Clin Oral Invest (2006) 10: 249–252
DOI 10.1007/s00784-006-0053-4

ORIGINAL ARTICLE

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Inhibition of enamel lesion formation by fluoridated milk assessed by laser fluorescence—an in vitro study

Received: 19 November 2005 / Accepted: 25 April 2006 / Published online: 16 May 2006
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Abstract The aim of this study was to investigate the effect of fluoridated milk on enamel lesion formation as assessed by laser fluorescence (LF). The material consisted of 18 extracted premolar teeth that were cut in mesial–distal direction and pairwise assigned to either test or control samples in an experimental caries model. The teeth were exposed to a low-pH 5% cellulose gel for 4 h, 5 days per week immediately followed by a 4-h period in either fluoridated (5 ppm, test) or nonfluoridated milk (control). In the meantime, the specimens were stored in pooled human-stimulated whole saliva in room temperature. All teeth were examined by visual inspection with a magnifying glass and by LF readings (DIAGNOdent) at baseline and after 2 and 4 weeks. The baseline LF readings ranged from 3 to 7 with a mean value of 5.6±0.9. The mean values increased with time in both groups but the increase was more marked in the control teeth, 8.7±2.3 vs 12.8±3.3 after 4 weeks, this difference being statistically significant ($p<0.01$). The visual examination could not distinguish between the test or control samples after 2 and 4 weeks, respectively. The findings indicated that fluoride added to milk may to some extent counteract enamel lesion formation as assessed by LF in an experimental caries model.

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Keywords Enamel demineralization · Enamel lesion · Fluoride · Laser fluorescence · Milk

Introduction

Milk contains compounds that reduce the risk for dental caries [4, 14], and this may be enhanced by the addition of fluoride. Therefore, fluoridated milk schemes for schoolchildren have been implemented, and field studies in Europe, Asia, and South America have shown a substantial decrease in caries increment [3]. In adjunct to the clinical studies, the pharmacokinetics of fluoride ingested with milk has been elucidated [13], and it has been claimed that milk with fluoride is well tolerated and safe from a toxic point of view. The local events in the oral cavity have however attained less attention. In our research group, a series of studies have been performed to investigate the concentration of fluoride in saliva and dental plaque in schoolchildren after a standardized intake of fluoridated milk [5, 18, 24]. We have also evaluated the impact of fluoridated milk on the salivary microbial composition and plaque acidogenicity [6, 7]. Collectively, the findings provide a background for the local anticaries events that occur in saliva and plaque after the intake of fluoride with milk. The next step would be to evaluate fluoridated milk's impact on enamel lesion formation. A number of in vitro and in situ studies have earlier been carried out with cow's milk with intrinsic or added fluoride [2, 10–12, 14, 19, 23], suggesting that milk with fluoride may prevent or reduce enamel demineralization and/or has a beneficial effect on remineralization. As a preparatory study for a clinical setup, we thought it would be of interest to evaluate the effect of fluoridated milk on enamel lesion formation and carry out the assessments with a sensitive device for early caries detection. Laser fluorescence (LF) is today commonly adopted by clinicians as a second opinion especially for occlusal caries [15] but it may also be used as an intermediate endpoint in clinical caries trials [1, 22]. The aim of the present in vitro study was thus to evaluate the use of a caries detection device based on LF to monitor

the effect of fluoridated milk on enamel lesion formation in an experimental caries model. The null hypothesis was that the DIAGNOdent readings would not differ from those obtained with nonfluoridated milk.

Materials and methods

Study group

The material consisted of 18 upper premolars from 11 adolescents, which were extracted on orthodontic indications, and the teeth were voluntarily donated by the patients to the research group. All teeth were extracted in a gentle way with the aid of elevators, and no forceps were used. After extraction and thorough cleaning, the buccal and lingual surfaces of the teeth were inspected with the aid of a magnifying glass, and only those with no visible signs of enamel demineralization, hypomineralization, or discoloration were included. The time that the included teeth had been erupted in the mouth varied from 3 months to 1.5 years. All patients came from a fluoride-deficient area with less than 0.3 ppm F in the piped drinking water.

Experimental caries model

Each tooth was hemisectioned in mesial–distal direction, and the buccal and lingual halves were and randomly designated in pairs as test or control samples. The teeth were then covered with an acid-resistant, transparent nail varnish with the exception of an exposed circular area of 2 mm diameter, approximately 3 mm from the enamel–cementum junction on the buccal or lingual surface. After baseline measurements with LF, the experimental teeth were placed and stored in 2 ml of thawed human whole saliva for 16 h a day. The saliva was previously collected by paraffin chewing during 15 min, pooled from 15 healthy nonmedicating young adults, and stored frozen in 2 ml aliquots at −18°C.

