

Fluoride concentration in whole saliva and separate gland secretions after topical treatment with three different fluoride varnishes

Svante Twetman, Kerstin Sköld-Larsson and Thomas Modéer

Departments of Pediatric Dentistry, Centre for Oral Health Sciences, Malmö University, Malmö, Sweden; Faculty of Odontology, Karolinska Institutet, Huddinge, Sweden; and Medical and Dental Health Center, Halmstad, Sweden

Twetman S, Sköld-Larsson K, Modéer T. Fluoride concentration in whole saliva and separate gland secretions after topical treatment with three different fluoride varnishes. *Acta Odontol Scand* 1999;57: 263–266. Oslo. ISSN 0001-6357.

Fluoride concentration in whole saliva and in separate gland secretions was determined after a single application of each of 3 different fluoride varnishes with contrasting levels of fluoride in a randomized crossover design. The study group comprised 8 healthy schoolchildren aged 10–12 years treated with A: Bifluorid[®] 12 (6% F); B: Duraphat[®] (2.26% F); and C: Fluor Protector[®] (0.1% F). Unstimulated and stimulated whole saliva, as well as stimulated parotid and submandibular-sublingual saliva, were collected at baseline and 1, 6, 12, and 24 h after the varnish treatments. The fluoride concentrations were determined with an ion-selective electrode. Time- and dose-dependent concentration curves were obtained in all the collected secretions, A > B > C. In whole saliva, the fluoride levels were significantly elevated ($P < 0.01$) 1 h after the A and B varnish applications compared with baseline, while the increase was insignificant for varnish C. Similar patterns were unveiled in the parotid and submandibular-sublingual secretions, although the increase in fluoride concentration was modest. The elevated levels did not exceed 6 h for any of the varnish tested. The results of this study suggest a correlation between the concentration of fluoride of the varnish and fluoride levels obtained in saliva after application. □ *Fluoride; preventive dentistry; saliva secretion; salivary glands; varnish*

S. Twetman, Department of Pediatric Dentistry, Medical and Dental Health Center, Länssjukhuset, SE-301 85 Halmstad, Sweden. Tel. +46 35 134020, fax. +46 35 215159, e-mail. svante.twetman@lthalland.se

Today, there is a body of evidence showing that preventive care rather than an operative approach should be used to control initial caries lesions (1). Local administration of fluoride varnish constitutes a non-surgical method decreasing the demineralization and enhancing the remineralization of enamel (2, 3). The clinical efficacy of fluoride varnish in the treatment of caries is well established (for reviews, see 4, 5) and, based on these findings, regimens with varnish administration every 3rd or 6th month have been recommended. However, there are presently several varnish brands of fluoride available on the European market, all with different contents, but data on the subsequent fluoride concentration in saliva are sparse (6). It is the responsibility of the clinician to minimize the risk for side effects, and the oral and total fluoride exposure after varnish applications is important information especially in small children. The aim of the present study was therefore to investigate the fluoride concentration in whole saliva and separate gland secretions in schoolchildren after professional treatment with each of three different commercial fluoride varnishes with contrasting levels of fluoride.

Material and methods

Subjects

Eight healthy girls, 10–12 years of age (mean age 11.1 years), were invited to participate in the study. The

children and their parents consented after verbal and written information on the aim and performance of the investigation. The children were residents in an area with a low natural fluoride content (0.1 ppm) in the piped drinking water and normally used a fluoridated toothpaste (1000–1500 ppm F) twice daily. The subjects had good oral health with no manifest caries lesions. The study protocol was approved by the Ethics Committee, Lund University, Sweden.

Study design

A randomized crossover (Latin square) design was used. The concentration of fluoride was determined in saliva at baseline and 1, 6, 12, and 24 h after a single topical application of each of 3 different fluoride varnishes. The test periods were separated by a washout period of 6 weeks.

