Remineralization by Fluoride Enhanced with Calcium and Phosphate Ingredients

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Abstract

The effectiveness of fluoride ions provided by toothpastes and mouthrinses in promoting remineralization can be limited by the low concentrations of calcium and phosphate ions in saliva. The purpose of this study was to determine whether improved remineralization can be obtained from toothpastes or mouthrinses that simultaneously deliver fluoride, calcium, and phosphate ions from dual-dispensing systems. Enamel specimens with artificial lesions between 60 and 90 minutes deep were cycled 15 times through demineralization for 30 minutes, treated for 5 minutes with an experimental or control fluoride toothpaste or mouthrinse, and remineralized for 60 minutes. In the toothpaste study, surface hardness increased by 11.5 ± 9.2 and 2.7 ± 3.6 Vickers hardness units, and enamel fluoride content was 5984 ± 521 ppm and 3971 ± 531 ppm for the experimental and control fluoride toothpastes, respectively. Remineralization was confirmed by x-ray microanalysis. In the mouthrinse study, surface hardness increased by 8.8 ± 7.7 and 2.2 ± 3.7 Vickers hardness units, and enamel fluoride content was 6111 ± 1078 ppm and 3160 ± 364 ppm for the experimental and control fluoride mouthrinses, respectively. Use of a non-fluoride control mouthrinse led to a decrease in surface hardness of 3.7 ± 5.2 Vickers hardness units despite a fluoride content of 402 ppm. The results demonstrate that calcium and phosphate supplementation in a toothpaste or mouthrinse can improve remineralization and increase fluoride uptake. (J Clin Dent 10:13–16, 1999.)

Introduction

Two processes—demineralization and remineralization—control the progression and reversal of carious lesions in teeth. Demineralization occurs when acids, generated by the breakdown of sugars by acidogenic bacteria, reduce the pH of plaque. Reduction of pH causes the plaque fluid to become unsaturated with respect to calcium and phosphate ions. Under these conditions, calcium and phosphate will be leached from the tooth until saturation of the plaque fluid is restored. The critical pH below which plaque fluid becomes undersaturated and remineralization occurs varies depending on the ionic concentrations of calcium and phosphate in the plaque. Lesions will remineralize when the plaque pH increases as a result of the influx of salivary buffers, and the plaque fluid becomes supersaturated with respect to calcium and phosphate ions. Under these conditions, calcium and phosphate ions will have a tendency to precipitate within lesions until the concentration has been reduced to saturation. Demineralization and remineralization occur in the mouth at different times. Thus, the two processes provide a dynamic ebb and flow of calcium and phosphate ions from and into tooth enamel. The formation of a cavity will be prevented if the average amount of demineralization that occurs is equal to or exceeded by the average amount of remineralization.

Saliva provides a natural source of calcium and phosphate ions for remineralization. However, in the absence of fluoride, saliva is not a very effective remineralizing medium. Increasing the fluoride content of saliva has been correlated with increased rates of remineralization and decreased caries incidence. It has been shown that even trace concentrations of fluoride ions are effective in promoting calcium hydroxyapatite formation from supersaturated solutions of calcium and phosphate. For this reason, fluoride is added to toothpastes, mouthrinses, and drinking water as an anticaries agent. However, fluoride's ability to promote remineralization in the oral environment is limited by the availability of calcium in saliva.

The addition of soluble calcium and phosphate to a fluoride-containing toothpaste or mouthrinse might be expected to enhance the efficacy of fluoride-mediated remineralization. Indeed, it has been shown previously that the apparent rate of remineralization of enamel increases with the quantity of calcium and phosphate ions in solution. However, the mixture of calcium salts with fluoride or phosphate in a toothpaste or mouthrinse would normally result in the precipitation of insoluble calcium fluoride or calcium phosphate. Both of these compounds are inactive, and, therefore, such combinations generally have been unavailable in oral care formulations until now.

We report the results of a study to evaluate a toothpaste and mouthrinse designed to enhance fluoride remineralization by supplementation with calcium and phosphate ingredients. The remineralization systems prevent direct mixture of calcium with fluoride or phosphate in storage until the ingredients are applied to the tooth. Once applied and mixed, the supersaturated concentrations of calcium, fluoride, and phosphate ions are temporarily stabilized in solution until penetration into the pores of the dental enamel has occurred.
Materials and Methods

Dual-dispensing toothpaste and mouthrinse prototypes containing fluoride, calcium, and phosphate ions were prepared. Calcium ions were supplied in one portion of each product, while phosphate and fluoride ions were present in the second part of the formulation.

The complete experimental toothpaste contained 1130 ppm of fluoride as sodium fluoride, about 8400 ppm of calcium ions as calcium sulfate, and about 25,000 ppm of ammonium phosphate. The control toothpaste contained 1150 ppm of fluoride as sodium fluoride.