The samples were exposed for a 5% cellulose gel (pH 4.5) for 4 h, 5 days a week during 4 weeks to develop an artificial caries-like subsurface lesion as previously described [17], and each sample was exposed to the gel in a separate jar. After each acid gel exposure and thorough rinsing in sterile saline, the teeth were transferred to 10-ml test tubes with either fluoridated (5 ppm F) or nonfluoridated low-fat milk (0.05%) for another 4-h period. During the rest of the day (16 h) and over the weekends, the samples were stored in the thawed saliva at room temperature. The fluoridated milk was prepared by adding a concentrated aqueous solution of sodium fluoride to fresh standard milk, and the same batch was used for 1 week. The saliva for storage was renewed every second day with the exception on weekends.

Laser fluorescence readings

Each tooth was thoroughly inspected visually with the aid of a 4.5× magnifying glass and scored according to the criteria of Andersson et al. [1]: 0 = no visible color change; 1 = slight white color change, only visible after air drying; 2 = slight color change with certain marked white areas; and 3 = white consistent color change. The LF recordings were carried out at baseline and after 2 and 4 weeks, respectively, with DIAGNOdent, a chair-side laser device from KaVo (Biberach, Germany) allowing reading values from 0–99. One single device and the same broad tip were used throughout the study. First, the instrument was calibrated using a ceramic standard provided by the manufacturer. The measurements were performed after

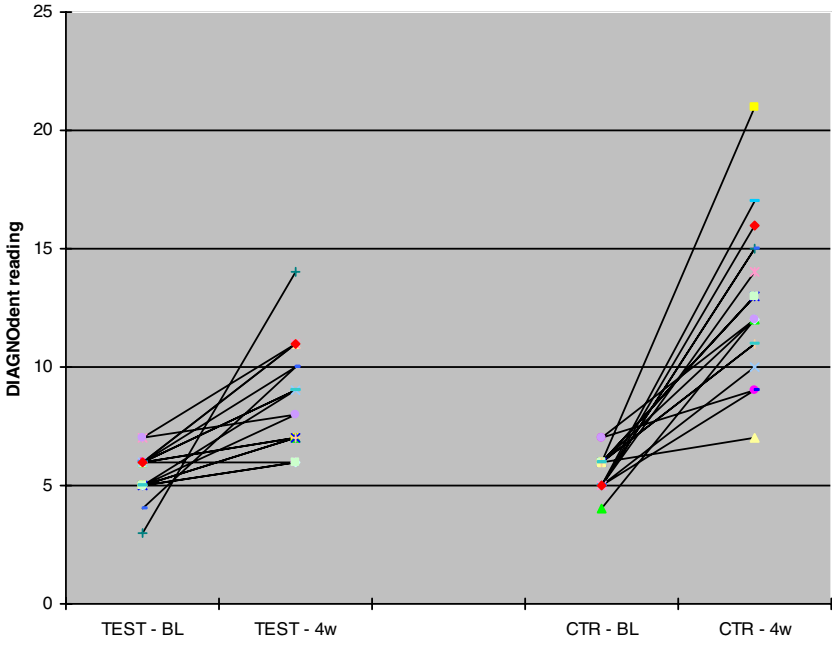

Fig. 1 The individual LF readings at baseline and after 4 weeks of samples subjected to a demineralization gel and immediately exposed to fluoridated (TEST, *n*=18) or nonfluoridated milk (CTR; *n*=18). *BL* denotes baseline and *4w* (4 weeks) is the end of the experimental period

5 s drying with compressed air, and a reference value from intact enamel was obtained. The tip was thereafter applied on the exposed test or control site and moved slightly to obtain the peak value. All teeth were measured twice, and the mean of two values was recorded. At the 4-week registration, the covered enamel area was cleaned from varnish by gentle scaling and measured as described above. All registrations were carried out by the same investigator who was blind to the test or control milk assignment. To verify the intra-examiner reproducibility, all samples were reexamined 7 days after termination of the experiment during which the teeth were stored in neutral saline.

Statistical methods

All data were processed with the Statistical Package for the Social Sciences software (version 12.0, Chicago, IL, USA). The follow-up values in the test and control groups were compared with the aid of nonparametric Wilcoxon paired test. Weighed Cohen's kappa statistics were used to analyze the intra-examiner reproducibility. The level of significance was set to 5% ($p<0.05$).