Fluoride varnish treatment

The following fluoride varnishes were used: Bifluorid[®] (6% F, Voco GmbH, Germany), Duraphat[®] (2.26% F, Inpharma, Germany), and Fluor Protector[®] (0.1% F, Vivadent, Liechtenstein). The varnishes were purchased and used in accordance with the manufacturer's recommendations. Subjects were asked to refrain from fluoride-rich food and drinks, fluoride rinses and tablets, and to use fluoride-free toothpaste for a period of 2 weeks prior to

Table 1. Fluoride concentration (mean ppm \pm SE) in unstimulated whole saliva in schoolchildren after application of each of 3 different fluoride varnishes

Time	Bifluoride [®]	Duraphat [®]	Fluor Protector [®]
Baseline	0.08 \pm 0.08	0.10 \pm 0.10	0.05 \pm 0.04
1 h	31.40* \pm 13.6	13.37* \pm 4.70	0.33 \pm 0.47
6 h	0.12 \pm 0.08	0.34 \pm 0.37	0.05 \pm 0.03
12 h	0.05 \pm 0.02	0.17 \pm 0.16	0.04 \pm 0.02
24 h	0.05 \pm 0.01	0.08 \pm 0.07	0.03 \pm 0.02

* Statistically significant ($P < 0.01$) elevation compared with baseline.

and throughout the test period. After the baseline samplings, but before the varnish applications, all teeth were professionally cleaned with a rubber cup and pumice without added fluoride. The interdental sites were cleaned with a dental floss. After air-spray drying, the varnishes were applied quadrant by quadrant in a thin layer on all teeth along the buccal and lingual gingival margin, interdentially and on the occlusal molar and premolar surfaces. The varnishes were then allowed to set for a few minutes and the children were asked to refrain from eating and drinking for the next 3 h and to omit toothbrushing during the 24-h test period. Approximately 0.5 ml of the Bifluoride and Fluor Protector varnishes were used for the single full mouth treatment, while the Duraphat applications required 0.75 ml of the varnish. This corresponded to a total dose of 30 mg F, 1 mg F, and 17 mg F, respectively.

Saliva sampling

On each sampling occasion, saliva was obtained in the following way: unstimulated whole saliva was collected by passive drooling into chilled test tubes during a 10-min period; paraffin-stimulated whole saliva was then collected for 5 min of active chewing; stimulated parotid saliva was thereafter collected by means of modified Carlson-Crittenden cups (7) and stimulated submandibular-sublingual saliva in an individual device according to the method of Nederfors and Dahlöf (8), during 5–10 min each. Parotid and submandibular-sublingual secretions were stimulated with 3% citric acid solution applied to the back of the tongue in a standardized way. The secretion rates were determined with a gravimetric method and expressed as ml/min. Thereafter, 1.0 ml aliquots of all samples were transferred to small fluoride-free plastic jars and immediately frozen and kept at -70°C until further processing.

Fluoride analysis

After thawing, the whole saliva samples were centrifuged for 3 min at $8,740 \times g$ (Microfuge B Centrifuge, Beckman Instruments Inc., Palo Alto, CA, USA). The concentration of fluoride in the saliva samples was determined with a fluoride-sensitive electrode (96–09,

Table 2. Fluoride concentration (mean ppm \pm SE) in stimulated whole saliva in schoolchildren during 24 h after application of each of 3 different fluoride varnishes

Time	Bifluoride [®]	Duraphat [®]	Fluor Protector [®]
Baseline	0.09 \pm 0.08	0.10 \pm 0.09	0.08 \pm 0.06
1 h	11.76* \pm 9.47	39.2* \pm 9.82	0.25 \pm 0.25
6 h	0.13 \pm 0.08	0.76 \pm 0.89	0.05 \pm 0.03
12 h	0.07 \pm 0.05	0.37 \pm 0.44	0.04 \pm 0.03
24 h	0.05 \pm 0.04	0.08 \pm 0.06	0.05 \pm 0.03

* Statistically significant ($P < 0.01$) elevation compared with baseline

Orion Research, Cambridge, MA, USA), standardized in the range of 0.1–5.0 $\mu\text{mol/l}$ F (9). All determinations were performed in duplicate. Several standard points were used below 1.0 $\mu\text{mol/l}$ in order to construct the non-linear part of the standard curve. Fifty microliters of 7.5 mol/l acetate buffer (pH 5.0) containing 2% CDTA was added to 450 μl of the standard solution of the sample. The precision of the method has been shown to be 8.3% and 13.3% (coefficient of variation) at 0.89 $\mu\text{mol/l}$ and 0.40 $\mu\text{mol/l}$, respectively (10).

