Anticaries effect of dentifrices with calcium citrate and sodium trimetaphosphate

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ABSTRACT

Because of the growing concerns regarding fluoride ingestion by young children and dental fluorosis, it is necessary to develop new dentifrices. Objective: The aim of this study was to evaluate the effect of dentifrices with calcium citrate (Cacit) and sodium trimetaphosphate (TMP) on enamel demineralization. Material and Methods: Enamel blocks (n=70), previously selected through surface hardness analysis, were submitted to daily treatment with dentifrices diluted in artificial saliva and to a pH-cycling model. The fluoride concentration in dentifrices was 0, 250, 450, 550, 1,000 and 1,100 μg F/g. Crest™ was used as a positive control (1,100 μg F/g). Cacit (0.25%) and TMP (0.25%) were added to dentifrices with 450 and 1,000 μg F/g. Surface hardness was measured again and integrated loss of subsurface hardness and fluoride concentration in enamel were calculated. Parametric and correlation tests were used to determine difference ($p<0.05$) and dose-response relationship between treatments. Results: The addition of Cacit and TMP did not provide a higher fluoride concentration in enamel, however it reduced ($p<0.05$) mineral loss when compared to other dentifrices; the dentifrice with Cacit and TMP and a low fluoride concentration presented similar results when compared to a dentifrice with 1,100 μg F/g ($p>0.05$). Conclusions: Dentifrices with 450 and 1,000 μg F/g, Cacit and TMP were as effective as a gold standard one.

Key words: Dentifrices. Dental enamel. Fluorides. Calcium citrate. Tooth demineralization.

INTRODUCTION

Scientific data from the last decades confirm fluoride dentifrices provide a substantial caries protective effect against carious lesions, inhibiting demineralization and enhancing remineralization[16]. However, the use of fluoride dentifrices during tooth development has been considered a risk factor for dental fluorosis in children[13]. Based on these considerations, the use of a dentifrice with a low fluoride concentration and as effective as a gold standard one (1,100 μg F/g) would be of great interest.

Clinical trials have been carried out to check the anticaries efficiency of low fluoride dentifrice compared to the conventional 1,100 μg F/g dentifrice[2]. Reed[15] (1973) found a lower anticaries effect for a low fluoride dentifrice when compared to the conventional dentifrice. On the other hand, Winter, et al.[21] (1989), Biesbrock, et al.[3] (2003) and Stookey, et al.[17] (2004) concluded that conventional and low fluoride dentifrices may have similar anticaries effects. A recent clinical trial[10] conducted for 12 months with children aged from 2 to 4 years showed the anticaries effect of a low fluoride dentifrice was similar to the conventional fluoride dentifrice when used by caries inactive children; in children with active caries lesions the low fluoride dentifrice was less effective than the 1,100 μg F/g dentifrice in controlling the progression of lesions.

In a recent study[18], a dentifrice with low

fluoride concentration (500 µg/g) and sodium trimetaphosphate (TMP) showed a similar effect to a standard dentifrice. The capacity of TMP to bind to crystal surfaces interferes with both dissolution and growth[9,14]. TMP adsorption to dental enamel increases the permselectivity facilitating the diffusion of cations into enamel[20]. As TMP, calcium citrate (Cacit) can also be adsorbed to hydroxyapatite (HA) and influence the surface charge distribution and precipitation of HA[11,12]. However, the effect of Cacit on demineralization and remineralization has not been tested yet.

The formulation of a dentifrice with a low fluoride concentration supplemented with calcium citrate and TMP could be as effective as a gold standard dentifrice and reduce fluoride ingestion by children, leading to a lower prevalence of dental fluorosis. The aim of the present study was to evaluate the effect of fluoride dentifrices with Cacit and TMP on enamel demineralization using a pH-cycling model.

MATERIAL AND METHODS

Experimental design

Enamel blocks (4x4 mm) were machined from bovine incisors, previously stored in 2% formaldehyde solution pH 7.0 for 1 month, and had their surface serially polished. Baseline surface hardness measurements (SH; 321.0 up to 357.0 KHN) were used to eliminate blocks with anomalous properties prior to further testing. Enamel blocks were randomized (according to their mean and interval of confidence) in 7 groups of 10 and submitted to pH cycling[19] for 5 days with treatment occurring twice a day with dentifrice slurries. The fluoride concentration in dentifrices was one of the following: 0, 250, 450, 550, 1,000 and 1,100 µg F/g. Crest™ (1,100 µg F/g, pH 7.0, Procter & Gamble, Cincinnati, Ohio, USA) was used as a positive control. Cacit (0.25%) and TMP (0.25%) were added to dentifrices with 450 and 1,000 µg F/g. After the pH-cycling, surface and cross-sectional hardness as well as fluoride concentration in enamel were analyzed.

