Inhibition of caries lesion formation by an MFP dentifrice and APF solution

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Abstract

An alternating demineralization-remineralization protocol was used to determine if intermittent treatment with two types of fluoride preparations could inhibit the formation of artificial caries and if the protocol could discriminate differences in the effectiveness of treatments.

Blocks of enamel were demineralized overnight in an acid calcium phosphate solution and then treated with a Sodium-monofluorophosphate (MFP) dentifrice, an acidulated-phosphate-fluoride (APF) solution, or a water placebo. These treatments were followed by 6 hr of remineralization. This demineralization-treatment-remineralization cycle was repeated for 5 days.

Quantitative microradiography of thin sections prepared from the enamel blocks showed that the APF treatment reduced the severity of the lesions by 97% compared to placebo. The MFP dentifrice treatments for 2 hr, 15 min, or 5 min reduced the severity by 96%, 88%, and 70%, respectively, showing that the procedure is able to discriminate between differences in treatment intensity.

In a recent study, Dijkman et al. found little difference in fluoride (F) levels acquired by sound enamel from various concentrations of F in APF gels. However, topically applied fluoride has been shown to exert its major cariostatic effect on initial carious lesions, rather than on sound enamel. There are numerous ways to test the effectiveness of fluoride preparations; one laboratory approach is to simulate the in vivo cariogenic challenge where pH changes cause cycling between demineralizing and remineralizing conditions. This allows fluoride treatments to be interspersed between demineralization and remineralization periods. In this case frequent acid challenge, similar to that which may occur in vivo, is presented to the enamel and later followed by a fluoride treatment. A continuing series of alternating acid challenge and treatment may be a more realistic and discriminatory means of evaluating the effectiveness of fluoride agents. In this manner the results of cumulative effects can be determined. Buonocore and Gwinnett used intermittent treatments with dentifrice between periods of demineralization. However, their design did not allow the influence of remineralization to be included in the comparisons.

The design of the present study includes a phase with remineralizing conditions in addition to the alternating demineralization and treatment phases employed in earlier studies. The purpose of this study was to: (1) develop artificial caries lesions using an alternating demineralizing/remineralizing protocol, (2) determine if fluoride products can inhibit the formation of lesions by this protocol, and (3) establish if this protocol is suitable for discriminating differences in the effectiveness of fluorides in inhibiting lesion formation.

Methods and Materials

Preparation of Enamel Blocks

Caries-free human teeth were cleaned and polished with a propylaxis paste. Areas of enamel, free of cracks and other defects, were selected with the aid of a dissecting microscope and cut into blocks approximately 20-25 mm². Duplicate zones, 1 mm wide, extending parallel to the gingival margin were masked with plastic tape. After painting the entire area with acid resistant varnish, the tape was removed leaving zones of enamel exposed. The blocks were mounted on wax-tipped plastic sticks and sealed with a soldering pencil so that only the enamel surface was exposed.

Treatments

Experiment 1. A daily cycle of demineralization, fluoride treatment and remineralization was repeated for a total of 5 days. The enamel blocks initially were subjected to demineralization for 16 hr (overnight) and then were treated with either dentifrice slurry, APF, or distilled water. Ten blocks were assigned
randomly to each of the treatment groups. After treatment the blocks were transferred to synthetic saliva for 6 hr of remineralization after which they were subjected again to the demineralizing solution for the remainder of the 24-hr cycle (16-18 hr). Between each phase of the cycle the blocks were rinsed in distilled water for 1 min. The demineralization solution contained 0.5 mM hydroxyapatite in 0.1 N lactic acid, at pH 4.6 and was used at 28°C. Each block treated with dentifrice was placed in 20 ml of a 20% aqueous slurry of an MFP-dicalcium phosphate dihydrate dentifricea (194 ppm F as PO4F2) for 2 hr each morning. The acidulated-phosphate fluorideb treatments (1.23% F) were given using 20 ml per enamel block for 4 min. The untreated blocks were placed in distilled water (20 ml per block) for 1 hr. All treatments were at 37°C.

The synthetic saliva contained 1.5 mM KH2PO4, 2.0 mM CaCl2, 2.5 mM urea, 8.3 mM NaHCO3, 4.8 mM NaCl, and 137 mM KCl. The pH was adjusted to 7.1 with HCl and the solution was used at 37°C and changed daily (P. Slater, personal communication).

Experiment 2. A second experiment was conducted under the same conditions except that treatments were with 50% slurries of the dentifrice for either 5 or 15 min twice daily. Five enamel blocks were used per treatment group in this experiment. The first daily treatment of each group was after overnight demineralization as usual and the second daily treatment was after 6 hr of remineralization. Otherwise, all aspects of treatment and analysis were the same as in the first experiment.

Preparation for Analysis

After completion of five days of the treatment cycle each enamel block was embedded in epoxy resin, cured overnight at 37°C and sectioned with a diamond saw to obtain three to five thin sections. The sections were bonded to a 175 μm thick polyester film using 1-2 μl of cyanoacrylate ester adhesive. Up to 40 sections were bonded to a single sheet of polyester film which was held by vacuum on the lapping jig of a polishing machine. The sections were reduced to a thickness of 100 μm using a 10% aqueous slurry of aluminum oxide abrasive (5 μm particle size).