The complete experimental mouthrinse contained 230 ppm of fluoride as sodium fluoride, about 850 ppm of calcium ions as calcium lactate, and about 4500 ppm of ammonium phosphate. The control mouthrinse contained 230 ppm of fluoride as sodium fluoride. A placebo mouthrinse, which contained no fluoride, calcium, or phosphate, was used as an additional, non-fluoride control.

The ability of the toothpaste and mouthrinse formulations to remineralize artificial lesions in enamel teeth specimens was determined by cycling the specimens through an in vitro treatment, remineralization, and demineralization regimen. Circular sections of sound human enamel were obtained from maxillary incisors and mounted in plastic rods. Uniform surfaces were prepared by grinding and polishing to remove the outer fluoride-rich 100 μm. Artificial lesions were formed by immersing the specimens for 72 hours in a 0.1 M lactic acid/0.2% Carbopol C907 solution, 50% saturated with calcium hydroxyapatite at pH 5.0 and a temperature of 37°C. The lesions formed were 60 μm to 90 μm deep. The micro-hardness of the surface of each specimen was then determined. To be acceptable for use in the study, the surface hardness had to be 25 to 45 Vickers hardness units. The specimens were distributed among the test groups in a balanced manner. One half of the exposed surface of the specimen was coated with nail varnish. The coated side of the specimen served as an untreated control for x-ray microradiographic comparisons of remineralization. Eight specimens were used for each test group.

Immediately before use, 5 g of the phosphate-containing portion of the toothpaste was diluted and mixed with 20 mL of whole human saliva. The slurry was divided equally into two beakers and, with stirring discontinued, 2.5 g of the calcium-containing portion of the toothpaste was added to each half of the treatment mixture. The resulting concentration of toothpaste in the saliva was 1:2. The control fluoride toothpaste was also diluted one part toothpaste to two parts saliva and divided equally into two beakers. Stirring was resumed and four enamel specimens were quickly immersed in each beaker.

The mouthrinses were used undiluted. In two beakers, 7.5 g of the phosphate-containing portion of the mouthrinse were directly added to 7.5 g of the calcium-containing portion. Two beakers contained a control mouthrinse with fluoride, and two beakers contained a control mouthrinse without fluoride. Stirring was started, and four enamel specimens were quickly immersed in each beaker.

The demineralization phase of the cyclic regimen was performed by immersing the specimens in a 0.1 M lactic acid/ carbopol solution, 50% saturated with calcium hydroxyapatite at pH 5.0 for 30 minutes. Specimens then were treated with the toothpaste or mouthrinse formulation as outlined above for 5 minutes. Remineralization then was performed by immersing the specimens in pooled whole human saliva for 60 minutes. Five cycles were performed each day, and the specimens were stored overnight with a coating of saliva. A total of 15 demineralization/treatment/remineralization cycles were completed.

Results

Treatment with the experimental remineralization toothpaste or mouthwash increased the hardness of the tooth enamel about four times more than did treatment with the fluoride controls (Table I). Treatment with the non-fluoride control mouthrinse actually resulted in a loss of hardness. The differences in hardness between the experimental and control formulations were statistically significant (p < 0.05, Fisher least significant difference).

Table I
Change in Hardness of Tooth Enamel Before and After Treatment with a Fluoride-containing Toothpaste or Mouthrinse with Control Toothpaste or Mouthrinse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vickers Hardness Numbers (VHN)</th>
<th>Before</th>
<th>After</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental toothpaste</td>
<td>39.2 (4.7)*</td>
<td>50.7 (8.77)</td>
<td>11.5 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Control toothpaste</td>
<td>38.7 (5.2)</td>
<td>41.4 (5.0)</td>
<td>2.7 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Experimental mouthrinse</td>
<td>38.6 (5.2)</td>
<td>47.4 (10.2)</td>
<td>8.8 (7.7)</td>
<td></td>
</tr>
<tr>
<td>Control mouthrinse</td>
<td>38.1 (5.8)</td>
<td>46.3 (8.0)</td>
<td>2.2 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Placebo mouthrinse</td>
<td>37.7 (6.2)</td>
<td>34.0 (7.6)</td>
<td>-3.7 (5.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean (SD); n = 8.
Italicized values do not differ significantly (experimental treatment vs. controls; p > 0.05).

The fluoride content of specimens treated with the experimental toothpaste and mouthrinse formulations was about 50% and 100% greater than the fluoride content of the specimens treated with the control fluoride toothpaste and mouthrinse, respectively (Table II). These differences were also statistically significant (p < 0.001).

