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ORIGINAL ARTICLE

Remineralization of initial carious lesions in deciduous enamel after application of dentifrices of different fluoride concentrations

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Abstract The aim of the present study was to evaluate the remineralization potential of five dentifrices with different fluoride concentrations. Initial caries lesions were created in 72 cylindrical enamel blocks from deciduous teeth. The specimens were randomly distributed among six experimental groups corresponding to six experimental periods. Each of the six volunteers carried two deciduous enamel specimens fixed in an intraoral appliance for a period of 4 weeks. They brushed their teeth and the enamel blocks at least two times a day with dentifrices containing 0 ppm (period 1), 250 ppm (period 2), and 500 ppm fluoride (period 3), respectively. A second group of volunteers (n=6) used dentifrices with a fluoride content of 0 ppm (period 4), 1,000 ppm (period 5), or 1,500 ppm (period 6). At the end of the respective period, the mineral content was determined by transversal microradiography (TMR). The use of dentifrices containing 500 ppm fluoride (38% MR), 1,000 ppm fluoride (42% MR), and 1,500 ppm fluoride (42% MR) resulted in a statistically significant higher mineral recovery compared to the control group (0 ppm fluoride). Mineral recovery was similar after use of dentifrices containing 0 and 250 ppm fluoride (24%; 25%). It is concluded that it is possible to remineralize initial carious lesions in deciduous enamel in a similar way as it has been described for enamel of permanent teeth.

Keywords Fluoride · Dentifrices · Carious lesions · Remineralization · Deciduous teeth

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Introduction

Fluoridated dentifrices are suggested to be an important factor for caries decline in children in many economically developed countries. Normally, fluoridated dentifrices contain 1,000–1,500 ppm fluoride. However, due to toxicological considerations, child formula toothpaste containing 250–500 ppm fluoride is recommended in some countries [8]. De- and remineralization efficacy of fluoride dentifrices have been evaluated in pH cycling models [13, 14]. With these laboratory-based experiments, it was shown that there is a positive relationship between the amount of fluoride administered and the existing cariostatic efficacy. Concerning the clinical use of dentifrices in a recently published Cochrane review, it has been argued that the caries reducing efficacy of low fluoridated dentifrices seems to be ambiguous, since well-performed randomized clinical trials comparing differently concentrated fluoridated toothpastes are lacking [9]. Particularly, studies aimed at remineralization capacity of fluoridated toothpaste in deciduous teeth are rare. It was therefore the aim of the present study to compare differently concentrated fluoride toothpastes in an in situ model concerning their relative remineralizing capacity in enamel of deciduous teeth.

Materials and methods

This institutionally approved, parallel-controlled, double-blind randomized in situ study was carried out in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the university's independent ethics committee. Seventy-two extracted deciduous teeth were gathered and cleaned thoroughly. The roots were cut off and from each tooth one specimen with a diameter of 2.5 mm was prepared with a trepan bur. The specimens were embedded in epoxy resin (Orthocryl, Dentaurum, Ispringen, Germany) and the enamel aspect was ground flat and polished with SiC paper up to FEPA P 2400, thereby removing minimum surface roughness. Then the enamel blocks were plasma sterilized. A defined area of intact enamel was covered with an adhesive tape. Subsequently, the specimens were kept in a demineralizing solution containing 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM KH_2PO_4, 50 mM CH_3COOH (acetic acid), 6 µM methylendiphosphonate, amounts of KOH to adjust the initial pH to 5.0, Thymol (traces) [5], and positioned on a shaking table for 3 days. After the demineralization period, a control slice was obtained and underwent a TMR (transversal microradiography) evaluation in order to check the demineralized specimens for minimum variation of mineral loss. Subsequently, the demineralized surfaces were partially covered with nail varnish to serve as control area.

Twelve adult volunteers in good oral health gave their written consent to participate in the study. For each volunteer, a mandibular appliance was fabricated carrying one enamel specimen in each buccal flange. The volunteers were equally distributed among two experimental groups and everybody shared three experimental periods.

Two enamel specimens each were mounted into the buccal aspects of intraoral appliances.

The first group of volunteers (n=6) used dentifrices containing 0, 1,000, and 1,500 ppm fluoride, while the second group received 0, 250, and 500 ppm fluoride-containing toothpaste (Olaflur, GABA, Therwil, Switzerland) during the respective experimental periods. The composition of the dentifrices did not differ except for the fluoride concentration. Each period lasted for 4 weeks. At the beginning and after each period, a washout phase of 1 week was fitted in. During that period, the teeth were brushed with fluoride-free toothpaste. During the whole study, the volunteers abstained from the use of other fluoride preparations (e.g., mouth rinses, fluoridated salt, fluoride-containing mineral water, fluoride gels, etc.) The volunteers brushed their teeth three times a day after the main meals with 1 ml toothpaste derived from a syringe. During the meals, the appliances were kept in 100% humidity.

