Effects of a sodium fluoride solution and a varnish with different fluoride concentrations on enamel remineralization in vitro

LIISA SEPPÄ

Faculty of Dentistry, University of Kuopio, Kuopio, Finland

Seppä L: Effects of a sodium fluoride solution and a varnish with different fluoride concentrations on enamel remineralization in vitro. Scand J Dent Res 1988; 96: 304–9.

Abstract – To study the efficacy of sodium fluoride varnishes and a NaF solution in remineralization of enamel, 120 slabs of non-carious human enamel enamel were presoftened for 6 h and randomly divided into six groups. The slabs were stored in synthetic saliva for 9 days, except for a daily 30-min immersion in 0.1 M lactic acid-NaOH buffer. During the 9-day period, one group of the slabs received no treatment, and the rest were treated once or three times with 2.3% or 1.1% sodium fluoride varnish Duraphat, or nine times with a 0.1% NaF solution. Finally, the slabs were demineralized for 1 h, and the amount of dissolved Ca and F was determined. Microhardness of enamel was determined initially, after presoftening, after the 9-day period, and after the 1-h demineralization. All fluoride treatments prevented enamel softening almost completely during the 9 days, but the control slabs softened markedly. Fluoride varnishes were more effective than NaF solution. Three applications of 2.3% Duraphat were slightly more effective than any of the other varnish treatments, but one treatment with 2.3% varnish was not more effective than treatments with 1.1% varnish. Enamel treated three times with 1.1% varnish showed the greatest acid resistance during the 1-h demineralization. The results suggest that the efficacy of the varnish was not proportional to the fluoride concentration but rather to the number of applications. Fluoride uptake by enamel was greatest with the most concentrated varnish. Enamel solubility was not, however, directly proportional to the fluoride content of enamel.

Key words: enamel, remineralization; fluoride, solution, varnish.

Faculty of Dentistry, University of Kuopio, P.O.B. 6, SF-70211 Kuopio, Finland.

Accepted for publication 3 February 1988.

In Scandinavia, the sodium fluoride varnish Duraphat is commonly used for caries prevention and remineralization of initial caries lesions. The regimen for remineralization varies; some dentists apply the varnish at an interval of 3–6 months, and others use repeated applications during a treatment period of a few weeks. Although Duraphat has proved effective in caries prevention (1), the efficacy of the different techniques in

enamel remineralization has not been studied. BODDE *et al.* (2, 3) have suggested that repeated applications and even a single application of concentrated fluorides may inhibit the long-term remineralization of enamel by inhibiting the in-depth penetration of calcium and phosphate.

The fluoride concentration of Duraphat is 2.26%. According to the modern concept, the caries preventive effect of fluoride is based on promotion of remineralization by small amounts of fluoride at the plaque-enamel interface rather than on an increase in the fluoride content of enamel, the latter requiring relatively high concentrations of fluoride (4). Based on this concept, the present fluoride concentration of the varnish seems unnecessarily high. In previous studies, the caries preventive effect of two fluoride varnishes did not continue after applications were discontinued, even though the fluoride content of enamel remained high (5–6). This suggests that increased fluoride concentration of enamel is not the main caries preventive mechanism of the varnishes. According to several studies, the efficacy of a fluoride regimen in enamel remineralization or caries inhibition does not increase proportionally with increasing concentration of fluoride in the preparation (7–11). Moreover, DIJKMAN *et al.* (12) found no difference in fluoride uptake by enamel from APF gels containing 0.11–1.23% fluoride.

The aim of the present investigation was to study remineralization of presoftened enamel by single and multiple applications of sodium fluoride with different fluoride concentrations.

Material and methods

120 slabs of noncarious enamel (about 5 × 5 mm) were cut from human molars and premolars. The outer enamel was polished flat with wet emery paper (240, 600, 1200 mesh). After polishing, the surface enamel was softened for 6 h in 0.1 M lactic acid-NaOH buffer, pH 5.0.

The slabs were randomly divided into six groups (20/group) and stored by groups in 50 ml of artificial saliva containing 10 g Na carboxymethylcellulose, 1.2 g KCl, 0.9 g NaCl, 0.26 g $CaCl_2 \cdot 2H_2O$, 0.458 g $K_2HPO_4 \cdot 3H_2O$, 0.05 g $MgCl_2 \cdot 6H_2O$, 0.15 mg NaF, H_2O ad 1000 ml (pH 7.0). Each group of slabs was given one of the following treatments:

1. No treatment (control slabs)
2. Application of 2.26% Duraphat (Woelm, FRG) on day 1
3. Applications of 2.26% Duraphat on days 1, 4, and 7
4. Application of 1.13% Duraphat on day 1
5. Applications of 1.13% Duraphat on days 1, 4, and 7
6. Daily 1-min treatment with 0.1% NaF (0.045% F) solution (nine treatments)

After 24 h, the varnish was removed with a scalpel. The saliva was renewed daily prior to the treatment procedures. Once a day, the slabs were removed from the saliva, immersed for 30 min in 50 ml of freshly prepared 0.1 M lactic acid-NaOH buffer, pH 5.0, rinsed with distilled water, and returned to the saliva. The experiment lasted for 9 days.