Toothpaste formulation and fluoride assessment

The experimental dentifrices were prepared by Sara Lee Household and Body Care Research (Sara Lee Household and Body Care Research, Utrecht, The Netherlands) and had the following ingredients: carboxymethylcellulose, sodium-methyl-p-hydroxybenzoate, flavor, sodium saccharin, peppermint oil, glycerol, sorbitol, Alfa Olefin Sulphonate, hydrated silica, titanium dioxide, trisodium phosphate and water. Sodium fluoride was added to all dentifrices except for placebo. Cacit (0.25%) and TMP (0.25%) were added to dentifrices with 450 and 1,000 µg F/g.

Fluoride measurements of experimental dentifrices followed the technique previously described by Delbem, et al.[6] (2009). After water dispersion, a sample from the suspension was treated with 2 mol L^{-1} HCl for total fluoride assessment and buffered with 1 mol L^{-1} NaOH. For ionic fluoride assessment, supernatants were obtained by centrifuge (906 xg; 20 min). The same volume of TISAB II ("total ionic strength adjustment buffer", Orion, Thermo Scientific Inc., Beverly, MA, USA) was added to the solutions. Fluoride measurements were performed with an ion-selective electrode Orion 96-09 (Orion, Thermo Scientific Inc.) and an ion analyzer Orion 720 A+ (Orion, Thermo Scientific Inc.), calibrated with standards containing between 0.125 and 4.0 µg F/mL was used.

For total phosphorus and calcium measurements in supplemented dentifrices (450 and 1,000 µg F/g), the sample was submitted to previous acid hydrolysis adding HCl 1 mol L^{-1} at a temperature just below the boiling point. The total phosphorus was measured according to colorimetric determination, as described by Fiske and Subbarow[8] (1925). For Ca dosage, a specific electrode (Orion 9720 BN, Thermo Scientific Inc.), attached to an ion analyzer (Orion 720 A+), was previously calibrated with 5 standards, using 20 µL de ISA (ionic strength adjustor, Orion, Thermo Scientific Inc.). The ionic phosphorus and calcium were measured after a 20-min centrifugation (906 x*g*) of the suspensions.

Toothpaste treatments and pH-cycling model

Enamel blocks (n=70) were submitted individually for 5 days to a pH-cycling model at 37°C, and remained in a fresh remineralizing solution for 2 days[19]. The treatment regime consisted of 1-min soaks of all blocks (2 mL/block) of each treatment group in fresh slurries of placebo or fluoride dentifrices (250, 450, 550, 1,000, 1,100 µg F/g and Crest™). Dentifrices were diluted (1:3 in weight) in artificial saliva (1.5 mmol L^{-1} $Ca(NO_3)^2$ $4H_2O$, 3.0 mmol L^{-1} NaH_2PO_4 H_2O and 7.5 mmol L^{-1} $NaHCO_3$, 0.05 µg F/mL, pH 7.0)[4]. The treatment was performed twice a day (before and after a demineralizing period) under agitation. The demineralizing period was 6 hours, and the solution (2.2 mL/mm^2) consisted of 2.0 mmol L^{-1} $Ca(NO_3)_2$ $4H_2O$ and 2.0 mmol L^{-1} NaH_2PO_4 H_2O in 0.075 mol L^{-1} acetate buffer, 0.04 µg F/mL at pH 4.7. The remineralizing period was set at 18 hours and the solution (1.1 mL/mm^2) consisted of 1.5 mmol L^{-1} $Ca(NO_3)^2$ $4H_2O$, 0.9 mmol/L NaH_2PO_4 H_2O, 0.15 mol L^{-1} KCl in 0.02 mol L^{-1} cacodylate buffer, 0.05 µg F/mL at pH 7.0. All blocks were rinsed before and after treatments.

