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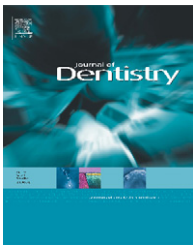

Effect of low pH on surface rehardening efficacy of high concentration fluoride treatments on non-cavitated lesions

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ABSTRACT

Objective: Professionally applied acidulated phosphate fluoride has been shown to reduce caries incidence. However, it has been suggested that its efficacy might be reduced in advanced non-cavitated lesions. This study aimed to compare the surface rehardening and fluoride uptake effect of 2%-NaF solutions at different pH on non-cavitated caries-like lesions with two different levels of demineralization.

Methods: Human enamel specimens were demineralized to create early and advanced non-cavitated lesions. Specimens for each type of lesion were divided into 3 groups, treated for four minutes with either 2%-NaF pH 3.5, 2%-NaF at pH 7.0, or neutral deionized water, and exposed to a pH cycling remineralization/demineralization model for five days. An additional treatment was then done as described above followed by five more days of cycling (total of 2 treatments, ten-day pH cycling). Specimens were analyzed for surface microhardness change and fluoride uptake.

Results: It was found that for both types of lesions, acidic pH fluoride treatment was significantly ($p < 0.05$) more effective than neutral pH treatment in rehardening the lesion surface and promoting fluoride uptake. Furthermore, the low pH vs neutral pH difference in rehardening was significantly larger in the less demineralized lesions ($p = 0.0001$). Water treatment resulted in no rehardening or fluoride uptake.

Conclusions: Results from this study suggest that high concentration fluoride treatments at acidic pH are more effective in rehardening the surface of non-cavitated caries lesions and promoting fluoride uptake than those at neutral pH. This effect appears to be greater in less demineralized lesions when compared to more advanced ones.

Clinical significance: The results of this investigation suggest that when no other attenuating circumstances are present (e.g., the possibility of damaging tooth-coloured restorations), high concentration fluoride treatments for high risk individuals might be more efficacious using products at low pH.

1. Introduction

Acidulated phosphate fluoride (APF) was introduced in the early 1960s by Brudevold et al.[1] Since then, several clinical studies have shown that regular professional application of APF gels can reduce caries incidence by approximately 21% (95% CI, 14% to 28%).[2]

Despite the number of studies conducted with APF, its mechanism of action is not well understood. It has been

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proposed that APF partially demineralizes enamel apatites creating spaces that increase lesion diffusion and are subsequently filled with CaF_2-like material. Later on, the fluoride would be released during undersaturation periods promoting remineralization and creating more resistant crystals.[3,4] This low pH mechanism of action would enhance the remineralization efficacy of fluoride treatments on sound enamel and non-cavitated (i.e., incipient) caries lesions, particularly at their earlier stages. On the other hand, in more advanced non-cavitated lesions, the demineralization caused by the APF treatment could damage crystals in advanced stage of demineralization reducing the number of available nucleation sites for remineralization affecting the efficacy of the fluoride treatment. It has been shown that APF might have limited efficacy preventing lesion progression[5] and could affect remineralization and fluoride uptake potential of fluoride toothpastes[6] when used in lesions with significant mineral loss.

We hypothesized that reducing the pH of products with high concentration of fluoride enhances their remineralization ability only in non-cavitated lesions with limited mineral loss (lesions at their early stages of demineralization). The aim of this study was to partially test the hypothesis proposed above by comparing the surface rehardening and fluoride uptake effect, as surrogates for lesion remineralization, of a 2% NaF solution at two different pH on non-cavitated caries-like lesions with two different levels of demineralization. The specific null hypothesis tested was: there is no difference in surface rehardening and fluoride uptake level of early and advanced non-cavitated lesions treated with low pH or neutral pH fluoride treatments. To our knowledge, no investigation has been conducted comparing directly the effect of pH in high concentration fluoride treatments on the remineralization of non-cavitated lesions with different levels of demineralization.