Table II
Fluoride Content of Tooth Enamel After Treatment with a Fluoride-containing Toothpaste or Mouthrinse with Control Toothpaste or Mouthrinse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>μgF/cm²</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental toothpaste</td>
<td>5964 (521)*</td>
<td></td>
</tr>
<tr>
<td>Control toothpaste</td>
<td>3971 (531)</td>
<td></td>
</tr>
<tr>
<td>Experimental mouthrinse</td>
<td>6111 (1078)</td>
<td></td>
</tr>
<tr>
<td>Control mouthrinse</td>
<td>3160 (364)</td>
<td></td>
</tr>
<tr>
<td>Placebo mouthrinse</td>
<td>402 (32)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean (SD); n = 8.
Differences between experimental and control treatments are significant (p < 0.001).

X-ray microradiography performed on the specimens treated with the remineralization toothpaste and the control fluoride.
Table III

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Delia Z* (μm)</th>
<th>Lesion Depth (μm)</th>
<th>Surface Volume % Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Experimental toothpaste</td>
<td>2670 (341)</td>
<td>2216 (261)</td>
<td>78.3** (6.1)</td>
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<td></td>
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<tr>
<td>Fluoride control toothpaste</td>
<td>2474 (282)</td>
<td>2180 (215)</td>
<td>80.0 (8.5)</td>
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</table>

*Mean (SD); n = 8.
**Indicated values do not differ significantly before and after treatment (p ≥ 0.05).

Toothpaste indicated that both formulations produced significant remineralization of the lesions (p < 0.05), as shown in Table III. The absence of mineral (ΔZ value) decreased by 17% with the experimental toothpaste and by 11.9% with the control fluoride toothpaste; these differences were not statistically significant. Lesion depth and surface volume % mineral were essentially unchanged by either treatment.

Discussion

The results show that the addition of calcium and phosphate to a fluoride toothpaste or mouthrinse has the potential to beneficially affect remineralization. Silverstone and colleagues previously reported that supplementation of calciy fluid with calcium ions at concentrations greater than those of saliva can actually reduce the degree of sub-surface enamel remineralization as a result of surface remineralization and blockage of surface pores. In that study, especially long remineralization periods consisting of ten consecutive 1-hour and 24-hour treatments were used. The results of our study may differ because the 5-minute treatment periods were much shorter, and the remineralization and treatment steps were separated by half-hour demineralization periods. The conditions of our study, therefore, might be expected to remove any blockage building up on the surface and allow remineralization to proceed in the subsurface.

Plaitz and Hicks also reported slightly greater lesion depth reductions with calcifying solutions containing lower calcium concentrations. They also noted the development of a much thicker remineralized surface zone in the enamel treated with the solutions containing higher concentrations of calcium. The presence of the thicker remineralized surface zone supports the theory that the blockage of surface pores can reduce remineralization of the body of a lesion. Although Plaitz and Hicks' remineralization periods of 1 minute were much shorter than those of Silverstone and colleagues, the remineralization periods were not cycled with demineralization periods as in our study. In our study, the surface volume % mineral of the lesions remained essentially unchanged after treatment with either the experimental or control fluoride formulations, indicating that blockage of surface pores is not significant with our regimen. While the exact cycle times in our study may not mimic the physiologic demineralization-remineralization process, we believe that the cyclic treatment regimen of our study better reflects the dynamic nature of the caries lesion progression and reversal process.

Ten Cate observed that partially demineralized crystallites must be present to act as a clean surface for mineral deposition for remineralization to occur. Thus, the demineralization phase is an important component of the remineralization process, and in moderation promotes subsurface remineralization. In the absence of demineralization, the evaluation of a product's remineralizing performance may not be relevant. Nevertheless, lesion progression ceases and the disease is at least dormant, if not completely absent.

Since the remineralization-to-demineralization time was quite low in our study, it might be argued that our model was weighted too heavily toward demineralization. However, a demineralizing pH of 5.0 is not very low. In fact, the pH can sometimes decrease not significantly below 4.5, a level at which the hydrogen ions in concentration is three times that of a pH 5 solution. Indeed, even the fluoride controls were able to remineralize the lesions. Since the purpose of supplementing fluoride toothpastes and mouthrinses with calcium and phosphate is to provide additional caries protection, it would be more relevant to evaluate these products under conditions that make a standard fluoride toothpaste unable to withstand a cariogenic challenge. It may be actually more valuable to conduct future studies under even more demanding conditions.

It is interesting to note that supplementation of calcium and phosphate ions also led to an increased enamel fluoride content. This was reported previously by Crall and Bjor, who demonstrated that pre-rinsing of tooth enamel with an acidified solution saturated with dicalcium phosphate dihydrate (pH 2.1) resulted in increased fluoride uptake from acidified phosphate-fluoride treatments.

Conclusion

Supplementation of salivary calcium and phosphate during treatment with a fluoride-containing product has the potential to promote remineralization efficacy in vitro. The clinical significance of this finding must be determined in human studies.

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References

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