Finally, to determine the acid resistance and fluoride uptake by the enamel, the slabs were covered with wax except for a round area (9.6 mm^2), and each slab was immersed for 1 h in 1 ml of the lactic acid-NaOH buffer. From the solution, the amount of dissolved calcium was determined by atomic absorption spectrophotometer (Perkin-Elmer Model 372) and the amount of fluoride by F-specific electrode (Orion Research Inc.). The thickness of the dissolved layer was calculated on the basis of dissolved calcium, assuming the calcium content of enamel to be 37.5% and its average density 3.0 (13–15).

For hardness measurements, a Leitz hardness tester with a Vickers diamond and 200 g load was used. Five indentations were made in each slab initially (A), after presoftening (B), after the 9-day demineralization and remineralization (C), and after the 1-h demineralization (D). The mean of the five indentations was converted to Vickers hardness (kg/mm^2).

Data were analyzed using the analysis of variance to detect significant differences and *t* statistics to compare the means. To make the fluoride content of enamel comparable between the groups, the values for dissolved fluoride were adjusted for

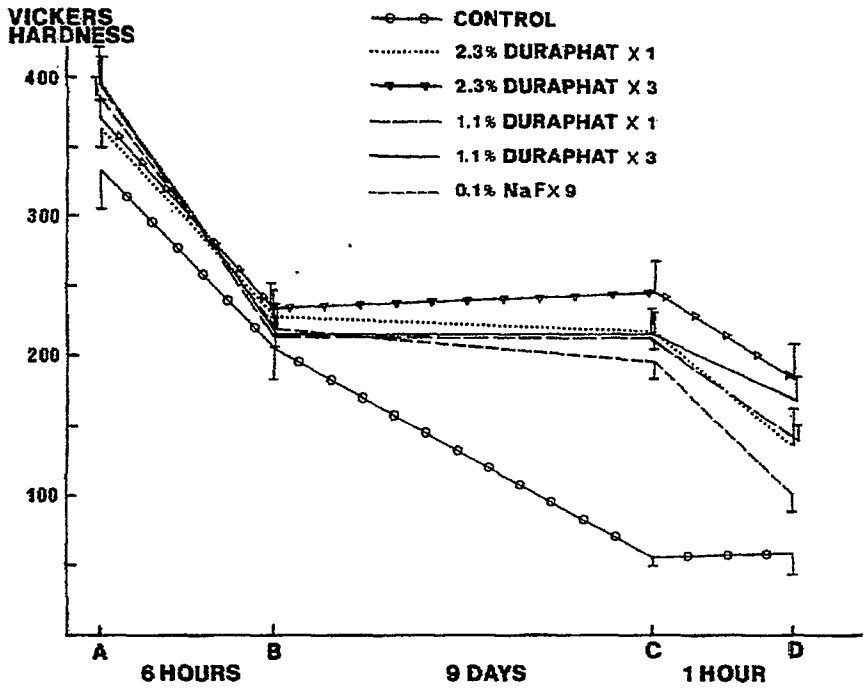

Fig. 1. Microhardness of enamel initially (A), after the presoftening (B), after the 9-day de- and remineralization (C) and after the 1-h demineralization (D).

depth using analysis of covariance, and the differences between the adjusted values were analyzed by analysis of variance.

Results

All fluoride treatments prevented softening of enamel effectively during the 9 days of demineralization and remineralization (Fig. 1). The overall differences between all groups and between the treatment groups during the 9 days of demineralization (hardness changes B–C) were statistically significant ($P<0.001$). Three treatments with 2.3% Duraphat were slightly more effective than the rest of the varnish treatments, but the difference was not statistically significant. The 0.1% NaF solution was least effective (compared to other groups, $P<0.05$).

During the 1-h demineralization, some dissolution of enamel occurred in all groups, although the already markedly softened control slabs showed no further softening (hardness changes C–D). Loss of calcium was significantly reduced by all treatments (Table 1). Enamel treated three times with 2.3% or 1.1% fluoride varnish showed the greatest acid resistance.

