Fluoride concentration in supragingival dental plaque after a single intake or habitual consumption of fluoridated milk

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The aim of this study was to evaluate the fluoride concentration in supragingival dental plaque after single and repeated intakes of fluoridated milk. The study group consisted of 22 schoolchildren, young adults and adults of both sexes, 8–41 years of age. After a 2-week fluoride depletion period and 3 days of plaque accumulation, 200 ml of fluoridated milk (1g F) was ingested along with a standardized lunch meal. Plaque samples were collected immediately before the intake and after 30, 120 and 240 min. From the adult participants ($n = 9$), additional samples were colleted after 12 and 18 h. After a fluoride-free washout period of at least 2 weeks, the whole experimental procedure was repeated after 4 consecutive daily intakes of fluoridated milk. The fluoride concentration was determined after micro-diffusion with a fluoride selective electrode. The results showed a statistically significant 3-fold increase of the plaque fluoride levels up to 4 h after the intake. At 12 and 18 h after the intake, the recorded levels went gradually back to baseline. There was no significant difference between the fluoride concentrations in the supragingival plaque after the single intake compared with the repeated intakes. In conclusion, the findings support the suggestion that milk is a suitable vehicle for local fluoride administration into the oral cavity, also when consumed together with a meal. □ *Fluoride; dental plaque; milk*

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The concept of the anti-caries mechanism of fluoride has undergone a paradigmatic shift during the past decade (1). The systemic effect has been downgraded and the local mode of action has been correspondingly upgraded. The current view is that fluoride has to be present in saliva and plaque fluid during the caries challenge to slow down the dissolution of the enamel and enhance the precipitation phase (2). After topical administration, calcium fluoride-like deposits are formed as a reservoir on the tooth surface as a pH controlled release system (3). Milk supplemented with fluoride may in that context be an excellent vehicle to retain fluoride in the oral tissues (4–6). It has been suggested that milk itself may have cariostatic properties (7, 8), and a recent in vitro study has indicated that fluoridated milk can increase pH in oral biofilms and reduce the proportion of mutans streptococci (9). Our present knowledge on the effective concentration of fluoride in the oral cavity is limited, however (10), and it is thus important to establish the concentration of fluoride in dental plaque following different modes of administration. In previous studies, the fluoride concentration in whole saliva, separate gland secretions and labial glands was determined after intake of fluoridated milk (11, 12). The results from those studies showed that fluoride ingested with milk was excreted through the salivary glands, suggesting that the bioavailability of fluoride from milk was equal to other vehicles. Furthermore, a comparative study on the fluoride concentration in whole saliva and dental plaque after rinsing with fluoridated milk and sodium fluoride solutions was performed in schoolchildren (13). Plaque samples were collected 2 h after the rinse and significantly elevated levels of fluoride compared with baseline were recorded. It was therefore of interest also to determine the fluoride concentration in dental plaque at normal milk intake conditions. The aim of this study was to determine the time curve of fluoride concentration in dental plaque collected at designated time intervals after a single intake and habitual consumption of fluoridated milk together with a regular meal.

Material and methods

The study group comprised 22 healthy volunteers of both sexes (8 M and 14 F) with a mean age of 15.5 years (range 8–41 years). After verbal and written information, consent was collected from all participants as well as from the parents of the children. The study protocol was approved by the Ethics Committee of Lund University, Sweden. All subjects were inhabitants in a community with low fluoride (0.1 ppm) content in the piped drinking water and used fluoridated toothpaste (1.000–1.500 ppm F) twice a day. The oral health of the participating subjects was non-compromised and they had at least 24 teeth erupted. During the study period, a 14-year-old boy dropped out, for personal reasons, and the final results are thus based on 21 subjects.