Hardness analysis

After pH-cycling, surface and cross-sectional hardness (CSH) measurements were conducted by the

operator who was blind to treatment groups. Surface hardness of the enamel blocks was measured again (SH_1) using a Shimadzu HMV-2000 microhardness tester (Shimadzu Corporation, Kyoto, Japan) with a Knoop diamond under a 50-g load for 10 s. Five indentations spaced 100 µm from each other and from the baseline (SH) were made. All blocks were then longitudinally sectioned through the center of the exposed enamel. To measure cross-sectional hardness (CSH), half of each block was embedded in acrylic resin and the cut surfaces were exposed and polished. Three rows of 8 indentations each were made, one in the central region of the exposed dental enamel and the other two spaced 100 µm from the first one, under a 25-g load for 10 s. The indentations were made at 10, 30, 50, 70, 90, 110, 220 and 330 µm from the outer enamel surface. The mean values at all three measuring points at each distance from the surface were then averaged. The integrated area above the curve (cross-sectional profiles of hardness into the enamel), using the hardness values (KHN), was calculated by trapezoidal rule (GraphPad Prism, version 3.02) in each depth (µm) from the lesion up to sound enamel. This value was subtracted from integrated area of sound enamel, to obtain the integrated area of the subsurface demineralized regions in enamel, which was named integrated loss of subsurface hardness (∆KHN)[6,18].

Analysis of fluoride concentration in enamel

Blocks (4x2 mm) were obtained from one of the halves of the longitudinally sectioned blocks. An enamel biopsy was performed by immersing the enamel blocks in 0.5 mol L^{-1} HCl for 90 s under agitation. The same volume of TISAB II modified with NaOH (20 g/L) was added to the solution. Fluoride measurements were performed using a fluoride specific electrode Orion 96-09 and an ion analyzer Orion 720 A+, previously calibrated with standards containing 0.125 up to 2.0 µg F/ mL and 0.25 up to 4.0 µg F/mL.

Statistical analysis

The software GMC version 2002[5] was used for the statistical analyses, and the significance limit was set at 5%. Data from SH, SH_1, ∆KHN and fluoride in enamel (µg F/cm^2) presented normal (Kolmogorov-Smirnov) and homogeneous (Cochran tests) distribution and were submitted to one-way analysis of variance followed by Bonferroni's test, considering the fluoride concentration in the dentifrices as fixed factor. Pearson's correlation was done to compare SH1 x Fluoride in enamel, ∆KHN x fluoride in enamel, and SH_1 x ∆KHN.

RESULTS

The values (mean±sd) of ionic and total fluoride concentration (µg F/g) in the experimental dentifrice (placebo, 250, 450, 550, 1000, 1100 and positive control) were, respectively: 17.4±3.3 and 17.2±1.4; 235.9±12.2 and 235.8±9.6; 449.9±36.8 and 411.9±12.8; 557.9±21.3 and 558.2±5.9; 888.4±20.9 and 972.2±79.7; 1,109.6±31.4 and 1,105.8±11.1; 1,115.1±71.2 and 1,109.4±29.2. The values (mean±sd) of ionic and total calcium concentration (µg Ca/g) in the experimental dentifrice with 450 µg F/g were 2.9±0.4 and 115.2±2.8 and for the dentifrice with 1,000 µg F/g were 2.6±0.4 and 73.3±3.6. The values (mean±sd) of ionic and total phosphorus concentration (µg P/g) in the experimental dentifrice with 450 µg F/g were 116.8±2.7 and 623.9±14.7 and for the one with 1,000 µg F/g were 131.1±24.6 and 551.8±6.7.

Figure 1 shows the results of surface hardness (SH and SH_1). No statistical differences were observed among the blocks for the SH, regardless of the treatments ($p>0.05$). All groups showed a significant decrease in SH_1 after pH cycling; the experimental dentifrice with 1,000 µg F/g showed the highest value of hardness ($p<0.05$). There was no significant difference ($p>0.05$) among groups 450, 1,100 µg F/g and positive control.

Figure 2 shows the results of ∆KHN. No difference was observed among groups 450, 1,000, 1,100 µg F/g and positive control ($p>0.05$). There was a negative correlation between SH_1 and ∆KHN ($r=-0.933$; $p<0.001$).

Figure 3 shows the fluoride concentration in enamel after pH-cycling. No difference was observed between group 450 µg F/g and 550 µg F/g ($p>0.05$). Although group 1,000 µg F/g presented similar results when compared to 1,100 µg F/g ($p>0.05$), it was different from the positive control ($p<0.05$). A positive correlation was observed between SH_1 and

