a Ca pre-rinse/F rinse [Vogel et al., 2005]. In any case, the results presented here suggest that administration of a moderately high concentration of Ca immediately before application of topical fluorides may increase the cariostatic effect. There are of course disadvantages in a two-step procedure but it is noteworthy that the Ca-containing dentifrice used with a short (10 s) F rinse (table 2) produced a 2.2 × increase in salivary F compared to a 60 s F rinse and a 2.8 × increase with respect to the use of a F dentifrice. Such a procedure is entirely comparable to the post-F dentifrice water rinsing which most of the public currently perform.

Acknowledgements

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