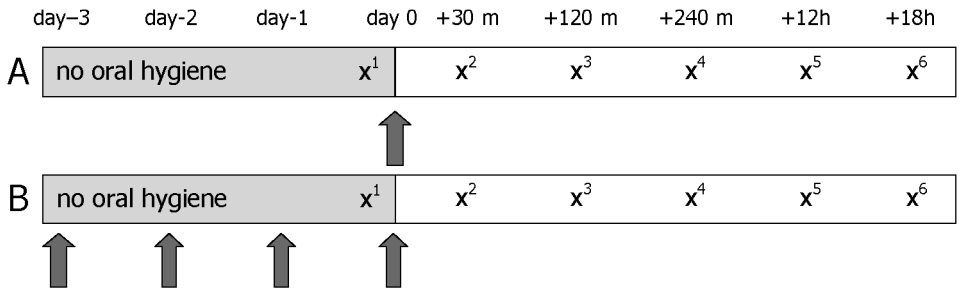

Fig. 1. Study design shown as flow-chart for fluoridated milk intake (arrows) and plaque sampling (x^n). The single (A) and habitual consumption (B) experiments were separated by a washout period of at least 2 weeks.

Study design

The investigation had a longitudinal crossover design (see Fig. 1). Two weeks prior to the experiments, the subjects were provided with fluoride-free toothpaste and instructed to use it during the whole study period, except for a run-in period of 3 days, with no oral hygiene before each experimental plaque-sampling day. The subjects were given a list of fluoride-rich food and liquids and asked to avoid that diet during the entire study period. A baseline plaque sample was collected from one randomly selected lower quadrant immediately before the single milk intake. After 30, 120 and 240 min, respectively, the plaque sampling procedure was repeated from one of each remaining quadrant. From 9 of the adolescent and adult participants, samples were also collected at 12 and 18 h after the milk ingestion. The same protocol was repeated after the 4-day consumption and the two regimens were separated by a fluoride-free washout period of at least 2 weeks.

Plaque sampling and fluoride measurements

Supragingival plaque samples from the buccal sites of the teeth were collected with a sterile curette and special care was taken to avoid gingival bleeding. The plaque from each quadrant was pooled in a plastic micro-container that was weighed before and after collection, on a microbalance, in order to determine the wet weight of the sample. The sample was then frozen and kept at −70°C until further analysis. The concentration of fluoride was determined with a fluoride-sensitive electrode (96-09; Orion Research, Cambridge, MA, USA) according to the micro-diffusion method described by Taves (14). After thawing, 1.0 ml of the sample was placed in a diffusion vessel with constant stirring for 6 h at room temperature in sealed Petri dishes together with 2.0 ml of 4 mol/l $HClO_4$ saturated with hexamethyldisiloxane (HF). HF was adsorbed to the lids that were spotted with 50 µl 0.5 mol/l NaOH. Following diffusion, the lids were dried with an exsiccator. The dried layer was dissolved with 50 µl 0.5 mol/l HCl and 100 µl HAc buffer (pH 5.0) and fluoride concentration was then measured with the electrode and expressed as ng F/mg wet weight (ppm). All determinations were made in duplicate. The precision of the method was verified by repeated measurements and was calculated to ±4% above 10 ng F, ±9% between 5–10 ng F and ±23% at concentrations below 5 ng F.

Preparation and intake of milk

The fluoridated milk was prepared by adding a concentrated aqueous solution of sodium fluoride to fresh standard milk (3% fat, 5% carbohydrates). For the single intake, 200 ml milk containing 1 mg F (5 ppm) was served in a drinking glass along with a standardized lunch meal consisting of macaroni and Swedish meatballs. During the habitual milk consumption, the same preparation and amount was given at lunchtime for 3 consecutive days prior to the experimental day.

Table 1. Fluoride concentration (mean ± SE) in supragingival dental plaque (ng F/mg wet weight) at designated times after a single intake or habitual consumption of fluoridated milk

Mode	Baseline $n = 21$	30 min $n = 21$	120 min $n = 21$	4 h $n = 21$	12 h $n = 9$	18 h $n = 9$
Single intake	5.9 ± 0.8	17.3[a] ± 2.5	18.3[a] ± 3.1	17.2[b] ± 4.3	10.4 ± 2.1	7.7 ± 2.0
Habitual consumption	6.1 ± 1.3	15.2[a] ± 2.4	17.3[a] ± 3.4	19.1[b] ± 4.6	11.9 ± 4.1	8.3 ± 1.8

[a] Statistically significant difference compared with baseline, $P < 0.01$.
[b] Statistically significant difference compared with baseline, $P < 0.05$.