Statistical methods

The data were processed using the non-parametric Wilcoxon paired test.

Results

The mean secretion rates in unstimulated and chewing stimulated whole saliva were 0.4 and 1.2 ml/min, respectively. The corresponding values for the stimulated parotid saliva were 1.0 ml/min and 1.3 ml/min for the submandibular-sublingual secretion. The baseline fluoride values ranged from 0.05 to 0.1 ppm in whole saliva and from 0.01 to 0.02 ppm in the separate gland secretions. The salivary fluoride concentration determined up to 24 h after a single topical application of each of the 3 different varnishes is presented in Tables 1 and 2 and Figs 1 and 2. In unstimulated whole saliva and in the separate gland secretions, time- and dose-dependent concentration curves were demonstrated, reflecting the fluoride content of the varnish. The fluoride increase was proportionally higher in whole saliva than in the separate gland secretions. After 1 h, a statistically significant ($P < 0.01$) increased fluoride concentration was found compared to baseline in stimulated and unstimulated whole saliva following the Bifluoride and Duraphat treatments. A slight increase was seen also after the Fluor Protector application, but the elevation was not statistically significant. Similar results were unveiled in the parotid and the submandibular-sublingual secretions, although the increase in fluoride concentration was modest. In general, all recorded values

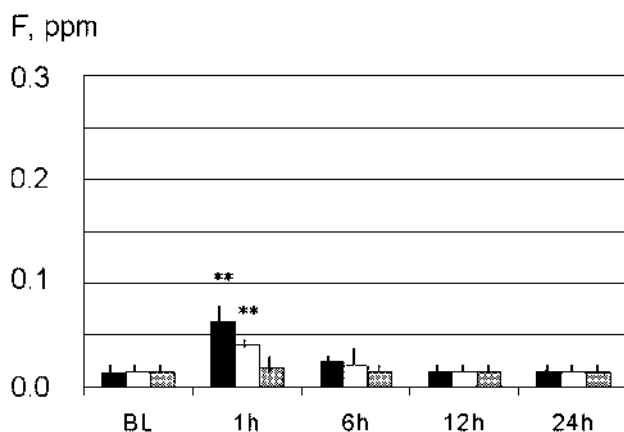


Fig. 1. Mean fluoride concentration (ppm F) in stimulated parotid saliva in schoolchildren after a single topical application of each of 3 different fluoride varnishes (Bifluoride ■; Duraphat □; Fluor Protector ▨). Vertical bars indicate the standard error of the mean (** $P < 0.01$).

were back to the baseline levels within 6 h after the topical professional varnish applications.

Discussion

The present study was performed to gain information on the fluoride concentrations in whole saliva and separate gland secretions after routine treatments with fluoride varnishes with contrasting levels of fluoride. The whole saliva represented the amount of fluoride locally present in oral tissues as well as its clearance after exposure. The separated gland secretions, on the other hand, reflected the amount of fluoride ingested and excreted. We were aware that not only the fluoride concentrations but also the amount of varnished used for the application differed, making a direct comparison between the 3 brands difficult. However, the purpose was to compare the fluoride levels obtained in the clinical situation. Variations in salivary flow rate and the frequent samplings were factors that may also have affected the results. The crossover study design, however, where all subjects were treated with the different varnishes, diminished the influence of secretory differences between the individuals. Unfortunately, the consistency and taste of the varnishes did not allow a blind study design. The participants, however, were not informed about either the brands or the sequence of the treatments.

The main finding of this study was that the fluoride levels in saliva correlated to the fluoride content of the varnish. The fluoride concentrations found in whole saliva were significantly higher than in the duct secretions, and, in agreement with a previous observation (11), higher levels were obtained in submandibular-sublingual saliva than in the parotid secretion. The time of elevated levels of fluoride in whole saliva was extended compared to

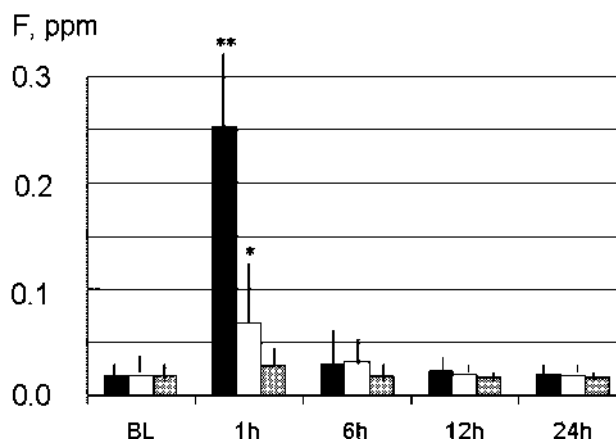


Fig. 2. Mean fluoride concentration (ppm F) in stimulated submandibular-sublingual saliva in schoolchildren after a single topical application of each of 3 different fluoride varnishes (Bifluoride ■; Duraphat □; Fluor Protector ▨). Vertical bars indicate the standard error of the mean (** $P < 0.01$; * $P < 0.05$).

previous findings after various home-care fluoride procedures (12, 13), but, notably, the fluoride levels were back to baseline values after 6 h. The relative short-term increase could possibly be explained by the fast-setting and slow-releasing properties characterizing the dental varnish formulas. Furthermore, the small alterations in fluoride concentration unveiled in the separate gland secretions were consistent with the assumption that the bioavailability of fluoride from ingested varnish is low (14, 15). Although the peak value may probably occur earlier than after 1 h, fluoride varnish applications with all the tested brands can be regarded as safe for schoolchildren from a toxicological point of view. It is noteworthy that no adverse effects or mucous reactions were seen in the present study.

The different fluoride levels demonstrated in saliva after the varnish applications were interesting in light of the clinical efficacy reported from various trials. Duraphat is the most investigated varnish and a meta-analysis has suggested an overall caries reduction of 38% in the permanent dentition (16). Previous studies with Fluor Protector have indicated a protective role but of less magnitude than Duraphat (17–19). The documentation of the efficacy of Bifluoride is still very limited (20). Ideally, the clinician must be encouraged to select or prescribe the least possible fluoride dose to achieve a maximal effect. In reality this is difficult, since the present knowledge on the effective concentration of fluoride in the oral environment is sparse (21). In vitro studies have suggested that very low levels of fluoride in saliva and plaque fluid can enhance remineralization (22–24). On the other hand, the minimal effective concentration that promotes remineralization might vary with respect to the caries activity of the individual and the pH drop in plaque after carbohydrate exposure (25). The use of fluorides in caries prevention regarding delivery frequency and concentration should

therefore be based on the severity of the caries problem of the individual.

A notable finding was that the Duraphat application resulted in the highest fluoride concentration in stimulated whole saliva after 1, 6, and 12 h. This can be explained by the fact that excessive material of this creamy varnish was lost and expectorated during chewing and saliva sampling. Normally, this problem is dealt with by asking the patient not to chew for 3 h after a topical treatment, and this message must be emphasized when using the Duraphat varnish.

In summary, the present study demonstrated that topical treatments with dental varnishes with contrasting fluoride levels resulted in time- and dose-dependent concentration curves in unstimulated whole saliva and in separate gland secretions. The duration of the elevated fluoride levels was slightly longer than in earlier reports of various home-care fluoride regimens, but did not exceed 6 h for any of the varnishes tested. A further and crucial issue is of course the levels of fluoride that are obtained in the plaque fluid after varnish applications and such studies are in progress.

Acknowledgements.—The authors are grateful to Mrs Anna-Karin Svensson for conducting the saliva samplings and Mr Can Yurdunuseven for his skilled laboratory work.