Contact microradiographs were made directly on the bonded specimens using high resolution film in a special clamping jig. The x-ray generator was operated at 40 kV and 2.8 ma for 1 hr at a source-to-film distance of 65 cm. Film was developed for 5.0 min at 68°C. Development conditions were monitored with preexposed control strips.

Analysis

Optical density scans of the radiographs of lesions in each polished section were made with a microdensitometer. Several scans were made of each lesion, each time ensuring that the scanning slit was parallel to the lesion surface. The scanning slit covered a 5 × 300 μm area of the specimen.

Each scan simultaneously was converted to per cent mineral data by a computer program and plotted for numerical analysis. The scans were corrected for the nonlinear response of the photographic film to mineral density and for variations in exposure and film processing by inclusion of an aluminum step series on each film. This step series previously was correlated to a primary standard prepared from a series of sections of known thicknesses of sound enamel. Mineral content therefore was expressed as a percentage of the value obtained for sound enamel. The plotted data also were normalized for variations in specimen thickness.

For each scan the lesion depth, area of the demineralized zone, and mineral content of the most severely demineralized point of the lesion were computed as illustrated in Figure 1. The area was calculated as the integral above the mineral curve using the surface layer maximum and sound enamel as limits (Figure 1). A mean value for each of these parameters was calculated from the replicate scans which were used to characterize each block. Grand means then were determined for each group.

Statistical comparisons between the grand means were done using Student's t test. These means also were used to calculate the per cent improvement relative to the water control. For the lesion minimum the following formula was used.

\[
\frac{\text{Control-treated}}{\text{Control}} \times 100 = \text{Relative } \% \text{ improvement}
\]

For parameters which would be expected to decrease with treatment (lesion depth and area), the terms in the numerator were reversed so a larger number would still indicate a more effective treatment.

Results

Figure 2 illustrates the marked difference in lesion formation observed between blocks treated with distilled water and blocks treated with dentifrice. Similar results were obtained from daily 4-minute APF treat-
TABLE 1. Mean Values ± S.D. For Lesion Characteristics After Treatment — Experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Depth, μm</th>
<th>Minima*</th>
<th>Area</th>
<th>Depth</th>
<th>Minima</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentifrice - 2 hr</td>
<td>17 ± 5*</td>
<td>97 ± 4*</td>
<td>66 ± 91*</td>
<td>79%</td>
<td>106%</td>
<td>96%</td>
</tr>
<tr>
<td>APF - 4 min</td>
<td>18 ± 5*</td>
<td>99 ± 1*</td>
<td>43 ± 31*</td>
<td>78%</td>
<td>111%</td>
<td>97%</td>
</tr>
<tr>
<td>Water - 1 hr</td>
<td>82 ± 5</td>
<td>47 ± 7</td>
<td>1710 ± 365</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Significantly different from water control (p < .01).
+ % mineral content at the point of most severe demineralization.

Table 2. Mean Values ± S.D. for Lesion Characteristics After Treatment — Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Depth, μm*</th>
<th>Minima*, +</th>
<th>Area*</th>
<th>Depth</th>
<th>Minima</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentifrice - 5 min</td>
<td>47 ± 8</td>
<td>80 ± 6</td>
<td>516 ± 184</td>
<td>43%</td>
<td>70%</td>
<td>70%</td>
</tr>
<tr>
<td>Dentifrice - 15 min</td>
<td>24 ± 16</td>
<td>92 ± 3</td>
<td>198 ± 120</td>
<td>70%</td>
<td>96%</td>
<td>88%</td>
</tr>
</tbody>
</table>

* Significant difference between groups (p < .05).
+ % mineral content at the point of most severe demineralization.

Figure 1. Mineral density scan of enamel showing parameters calculated by computer.

Figure 2. Mineral density scans of enamel treated with water or dentifrice for 2 hr and given a cariogenic challenge daily for 5 days.

Figure 3. Mineral density scans of enamel treated with water or APF for 4 min and given a cariogenic challenge daily for 5 days.

Examples typical of the scans obtained within each group.

The mean values for the lesion parameters from Experiment 1 are shown in Table 1. The lesion parameters of the dentifrice and APF groups were significantly different from those of the water control (p < .01). From these values the relative per cent improvement was calculated with respect to the control (treatment with distilled water). For the dentifrice and APF, the mean lesion depth was reduced by 79% and 78%, respectively, and the mean area of the demineralized zone reduced by 96% and 97%, respectively. The mineral content of the lesion minima was increased by 106% and 111% by the dentifrice and APF, respectively.
Figure 4 illustrates the results obtained after the 5- and 15-minute treatments given twice daily with 50% slurries of dentifrice. The per cent improvement of the measured parameters for this experiment was calculated based on the control group of Experiment 1, as the demineralization procedures were sufficiently similar. The two treatments produced differing results with mean lesion depths reduced by 43% and 70%; the mineral content of the lesion increased by 70% and 96%, and the area of the demineralized zone reduced by 70% and 88% for the 5- and 15-minute treatments, respectively (Table 2). These differences with respect to the water control were significant (p < .01). These treatments were also significantly different from each other in each lesion parameter (p < .05).