After tooth brushing, the volunteers had to spit out once, but they did not rinse with water, i.e., they retained some excess of toothpaste–saliva slurry in the oral cavity. The intraoral appliances were gently cleaned with a separate toothbrush without dentifrice and inserted into the oral cavity. Then the volunteers rinsed their mouth with the retained toothpaste–saliva suspension and spat out again.

For mineral analysis, the specimens were sectioned to a thickness of 100±10 µm and processed for TMR as described by Buchalla et al. [4]. Mineral recovery (MR) was calculated from the integrated mineral loss of the lesion before and after remineralization as (DZ demin−DZ remin)/DZ demin and expressed as %. Change of lesion depth (Ld) was calculated accordingly.

For each subject, the results from two TMR analyses were averaged and the average was used for statistical analysis. For confirmation of statistical testing, an analysis of variance (ANOVA) model was used. In the case of

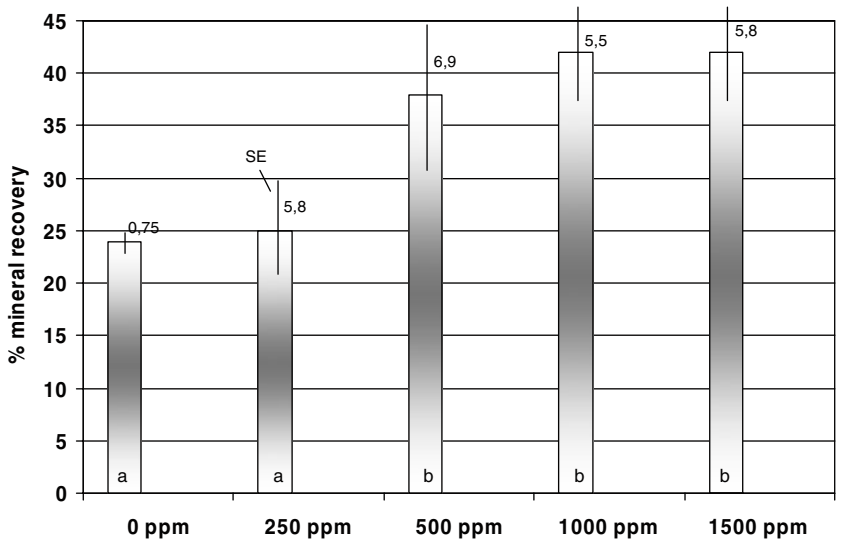

Fig. 1 Percent mineral recovery (vol.%·µm) in initial carious lesions in deciduous enamel after application of 0, 250, 500, 1,000, and 1,500 ppm F^- toothpaste (*bars*=standard errors of the means, SE; *different letters* indicate significant difference between the experimental groups)

Table 1 Mean mineral recovery (vol.%·µm) and mean reduction of lesion depth of initial carious lesions in deciduous enamel

n=number of volunteers, each volunteer carried two enamel specimens

*Mean±standard error of the mean

Groups	Mineral recovery (vol.%·µm)	Reduction of lesion depth (µm)
0 ppm F^- (*n*=12)	269.60±44.74*	6.9±0.41
250 ppm F^- (*n*=6)	264.80±25.69	6.3±0.59
500 ppm F^- (*n*=6)	674.70±194.56	14.2±2.19
1,000 ppm F^- (*n*=6)	856.90±186.58	13.2±2.07
1,500 ppm F^- (*n*=6)	806.10±123.26	15.5±1.53

significant differences by ANOVA, groups were statistically compared using the paired *t* test.

Results

All specimens showed a different but roughly comparable mineral loss after initial demineralization. The mineral loss accounted for 1,483±499 µm vol.% corresponding to an average lesion depth of 51±13.8 µm. After the respective in situ phase, all specimens revealed a distinct mineral recovery. Since mineral recovery and reduction of lesion depth were not statistically significant for the two placebo dentifrices (0 ppm F^-), data were pooled and only one control group is presented.

Figure 1 shows the relative mineral recovery for the different groups, while Table 1 gives the absolute numbers. It can be seen that the use of 0 and 250 ppm fluoride-containing toothpaste led to similar results of 24±0.75% and 25±5.8% mineral recovery, respectively. The application of 500, 1,000, and 1,500 ppm fluoride-containing dentifrices led to a significantly higher mineral recovery compared to the lower concentrated fluoride dentifrices. After the use of 500 ppm F toothpaste, the relative mineral recovery amounted to 38±6.9%, for the 1,000 ppm and 1,500 ppm F^- containing dentifrices, a mineral recovery amounted to 42±5.5% and 42±5.8%, respectively. However, between the three groups, no statistically significant differences could be observed. An almost similar behavior could be found for reduction of lesion depth (Fig. 2 and Table 1). Lesion depth was reduced by 13±3.2% and 13±2.3% in the 0 ppm and 250 ppm groups. The use of 500 ppm, 1,000 ppm, and 1,500 ppm dentifrices resulted in a reduced lesion depth of 23±4.0%, 23±2.3%, and 29±4.0%, respectively. Only the latter group differed significantly from the four other groups.