Results

The weighed Kappa value for intra-examiner agreement of the LF recordings was 0.86. By visual inspection, most samples seemed intact after the experimental period. Six tooth pairs exhibited a slight white color change visible with or without air spray (score 1–2) after 4 weeks but no marked difference was displayed between the test and control teeth. The mean LF values at baseline and after 2 and 4 weeks for the test and control sites are shown in Table 1. There was no difference between the test and control sites at baseline. The mean DIAGNOdent values increased by time at most test and control sites, which was seen as an indication of an increasing lesion formation. The increase was however more marked among the controls, and the difference between the test and control sites was statistically significant after 2 and 4 weeks ($p<0.05$ and $p<0.01$, respectively). The individual scores at baseline and after 4 weeks are illustrated in Fig. 1. When the paired samples were compared after 4 weeks, lower laser fluorescent readings were recorded for the fluoridated milk-treated samples when compared with the controls in all cases but two.

Discussions

The present study was undertaken to evaluate whether a caries detection device based on LF could be used to monitor the effect of fluoridated milk on enamel lesion formation in an experimental model. The detection is based on the fact that emitted laser light is absorbed by both inorganic and organic tooth substances and by metabolites from oral bacteria [8]. A recent study has suggested that this device would be a valuable tool for monitoring the outcome of preventive interventions [22]. The intra-examiner reproducibility on the experimental smooth surfaces was found to be excellent in the present study which was in harmony with previous findings [20], and we used one single device to avoid reported variations between the instruments. It must however be stressed that the device was measuring the organic components of a lesion rather than the actual mineral loss [8, 21]. Milk, as well as saliva, contains a number of organic components and bacteria, and it was therefore likely that both the milk and the storage media may have influenced and enhanced the obtained laser fluorescent readings by diffusion into the early porous enamel lesions.

The first and obvious finding of this investigation was that it was possible to verify an inhibition of enamel lesion formation by fluoridated milk already after 40 h of acid exposure and before any clearly visible clinical signs of demineralization were seen. The null hypothesis could therefore be rejected, and the results confirmed those of previous in vitro studies [2, 12, 14, 23]. Secondly, the findings support the assumption that fluoride is additive to the beneficial effects of milk per se. A previous study has shown that demineralization of enamel in an acid buffer was reduced by intermittent exposure to milk when compared with saline and that milk aided the remineralization of demineralized enamel [16]. One should however bear in mind that in vitro lesions are only simulations and quite far from the lesions formed in the real-life situation when the enamel surface is covered by a complex biofilm [9]. Therefore, the findings must be regarded with caution. Because the LF diagnostic system is primarily developed for chair-side use, our present results justify a clinical setup in which not only the effect of fluoridated milk on smooth

Table 1 Mean values of DIAGNOdent readings in permanent premolars (n=18) exposed to either fluoridated (test) or nonfluoridated milk (control) in an experimental caries model at baseline and after 2 and 4 weeks

Time	Test mean±SD (range)	Control mean±SD (range)	Reference[a] mean±SD (range)
Baseline	5.5±1.0 (3–7)	5.6±0.8 (4–7)	NM
2 weeks	6.6±1.3 (5–9)	7.9±2.0[b] (7–12)	NM
4 weeks	8.7±2.2 (6–14)	12.8±3.3[b] (7–21)	5.5±0.8 (4–7)

[a]Enamel measurement after removal of covering varnish
[b]Statistically significant difference between test and control ($p<0.05$), paired Wilcoxon test
NM Not measured

surfaces (white spot lesions) on first hand, albeit also occlusal surfaces can be evaluated.

Two of the included teeth pairs (#8 and #12) exhibited a somewhat different pattern as they exhibited higher LF values on the test samples than on the corresponding controls. They were not from the same individual, and we have at the moment no good explanation for these unexpected findings but they clearly illustrate the fact that there is an individual response to all interventions even in the laboratory setting.

In conclusion, the present in vitro findings suggest that the presence of fluoride in milk may counteract enamel lesion formation as assessed by an LF caries detection device. The findings confirm that milk seem to be a suitable vehicle for fluoride administration and suggest that LF is a simple and rapid method to quantify a preventive measure over time. Further investigations with LF caries detection are however required to elaborate the effect of fluoridated milk on lesion formation in vivo.

Acknowledgement This study was supported by a grant from the Borrow Foundation, Cowplain, England.

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