Discussion

The control group results indicate that the alternating scheme of demineralization/remineralization produces lesions typical of those generated by constant exposure to acidified calcium phosphate solutions. However, this method more closely resembles the situation in vivo, where acid challenges are cyclic with intervening periods of remineralization by saliva. This method also provides better in vitro simulation of actual oral conditions than it allows for fluoride treatments to be interspersed between demineralizing and remineralizing periods.

In the first experiment the values for both the dentifrice and APF treatments are quite similar and show nearly complete inhibition of white spot formation. The minimum mineral content of the lesion was maintained by the treatments to within 1-3% of sound enamel, and the lesion area to within 3-4% of sound enamel. The enamel soundness, as measured by each of these parameters was maintained to a greater extent than the lesion depth. Both treatments restricted depth by about 80% which indicated that although the acid penetrated to about 18 μm, it was able to dissolve only a small amount of mineral. The values calculated for the lesion areas are probably more significant because they represent the total mineral lost from the lesion. It is interesting that the brief (4 min) high fluoride concentration (12,300 ppm F) treatment with APF produced results similar to the long-term (2 hr), low fluoride concentration (194 ppm) of diluted dentifrice.

In the second experiment, the effect of 50% slurries of dentifrice was tested using different time periods, and the treatments were given twice daily. In this situation, differences were observable between the two treatments used. As in Experiment 1, the diminution of mineral loss as shown by the minimum mineral content of the lesion was greater than the reduction of the lesion depth. The mineral content throughout the lesion is probably more important than lesion depth, as a sufficiently low mineral content would allow the surface of a white spot to collapse and form a cavity. An important finding is that the reduction in the lesion scan areas from blocks given the two treatments differed by 18 percentage points, showing that this procedure easily can detect differences in treatments (p < .05). This finding suggests that treatment times similar to or even shorter than those used in Experiment 2 might be employed to reveal differences between dentifrices or other fluoride preparations.

In addition to the finding that the treatment protocol used in the present experiments can differentiate between different fluoride exposures, this work also points out the importance of frequent fluoride treatments in the suppression of caries formation. Although a 5- or 15-minute treatment with a fluoride dentifrice is obviously excessive when compared to normal brushing habits, the repeated 16-hour cariogenic challenges also must be considered to be extremely severe. In spite of this, caries formation was eliminated almost entirely. Fluoride exposures immediately prior to, during, or shortly after a caries attack should be very effective in reducing the amount of mineral ultimately lost from the tooth. These findings support the concept that an MFP/dicalcium phosphate dihydrate dentifrice (or frequent APF exposures) inhibits caries formation at a very early stage when it promotes remineralization and enhances the acid resistance of the remaining enamel. This is in addition to the effects it also might have on sound enamel and previously formed lesions.

Conclusions

Blocks of human enamel treated intermittently with APF or an MFP/dicalcium phosphate dihydrate dentifrice between periods of a cariogenic challenge, exhibited significantly less caries formation than control
blocks treated only with water. Lesion formation in blocks treated with APF was 97% less severe than in water-treated blocks. Dentifrice treatments inhibited lesion formation by 70-96% depending upon the treatment duration.

This experiment shows that the effectiveness of different fluoride treatments can be differentiated and suggests that fluoride exposure might be most effective when given in close proximity in time to the caries challenge.

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**Quotable quotes: making medical mistakes**

Developments in modern medicine have provided doctors with more knowledge of the human body, more accurate methods of diagnosis, and more sophisticated technology to help in examining and monitoring the sick. All of that means more power to intervene in the disease process. But modern medicine — with its invasive tests and potentially lethal drugs — also has given doctors the power to do more harm.

Yet, precisely because of its technological wonders and near-miraculous drugs, modern medicine has created for the physician an expectation of perfection. The technology seems so exact that error becomes almost unthinkable. We are not prepared for our mistakes and we don't know how to cope with them when they occur.

Doctors are not alone in harboring expectations of perfection. Patients expect doctors to be perfect, too. Perhaps patients have to consider their doctors less prone to error than other people: How else can a sick or injured person, already afraid, come to trust the doctor? Further, modern medicine has taken much of the treatment of illness out of the realm of common sense; a patient must trust a physician to make decisions that he, the patient, only vaguely understands. But the degree of perfection expected by patients is no doubt also a result of what we doctors have come to believe about ourselves or, better, have tried to convince ourselves about ourselves.

This perfection is a grand illusion, of course, a game of mirrors that everyone plays. Doctors hide their mistakes from patients, from other doctors, even from themselves. Open discussion of mistakes is banished from the consultation room, from the operating room, from physicians' meetings. Mistakes become gossip, and are spoken of openly only in court.

Unable to admit our mistakes, we physicians are cut off from healing. We cannot ask for forgiveness, and we get none. We are thwarted, stunted; we do not grow.

Hilfiker D: Making medical mistakes.