Discussion

The cariostatic efficacy of fluoridated dentifrices is indisputable. However, it has been reported that their unsupervised use in small children may result in an undesirable high fluoride intake [6]. Consequently, dentifrices with a lower fluoride content dedicated to young children have been developed. The clinical efficacy of these toothpastes in deciduous teeth is a matter of dispute and particularly whether a lower caries preventive effect can be determined for fluoride concentration below 500 ppm. Twetman et al. [15] stated in a recent review that inconclusive evidence was found regarding the effect of fluoride toothpaste in primary dentition. In contrast to our study, for permanent teeth, Dunipace et al. [7] and Wefel et al. [16] found that mineral acquisition by initial white spot lesions increased with an increasing fluoride level.

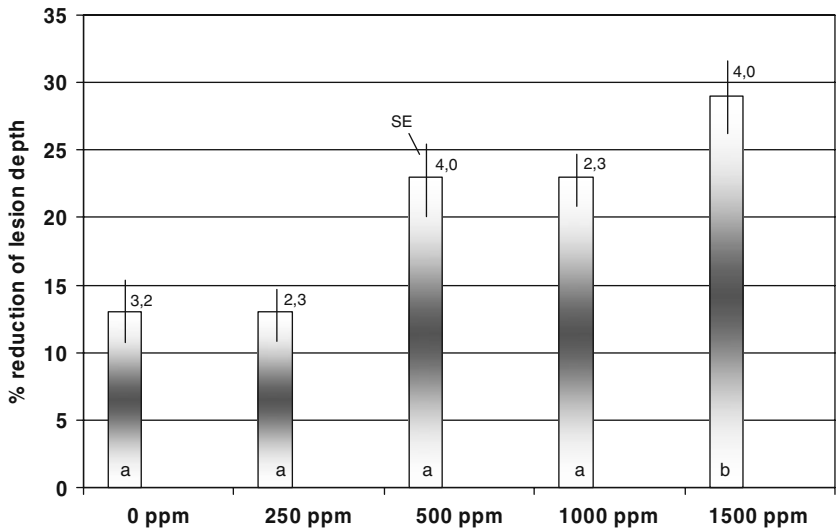

Fig. 2 Percent reduction of lesion depth (µm) in initial carious lesions in deciduous enamel (*bars*=standard error of the means, SE; *different letters* indicate significant difference between the experimental groups)

Recent studies showed that 500 ppm F^- containing dentifrices led to a similar caries reduction compared to standard toothpastes in permanent teeth [12]. It has also been proven in vitro that at least 500 ppm are necessary to protect the enamel of permanent teeth from net demineralization [13]. Bjarnason and Finnbogason [3] found in a randomized clinical study that the progression of already existing carious lesions was similar, after use of 250-ppm- or 1,000-ppm-containing fluoride dentifrice. On the other hand, Peterson et al. [10] could show that 250-ppm-containing toothpaste did not inhibit lesion development when a high number of acid-producing bacteria are present on the respective enamel surfaces. Thus, inherent information about the effectiveness of using a low concentrated fluoride dentifrice is available. Additionally, deciduous teeth exhibit a different behavior according to de- and remineralization [11]. Therefore, the question arose how deciduous enamel behaves concerning remineralization after use of differently concentrated toothpastes. In the present study, it could be shown that the application of a 250-ppm-containing toothpaste did not result in a significantly higher mineral uptake compared to placebo. In a systematic review, Ammari et al. [1] reported about randomized controlled trials comparing low fluoride toothpastes containing 600 ppm F or less with toothpastes containing 1,000 ppm or more in children and adults that 250 ppm fluoride dentifrices are not as effective in caries prevention as dentifrices containing 1,000 ppm fluoride. The results of our study are in accordance with these clinical studies. The cariostatic efficacy of the dentifrice containing 250 ppm fluoride was not superior to that found for 0 ppm F. This may be due to the model chosen for the experiments. In fact, almost 25% of the mineral loss is recovered by salivary exposure alone, with no added fluoride. In a model with demineralization episodes, different results could have occurred. However, the results of the present experimental set up shows that 500 ppm F^- is needed to see a response and this response is not significantly improved with higher F-concentrated products. Nevertheless, it is not clear if mineral recovery has reached a plateau or if an increased experimental time would have resulted in a continued effect. When using 500 ppm fluoride-containing toothpaste, a significantly higher remineralization took place. It was comparable to the mineral uptake in the 1,000 and 1,500 ppm fluoride groups. These results are in accordance with those of Biesbrock et al. [2] who performed placebo-controlled dose response study in 657 subjects. They did not find any evidence of a dose response (1,450 ppm F versus 500 ppm F) according to caries reduction.

Conclusion

From the present study, it can therefore be concluded that it is possible to remineralize initial carious lesions in deciduous enamel in a similar way as it has been described for enamel of permanent teeth and that 500 ppm F^- is needed to see a response.

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Conflict of interest The authors declare that they have no conflict of interest.

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