2. Materials and methods

2.1. Study design

Enamel specimens were demineralized to create non-cavitated caries-like lesions with two different levels of mineral loss at the tooth surface: early (EL) and advanced (AL) lesions based on the level of surface mineral loss. Specimens for each type of lesion were divided into 3 groups (6 groups total, $n = 12$). For each lesion type, groups were treated for 4 min with either 2% NaF at pH 3.5, 2% NaF at pH 7.0, or neutral deionized water and then exposed to a pH cycling remineralization/demineralization model for five days. After the five days of cycling, an additional treatment was done as described above followed by five more days of pH cycling. After the two five-day cycling periods (a total of 2 treatments and ten days of cycling), specimens were analyzed for surface microhardness change and fluoride uptake.

Plano-parallel enamel specimens (3 mm × 3 mm) obtained from human permanent teeth stored in 0.1% thymol solution since extraction were ground and polished (Struers RotoPol 31/RotoForce 4) using an ascending grit series of silicon carbide paper to a 4000-grit paper followed by a cloth with 1 μm diamond polishing suspension. Specimens were then analyzed for surface Vickers microhardness (Leco LM-247-AT/Confident measuring system; 200 g load for 15 s; 4 indents per specimen). Specimens were selected based on their sound Surface Vickers microhardness number (SVMHN). Specimens with an average SVMHN between 300 and 350 were accepted for the study.

Caries-like subsurface lesions were created in the enamel specimens by a ~13 (for EL) or ~90 h (for AL) immersion in a 37 °C solution of 0.1 M lactic acid and 0.2% (wt/vol.) Carbopol C907, 50% saturated to hydroxyapatite and adjusted to pH 5.0.[7] The SVMHN of the demineralized specimens was determined, as described before. Specimens with SVMHN between 100 and 125 we assigned to the EL groups and those with SVMHN between 25 and 45 for the AL groups. For each lesion type, specimens were balanced into the different groups by their demineralized SVMHN values ($n = 12$).

A 50:50 mixture of pooled stimulated human (10–15 individuals) and artificial saliva (gastric mucin 2.20 g/L, NaCl 0.381 g/L, $CaCl_2{\cdot}2H_2O$ 0.213 g/L, KH_2PO_4 0.738 g/L, KCl 1.114 g/L; pH 7.0) was prepared daily as the remineralization solution during the cycling period and for the formation of an initial pellicle on the specimen's surface before the first treatment each of the two weeks of treatment.

On the first day of each of two consecutive weeks, specimens were incubated in the saliva mixture for 2 h (37 °C) to allow for a young pellicle to form and then submerged for 4 min in a stirring solution (350 rpm) of 2% NaF at pH 3.5 (0.1 M H_3PO_4) or at pH 7.0, or with deionized water at pH 7.0. After treatment, specimens were placed in the saliva mixture for three hours followed by incubation in the demineralization solution for four hours. After the demineralization period, specimens were incubated in fresh saliva mixture until next day's 4 h demineralization period. The following four days of incubation consisted of 20 h of saliva mixture and 4 h of demineralization solution incubation periods. The same exact five-day treatment and pH cycling was repeated. All the steps, except for pellicle formation, were done at room temperature.

At the end of the ten-day treatment/pH cycling regimen, specimens were rinsed with deionized water and analyzed for SMH as described above, placing new indentations next to the previous ones. At the end of the SMH analysis, the fluoride content of each enamel specimen was determined using a microdrill technique to a depth of 100 μm.[8] Fluoride content was calculated based on the amount of fluoride obtained divided by the diameter of the enamel hole and expressed as $\mu g\ F/cm^2$.

The effects of lesion size (AL vs EL) and treatment (2% NaF at pH 3.5, 2% NaF at pH 7.0, or neutral deionized water) on microhardness change and fluoride uptake were analyzed using two-way ANOVA models at 5% significance level. Using the ANOVA models we were also able to assess whether the difference between treatments at acidic and neutral pH varied between the lesion sizes. Analyses used the ranks of the data for a nonparametric test due to the non-normal distributions of the data. Analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC).