Effect of an Anticalculus Toothpaste Containing Pyrophosphate on Pyrophosphatase Activity and the State of Calcium Phosphate in Saliva

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Key words: anticalculus toothpaste, pyrophosphate, pyrophosphatase, salivary calcium, phosphate

Abstract

Experiments were performed to determine if a pyrophosphate-containing anticalculus toothpaste could affect certain salivary constituents involved in the mineralization of dental plaque. The findings indicated no obvious changes in acid and alkaline pyrophosphatases, ionized calcium, total calcium, inorganic phosphate or pH in saliva for 1-135 min after brushing the teeth with the anticalculus toothpaste. Data obtained by brushing with the toothpaste three times daily for two weeks also confirmed these results. Our findings clearly indicate that pyrophosphate-stabilizing agents in the anticalculus toothpaste are not fully effective in the oral cavity. In addition, the pyrophosphate-containing toothpaste has no influence on the state of calcium and phosphate in saliva.

Introduction

Although it is clear that dental plaque is the main etiologic factor in periodontal disease, the formation of mineralized dental plaque, dental calculus, in the oral environment is also a problem of considerable importance¹. Dental calculus provides an enlarged surface for dental plaque to make contact with the gingiva. Several investigations have been carried out on the pharmacological removal of dental calculus or inhibition of its formation. Incorporation of anticalculus agents into toothpaste is one such approach. The two most widely tested anticalculus agents are soluble pyrophosphate⁶⁻⁹ and zinc salts⁶⁻⁹.

Pyrophosphate has received particular attention in view of its proposed role in inhibition of crystal growth¹⁰⁻¹⁴. Nevertheless, it is unlikely that pyrophosphate in toothpastes will persist for a reasonable length of time in the mouth, because of the presence of considerable amounts of pyrophosphatase enzymes in saliva¹⁵,¹⁶ and oral bacteria¹⁷⁻¹⁹. Pyrophosphate, when introduced into a simulated oral environment, is hydrolyzed by various phosphatases with concomitant loss of its inhibitory activity for calcification in vitro²⁰. To overcome this problem, a copolymer of vinyl methyl ether maleic anhydride (Gantrez) and NaF has been introduced in order to inhibit the hydrolysis of pyrophosphate. Animal and clinical studies have shown that toothpaste containing a combination of pyrophosphate, NaF and Gantrez, was highly effective in retarding supragingival calculus formation⁶⁻⁹,²¹⁻²³. As yet, no data are available concerning the effect of these active agents on pyrophosphatase activity under known experimental conditions in vivo. For this reason, it is of interest to assess the ability of a toothpaste containing pyrophosphate and pyrophosphatase inhibitors to decrease the activities of pyrophosphatases.

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under physiological conditions. Since the state of calcium and phosphate in saliva is related to supragingival calculus formation\textsuperscript{24–26}, it would also be useful to determine whether anticalculus toothpaste could affect the state of these ions in saliva.

**Materials and Methods**

**Subjects**

Eighteen subjects of both sexes were selected from the staff and dental students of the Faculty of Dentistry, Mahidol University, Bangkok, Thailand. The age range was 21-35 yrs. Participants with severe periodontal diseases, gingival recession or stained teeth were excluded. All the subjects were asked to avoid using commercial mouthwashes or any toothpastes which contained anticalculus agents for at least 1 week before the experiment.

**Toothpastes**

The test toothpaste contained 0.22\% NaF, 1.5\% tetrasodium pyrophosphate, 4.5\% tetrapotassium pyrophosphate, Gantrez and silica abrasive. The placebo toothpaste was identical except that it did not contain pyrophosphates. Both toothpastes were packed in identical plain white tubes, so that the subjects were unaware of the product assigned to them.

**Study designs**

**Experiment 1: Study on short-term effects**

Nine faculty members participated in this study. Unstimulated mixed saliva from the subjects was first collected to provide baseline data. Saliva was collected between 800 and 1000 h after normal oral hygiene or intake of food or drink. Subjects brushed their teeth under supervision for 1 min with approximately 1 g of the placebo toothpaste. Residual toothpaste was removed by rinsing with 10 ml deionized water. Further samples of unstimulated saliva were taken at 1, 15, 30, 45, 75 and 135 min after brushing. In a second test performed at least 1 week after the first, each subject was tested with the anticalculus toothpaste using a similar procedure to that above.

**Experiment 2: Study on long-term effects**

In order to confirm the results of the short-term experiment, studies were carried out on the cumulative effect of the anticalculus toothpaste in 9 dental students. They brushed their teeth three times daily for 1 min with the placebo toothpaste and soft-bristled toothbrush provided. Unstimulated saliva was taken from each subject at the first-week recall, and the subjects were asked to use the anticalculus toothpaste instead of the placebo toothpaste for the next two weeks. Saliva samples were also collected weekly.