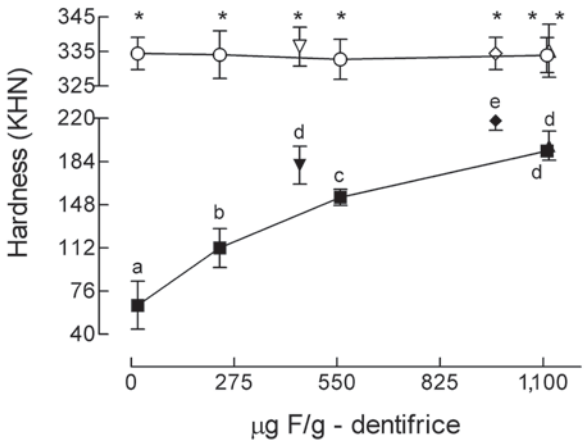

Figure 1- Surface hardness according to dentifrice. "o" indicate SH; "■" indicate SH_1; "△" and "▲" indicate SH and SH_1 for Crest; "▽" and "▼" for 450 µg F/g; "◊" and "◆" for 1,000 µg F/g (mean±standard deviation; n=10). Means followed by distinct letters are statistically different (ANOVA; $p<0.05$); *not statistically different

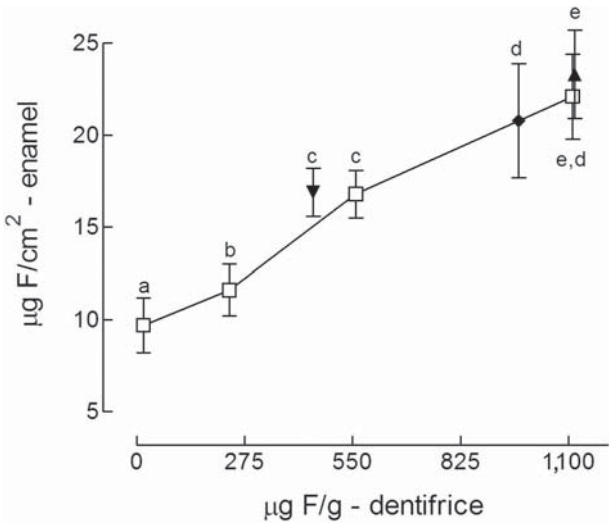

Figure 3- Fluoride concentration (mean±standard deviation; n=10) in enamel according to dentifrice. "□" indicate dentifrice placebo, 275, 550 and 1,100 µg F/g; "▲" indicate Crest; "▼" indicate 450 µg F/g; "◆" indicate 1,000 µg F/g. Distinct letters represent statistically significant differences among groups (ANOVA; p<0.05)

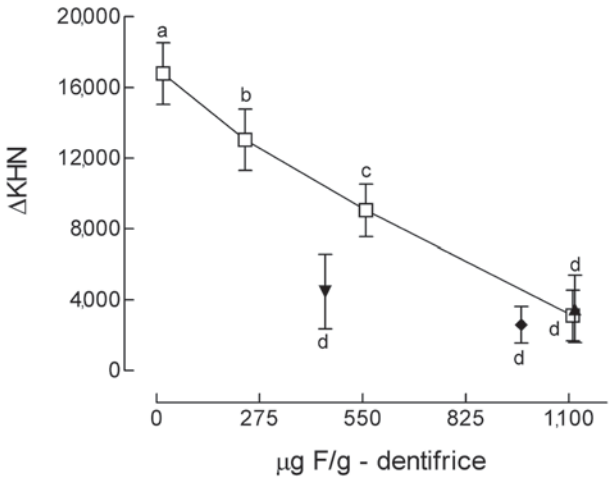

Figure 2- Values (mean±standard deviation; n=10) of integrated loss of subsurface hardness (△KHN) according to dentifrice. "□" indicate dentifrice placebo, 275, 550 and 1,100 µg F/g; "▲" indicate Crest; "▼" 450 µg F/g; "◆" indicate 1,000 µg F/g. Distinct letters represent statistically significant difference among groups (ANOVA; p<0.05)

fluoride in enamel (r=0.885; p<0.001); there was a negative correlation between ΔKHN and fluoride (r=-0.909; p<0.001).

DISCUSSION

Because of the growing concerns regarding fluoride ingestion by young children and dental fluorosis, there is a need to develop novel dentifrices with ingredients that would inhibit demineralization and enhance remineralization. This study evaluated the effect of two dentifrices supplemented with Cacit and TMP on enamel demineralization. The dentifrices were diluted in artificial saliva, aiming to supply calcium and phosphate ions to all groups to simulate what happens with human saliva[1].