Statistical methods

The fluoride concentrations in the post-ingestion samples were compared with the baseline value within each regimen with ANOVA for repeated measures using the SPSS 10.0 software program. Analysis of variance was also used to test the possible influence of age.

Results

The concentration of plaque fluoride at baseline and at the designated time intervals after intake of fluoridated milk is presented in Table 1. The baseline levels were low (5.5–6.4 ng F/mg wet weight), but a statistically significant 3-fold increase to over 15 ng F/mg wet weight was evident after 30, 120 and 240 min. After 12 h, the fluoride levels were still insignificantly elevated, but almost back to the baseline values again after 18 h. There was no statistically significant difference between the fluoride concentrations following the single intake compared with the habitual intakes of milk at any of the sampling occasions. There were considerable and consistent inter-individual variations in the plaque fluoride concentrations, but no age-related differences were disclosed within the study group.

Discussion

The present study was undertaken to establish a time-curve of fluoride concentration in plaque after a single intake of fluoridated milk and to evaluate whether or not an accumulation of fluoride took place following habitual milk consumption. Prior to the samplings, plaque was allowed to accumulate during a 3-day period without any self-performed oral hygiene measure. The study group consisted mainly of schoolchildren, as they are the primary target group for a fluoridated milk scheme. In order to be able to monitor the plaque fluoride concentration over a 24-h period, a number of young adults and adult persons were included. The age variation did not affect the results, however, since no age-related differences in plaque fluoride levels were unveiled. The amount of fluoride in the milk was chosen since several clinical anti-caries studies have been conducted with this regimen (15, 16). The precision of the fluoride assays was acceptable ($<\pm 10\%$) within the range of most samples.

In agreement with our previous findings (13), a statistically significant increase in the fluoride concentration in dental plaque was evident after a single intake of fluoridated milk and the levels were of the same magnitude as a water-based mouth rinse with the same amount of fluoride. The novel finding was that the elevation was present 30 min after the intake and that it remained so for several hours. After 12 and 18 h, the plaque fluoride concentration gradually approached the baseline levels again. It should be stressed, however, that the individual variations were considerable, which could be explained by factors such as variations in diet, saliva secretion rate and composition, plaque-forming rate and microbial composition. The baseline values represented a fluoride-depleted plaque, since all sources of fluoride were avoided for a 2-week period prior to the experiments. The "normal" fluoride concentration in plaque, from individuals living in an area with suboptimal fluoride levels, is reported to be around 10 ng F/mg (17). Interestingly, the post-ingestion levels of fluoride in the supragingival plaque in this study were of the same magnitude as in our previous study (13) although the mode of intake differed substantially. In that experiment, the subjects were asked to actively rinse the mouth with the milk, while in this study, the milk was taken without any particular instructions in connection with a regular meal. This finding strongly indicates that the mode of intake is not crucial and that a milk delivery program for schools seems highly appropriate (18).

The finding that the levels were comparable after a single intake and habitual consumption was important. This might indicate that the 3-day supragingival plaque was saturated with fluoride shortly after the ingestion and obviously bound in a reversible way. It has also been suggested that the oral mucosa may act as a fluoride reservoir that can be slowly released and recharge the dental plaque (19). This was supported by the fact that no peak value was disclosed in the plaque but rather prolonged, continuously elevated values for more than 4 h. From a clinical point of view, our results underline that fluoridated milk must be administrated daily to secure caries-protective levels in the oral cavity. This is worth consideration in the planning and implementation of school-based dental milk protocols. Extended summer holidays or low attendance of pupils are factors that might influence the effectiveness of such programs.

In conclusion, the present findings showed that significantly elevated fluoride levels in supragingival dental plaque were evident for at least 4 h after intake of fluoridated milk. Therefore, the results are supportive to the suggestion that milk, when consumed together with a meal, is a suitable vehicle for local fluoride administration into the oral cavity.

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