**Analysis of saliva**

Salivary pH was measured immediately after collection using a pH meter (model 28, Radiometer, Copenhagen, Denmark). The ionized calcium was determined using an Orion calcium ion electrode (Cat. No. 93-20-01) connected to a microprocessor ion analyzer (Orion model 901, Orion Research Incorporated, Cambridge, Mass., U. S. A.). The measurements were performed as described in the instrument manual. Free fluoride was determined using an Orion fluoride electrode\textsuperscript{27}. Total calcium was estimated by atomic absorption spectroscopy (Shimadzu AA 650, Shimadzu Corporation, Kyoto, Japan). Inorganic phosphate (Pi) was determined spectrophotometrically using ammonium molybdate as an indicator\textsuperscript{28}. Protein was estimated by the biuret method\textsuperscript{29} with bovine serum albumin as a standard. Acid pyrophosphatase (acid PPase) and alkaline pyrophosphatase (alkaline PPase) activities were assayed by incubation of saliva samples with pyrophosphate\textsuperscript{10}. Phosphate liberated by the reaction was measured colorimetrically\textsuperscript{28}. Enzyme activities were expressed as nM Pi formed per mg salivary protein per hour.

**Statistical analysis**

The data were analyzed statistically using the Systat Statistical Package (Systat Inc., Evanston, Ill. U. S. A.). Median and range were represented as descriptive statistics. Friedman
analysis of variance and multiple comparison were used to compare the difference in levels of each salivary parameter before and after brushing with the placebo or active toothpaste at a significance level of 0.01.

**Results**

The results of short-term and long-term usage of the toothpastes are shown in Tables 1, 2 and 3. Table 1 shows the median values and ranges for acid PPase, alkaline PPase, ionized calcium, total calcium, Pi and pH for resting whole saliva before and after brushing with the placebo toothpaste. Comparison of these levels with the baseline values revealed no significant differences except for ionized calcium and fluoride. Similar results were also observed in the case of brushing with the anticalculus toothpaste (Table 2). It was also seen that none of the salivary parameters changed significantly (p<0.01) after brushing with the placebo and anticalculus toothpastes for 1 and 2 weeks, respectively (Table 3).

**Discussion**

The present study showed that pyrophosphate-containing toothpastes do not affect acid and alkaline PPase activities in saliva, thus suggesting that the pyrophosphate-stabilizing agents NaF and Gantrez might not effectively inhibit pyrophosphatase under physiological conditions. Our data are in contrast to those obtained in vitro by GAFFAR et al. [20] The difference might be due to the fact that they used consistently higher concentrations of NaF (100-1000 ppm) than those of salivary fluoride released after tooth-brushing with toothpaste containing 1000 ppm F [30,31]. In addition, they demonstrated that the use of Gantrez alone could not inhibit pyrophosphatases within a 2-h observation period. Therefore, high fluoride concentrations might be responsible for such initial inhibition. However, it should be noted that under physiological conditions, salivary fluoride levels, with or without tooth-brushing, are always low and within the non-inhibitory range [32].

The data obtained for salivary inorganic phosphate and calcium indicated no difference in the concentrations before and after brushing with either toothpaste. However, lower amounts of ionized calcium were found in saliva of both groups after 1 min of tooth-brushing. The decrease in salivary calcium was not influenced by pH [30] because no change in salivary pH was observed in this study (Tables 1 and 2). We believe that the lower calcium ion concentration was not due to formation of a calcium pyrophosphate complex because such reduction was also seen in the control group. It seems more probable that the immediate fall in the calcium ion concentration is a consequence of salivary stimulation by tooth-brushing [34].

Brushing of teeth with pyrophosphate-containing toothpaste for two weeks did not induce any detectable changes in salivary constituents (Table 3), thus confirming the short-term effect described in Tables 1 and 2. There are a few reports on the clinical efficacy of pyrophosphate-containing toothpastes in retarding dental calculus formation. However, the results reported here do not provide any evidence that such toothpaste can affect the levels of most salivary constituents involved in calculus formation except for a transient change in fluoride and ionized calcium. Hence, the role of pyrophosphate-containing toothpaste in inhibiting dental calculus formation is not clear. However, if pyrophosphate is the agent responsible for such inhibition, it should be stable in the oral cavity for a certain period after tooth-brushing [35]. At present, there is no direct evidence from any previous work to indicate that the use of such toothpaste could effectively increase pyrophosphate concentrations in the oral cavity. Preliminary findings (our unpublished observations) suggest that there is no marked change in salivary pyrophosphate after brushing with anticalculus toothpaste. This might be the result of rapid pyrophosphate degradation by pyrophosphatases [15,16] and phosphatase [36–39] in saliva and/or rapid adsorption of pyrophosphate on tooth surfaces [40,41]. Furthermore, the limited sensitivity of the spectrophotometric method [41] used in our study for measuring the very low level of pyrophosphate
**Table 1** Experiment 1: Short-term effects of tooth-brushing with the placebo toothpaste

<table>
<thead>
<tr>
<th>Salivary parameter</th>
<th>0</th>
<th>1</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>75</th>
<th>135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid PPase (nMPL/h/mg protein)</td>
<td>0.60</td>
<td>0.88</td>
<td>0.88</td>
<td>0.64</td>
<td>0.22</td>
<td>1.24</td>
<td>0.69</td>
</tr>
<tr>
<td>Alkaline PPase (nMPL/h/mg protein)</td>
<td>18.08</td>
<td>25.85</td>
<td>22.40</td>
<td>15.14</td>
<td>18.17</td>
<td>30.94</td>
<td>15.45</td>
</tr>
<tr>
<td>Ionized calcium (mM)</td>
<td>0.44</td>
<td>0.29</td>
<td>0.38</td>
<td>0.40</td>
<td>0.41</td>
<td>0.43</td>
<td>0.59</td>
</tr>
<tr>
<td>Total calcium (mM)</td>
<td>1.06</td>
<td>1.19</td>
<td>1.20</td>
<td>1.08</td>
<td>1.08</td>
<td>1.14</td>
<td>1.21</td>
</tr>
<tr>
<td>Inorganic phosphate (mM)</td>
<td>5.98</td>
<td>4.48</td>
<td>6.77</td>
<td>5.81</td>
<td>6.45</td>
<td>6.72</td>
<td>7.15</td>
</tr>
<tr>
<td>Ionized fluoride (mM)</td>
<td>0.012</td>
<td>0.058</td>
<td>0.015</td>
<td>0.020</td>
<td>0.013</td>
<td>0.010</td>
<td>0.013</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.2</td>
<td>6.9</td>
<td>6.8</td>
<td>7.1</td>
<td>7.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*: Each value represents median for 9 subjects with the range in parentheses.
**: Statistically significant difference from baseline value (p<0.01)

**Table 2** Experiment 1: Short-term effects of tooth-brushing with the anticalculus toothpaste

<table>
<thead>
<tr>
<th>Salivary parameter</th>
<th>0</th>
<th>1</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>75</th>
<th>135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid PPase (nMPL/h/mg protein)</td>
<td>0.94</td>
<td>0.74</td>
<td>0.16</td>
<td>0.40</td>
<td>0.69</td>
<td>1.90</td>
<td>0.22</td>
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<tr>
<td>Alkaline PPase (nMPL/h/mg protein)</td>
<td>22.94</td>
<td>42.42</td>
<td>26.84</td>
<td>14.33</td>
<td>17.54</td>
<td>17.72</td>
<td>22.94</td>
</tr>
<tr>
<td>Ionized calcium (mM)</td>
<td>0.41</td>
<td>0.24*</td>
<td>0.36</td>
<td>0.40</td>
<td>0.44</td>
<td>0.49</td>
<td>0.52</td>
</tr>
<tr>
<td>Total calcium (mM)</td>
<td>1.09</td>
<td>1.13</td>
<td>1.25</td>
<td>1.30</td>
<td>1.28</td>
<td>1.29</td>
<td>1.31</td>
</tr>
<tr>
<td>Inorganic phosphate (mM)</td>
<td>3.91</td>
<td>5.51</td>
<td>7.26</td>
<td>6.28</td>
<td>6.44</td>
<td>7.46</td>
<td>7.47</td>
</tr>
<tr>
<td>Ionized fluoride (mM)</td>
<td>0.011</td>
<td>0.065*</td>
<td>0.015</td>
<td>0.014</td>
<td>0.014</td>
<td>0.009</td>
<td>0.012</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.2</td>
<td>6.9</td>
<td>6.8</td>
<td>7.1</td>
<td>6.9</td>
<td>6.9</td>
</tr>
</tbody>
</table>

*: Each value represents median for 9 subjects with the range in parentheses.
**: Statistically significant difference from baseline value (p<0.01)

**Table 3** Experiment 2: Long-term effects of tooth-brushing with the placebo toothpaste for 1 week and anticalculus toothpaste for 2 weeks

<table>
<thead>
<tr>
<th>Salivary parameter</th>
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<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid PPase (nMPL/h/mg protein)</td>
<td>0.45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline PPase (nMPL/h/mg protein)</td>
<td>19.06</td>
<td>27.80</td>
<td>25.47</td>
</tr>
<tr>
<td>Ionized calcium (mM)</td>
<td>0.52</td>
<td>0.68</td>
<td>0.57</td>
</tr>
<tr>
<td>Total calcium (mM)</td>
<td>1.04</td>
<td>1.14</td>
<td>0.98</td>
</tr>
<tr>
<td>Inorganic phosphate (mM)</td>
<td>6.17</td>
<td>6.05</td>
<td>5.61</td>
</tr>
<tr>
<td>Ionized fluoride (mM)</td>
<td>0.016</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>7.1</td>
<td>6.9</td>
</tr>
</tbody>
</table>

*: Each value represents median for 9 subjects with the range in parentheses.
(5-40 nmol) cannot be ruled out. However, the preliminary data obtained from whole saliva may not directly reflect alterations at tooth surfaces. Further studies of tooth/plaque/calculus/surfaces are required in order to confirm our negative findings.

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