Since this study verified if dentifrices with Cacit and TMP would produce a synergic effect when associated to a low or a high concentration of fluoride against demineralization, the following matters were of great importance: the pH-cycling model had to be able to differ the efficacy with the increase of fluoride concentration in the product; and any benefit from Cacit and TMP had to be detected. Dentifrices with 0, 275, 550 e 1,100 µg F/g were included in the study and a fluoride dose response was demonstrated by this methodology. The increase of fluoride concentration in enamel presented correlation with SH_1 (r=0.885) and ΔKHN (r=-0.909). Mineral loss was lower and a higher fluoride concentration in enamel was observed with the increase of fluoride concentration in dentifrices. The inclusion of a commercial gold standard dentifrice was important to verify the efficacy of dentifrices with Cacit and TMP.

Although dentifrices with 450 and 1,000 µg F/g showed similar fluoride concentration in enamel (p>0.05) when compared to dentifrices with 550 and 1,100 µg F/g (Figure 3), respectively, the hardness measurements (SH_1; Figure 1) of groups 450 and 550 µg F/g, and groups 1,000 and 1,100 µg F/g were significantly different (p<0.05). The results of group 450 (Cacit/TMP) of this study was similar to the group 500 µg F/g 0.25% TMP of the study of Takeshita, et al.[18] (2009). Additionally, in this study, group 450 µg F/g (Cacit/TMP) showed a lower fluoride concentration in enamel and similar results of surface hardness when compared to group 1,100 µg F/g or to positive control (Figure 1 and Figure 3). As TMP presents a great affinity by enamel[20], it is believed that these results were promoted mainly by adsorption of TMP on the enamel surface. TMP adsorbed on enamel alters the selective permeability and the ions diffusion into the lesion cavity, mainly calcium[21] or it may reduce enamel demineralization[8,9]. Based on surface hardness values, the ability of the dentifrice with 450 µg F/g to inhibit demineralization was 17% better when compared to 550 µg F/g; dentifrice with 1,000 µg F/g was only 10% better than groups 1,100 µg F/g and positive control.

The dentifrice with 450 µg F/g showed a similar effect with regards to lesion area (Figure 2) when compared to group 1,100 µg F/g and positive control (p>0.05), and it was better (50%) than group 550 µg F/g (p<0.05). It may be assumed that TMP concentration in dentifrice was enough to inhibit hydroxyapatite demineralization showing a diminutive subsurface lesion area. In the study of

Takeshita, et al.[18] (2009), the addition of 0.25% of TMP produced similar results when compared to the 1,100 µg F/g dentifrice. An analysis of the lesion area shows that increase of fluoride concentration from 450 to 1,000 µg/g in dentifrices with Cacit and TMP did not improve its efficacy when compared to groups 1,100 µg F/g and positive control. Cacit, TMP and fluoride have affinity by hydroxyapatite and may compete for the same sites, interfering with their mode of action. The increase in fluoride concentration may have reduced the effect of Cacit and TMP, as it is an element of greater reactivity. Another factor that may have influenced is the low solubility of Cacit, an organic salt, in water-calcium concentration in dentifrices with 450 and 1,000 µg F/g with Cacit and TMP was around 0.1 to 4.6% of the total (2,500 µg Ca/g).

It is most likely that TMP may have assisted the diffusion of calcium ions to the inner of enamel or reduced their loss to the solutions, since dentifrice with 450 µg F/g presented similar results of mineral loss (Figures 1 and 2) and different values of fluoride concentration in enamel (Figure 3) when compared to dentifrice with 1,100 µg F/g. The low availability of calcium from Cacit did not lead to better results than the ones obtained by Takeshita, et al.[18] (2009) with 0.25% of TMP. There was an expectation that the increase of fluoride concentration in dentifrice (1,100) associated to Cacit and TMP would result in improved values when compared to dentifrices with 450 and 1,100 µg F/g. Data from pilot studies (data not shown) have shown that TMP proportion with regards to fluoride concentration is important for an improved efficacy of the product. Thus, the concentration of 0.25% was not enough to make dentifrice with 1,000 µg F/g better than dentifrice with 1,100 µg F/g but it was an adequate amount to make dentifrice with 450 µg F/g similar to dentifrice with 1,100 µg F/g.

Even knowing the limitations of this *in vitro* study, i.e., lack of dental biofilm and the fact that artificial saliva may present lower calcium and phosphate concentration, the experimental dentifrice may bring benefits for prevention of new lesions or as a therapy of remineralization, mainly for young children, due to fluoride ingestion and risk of fluorosis development.

CONCLUSION

Dentifrices with 450 and 1,000 µg F/g, Cacit and TMP were as effective as a gold standard dentifrice.

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