References

- Anusavice KJ. Efficacy of nonsurgical management of the initial caries lesion. *J Dent Educ* 1997;61:895–904.
- Fejerskov O, Thylstrup A, Larsen AJ. Rational use of fluorides in caries prevention. A concept based on possible cariostatic mechanisms. *Acta Odontol Scand* 1981;39:241–9.
- Øgaard B, Duschner H, Ruben J, Arends J. Microradiography and confocal laser scanning microscopy applied to enamel lesions formed in vivo with and without fluoride varnish treatment. *Eur J Oral Sci* 1996;104:378–83.
- Petersson LG. Fluoride mouth rinses and fluoride varnishes. *Caries Res* 1993;27 Suppl 1:35–42.
- Petersson LG, Twetman S, Pakhomov GN. Fluoride varnish for community-based caries prevention. World Health Organization WHO/NCD/ORH/FV/97.1, Geneva 1997.
- Petersson LG, Ludvigsson N, Ullbro C, Gleerup A, Koch G. Fluoride clearance of whole saliva in young schoolchildren after topical application. *Swed Dent J* 1987;11:95–101.
- Carlson AJ, Crittenden AC. The relation of ptyalin concentration to the diet and to the role of secretion of saliva. *Am J Physiol* 1910;26:169–77.
- Nederfors T, Dahlöf C. A modified device for collection and flow-rate measurements of submandibular-sublingual saliva. *Scand J Dent Res* 1993;101:210–4.
- Ekstrand J. A micromethod for the determination of fluoride in blood plasma and saliva. *Calcif Tissue Res* 1977;23:225–8.
- Oliveby A, Twetman S, Ekstrand J. The diurnal fluoride concentration in whole saliva in children living in a high and a low fluoride area. *Caries Res* 1990;24:44–7.
- Twetman S, Nederfors T, Petersson LG. Fluoride concentration in whole saliva and separate gland secretions in schoolchildren after intake of fluoridated milk. *Caries Res* 1998;32:412–6.
- Zero DT, Raubertas RF, Pedersen AM, Fu J, Haynes AL, Featherstone JDB. Fluoride concentrations in plaque, whole saliva and ductal saliva after applications of home-use fluoride agents. *J Dent Res* 1992;71:1768–5.
- Seppä L, Salmenkivi S, Hausen H. Salivary fluoride concentration in adults after different fluoride procedures. *Acta Odontol Scand* 1997;55:84–7.
- Ekstrand J, Koch G, Petersson LG. Plasma fluoride concentration and urinary fluoride excretion in children following application of the fluoride containing varnish Duraphat. *Caries Res* 1980;14:185–9.
- Rölla G, Øgaard B, Cruz R. Topical application of fluorides on teeth. New concepts of mechanisms of interaction. *J Clin Periodontol* 1993;20:105–8.
- Helffenstein U, Steiner M. Fluoride varnishes (Duraphat): a meta-analysis. *Community Dent Oral Epidemiol* 1994;22:1–5.
- De Bruyn H, Arends J. Fluoride varnish—a review. *J Biol Buccale* 1987;15:71–82.
- Twetman S, Petersson LG, Pakhomov GN. Caries incidence in relation to salivary mutans streptococci and fluoride varnish applications in preschool children from low- and optimal-fluoride areas. *Caries Res* 1996;30:347–53.
- Petersson LG, Twetman S, Pakhomov GN. The efficiency of semiannual fluoride varnish applications: a two-year clinical study in preschool children. *J Public Health Dent* 1998;58:57–60.
- Borutta A, Künzel W, Rübsam F. Kariesprotektive Wirksamkeit zweier Fluoridlacke in einer klinisch kontrollierten Zweijahrestudie. *Dtsch Zahn-Mund-Kieferheilk.* 1991;79:543–9.
- Ismail AI. What is the effective concentration of fluoride? *Community Dent Oral Epidemiol* 1995;23:245–51.
- Borsboom PCF, van der Mei HC, Arends J. Enamel lesion formation with and without 0.12 ppm F in solution. *Caries Res* 1985;19:396–402.
- Page DJ. A study of the effect of fluoride delivered from solution and dentifrices on enamel demineralization. *Caries Res* 1991;25:251–5.
- Featherstone JDB. Prevention and reversal of dental caries: role of low level of fluoride. *Community Dent Oral Epidemiol* 1999;27:31–40.
- Lammers PC, Borggreven JPM, Driessens FCM. Influence of fluoride and pH on in vitro remineralization of bovine enamel. *Caries Res* 1992;26:8–13.

Received for publication 28 June 1999

Accepted 31 August 1999