Due to the minimal etching depths, adjusting the values of dissolved fluoride for depth modified them very little (range of change 0.001–0.003 μg). Therefore, only unadjusted values are presented (Table 1). Fluoride uptake by enamel increased with

Table 1

Reduction of enamel microhardness, total amount of dissolved calcium and fluoride, and etching depth in the last 1-h demineralization

Group	Hardness reduction		Ca (μg)		F (μg)		Depth (μm)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Control	–		5.12	0.33	0.029	0.005	0.218	0.10
2.3% Duraphat × 1	83.0	5.7	1.81	0.14	0.337	0.018	0.168	0.12
2.3% Duraphat × 3	64.1	5.9	1.59	0.15	0.390	0.016	0.146	0.13
1.1% Duraphat × 1	73.9	2.0	2.35	0.11	0.135	0.005	0.218	0.01
1.1% Duraphat × 3	44.3	4.2	1.53	0.08	0.116	0.005	0.140	0.01
0.1% NaF × 9	93.9	4.1	2.37	0.12	0.085	0.002	0.222	0.11

increasing concentration of fluoride in the preparation. With the more concentrated varnish, fluoride uptake was slightly greater in enamel treated three times, whereas using 1.1% varnish, increased number of applications did not increase the fluoride uptake. There was a trend towards reduced solubility with increasing fluoride uptake. However, the enamel treated three times with 1.1% Duraphat having considerably lower fluoride content than those treated with the more concentrated varnish, dissolved least during the last 1-h demineralization.

Discussion

The measurement of enamel microhardness used in the study has been found to accurately reflect the degree of demineralization of the lesion (16–18). In severely softened enamel, however, the method is imprecise, which explains why the control slabs did not show further softening during the last 1-h demineralization, in spite of marked loss of calcium. The alternating demineralization and remineralization of the enamel mimicks in vivo conditions (19, 20), although in vivo, pH changes occur more frequently than here.

The loss of calcium and decrease in enamel microhardness in the 1-h demineralization show that, during the 9-day period, the surface enamel was dissolved in each demineralization; and during storage in synthetic saliva, it remineralized again. This remineralization was due to fluoride treatments and not to the small amount of fluoride in the synthetic saliva, since the control slabs dissolved markedly during the 9-day period.

Three treatments with 2.3% Duraphat were slightly more effective in enamel remineralization than any of the other varnish treatments. But as no difference was found between enamel treated once with 2.3% varnish and once or three times with 1.1% varnish, and enamel treated three times with 1.1% varnish showed the greatest acid resistance during 1-h demineralization, the efficacy of the treatment would appear to be due not mainly to the high fluoride concentration but rather to multiple applications.

It is surprising that a single application of Duraphat was able to promote remineralization during the whole 9-day period. Application of concentrated fluorides is known to deposit a layer of soluble fluoride, mainly CaF_2, on enamel (21). This slowly dissolving fluoride reservoir is obviously able to promote remineralization for long periods. The highly increased fluoride content of enamel found after varnish treatment may contribute to caries prevention by releasing fluoride into the oral environment during acid at-

tack. Thus infrequent applications of concentrated fluorides may result in the continuous presence of small amounts of fluoride at the plaque-enamel interface, i.e. the same as is achieved by frequent rinses with dilute fluoride solutions. This may explain why Duraphat was somewhat more effective than the NaF solution, a finding which at first seems surprising, keeping in mind the importance of frequent exposure to fluoride. Daily rinses with a 0.2% sodium fluoride solution have been shown to give a nearly total caries protection during a high caries challenge (22). However, the present results are in agreement with a previous study in which Duraphat was more effective than fortnightly rinses with 0.2% NaF solution (23).

The thickness of the enamel layer dissolved during the final demineralization was calculated using the calcium content and density of sound enamel. In softened enamel, these values may vary according to the degree of demineralization. However, this did not affect the amount of dissolved fluoride, since the values of fluoride were not adjusted for the etching depth.

Fluoride uptake from the 2.3% varnish was much greater than from the 1.1% varnish or from the NaF solution. Repeated applications did not essentially increase fluoride uptake. This agrees with the findings of a previous clinical trial, in which the amount of permanently bound fluoride in enamel found after one application was not increased by additional semi-annual applications of Duraphat (6). As is also obvious from the present results, however, reduction of enamel solubility is not directly proportional to fluoride content of the enamel.

These results suggest that sodium fluoride varnish with a fluoride concentration half that used at present effectively remineralizes enamel, and that three applications of varnish may be slightly more effective than one application. Thus the results do not support the findings of Bodde *et al.* (2, 3) who reported that multiple treatments with concentrated fluorides may prevent remineralization. However, the real value of a preventive measure can only be evaluated in clinical follow-up, and the present in vitro results must be interpreted with caution.

Acknowledgment – The excellent technical assistance of Mr. Arja Korhonen is gratefully acknowledged.

References

1. De Bruyn H, Arends J. Fluoride varnishes – a review. *J Biol Buccale* 1987; 15: 71–82.
2. Bodde HE, Arends J. The influence of a single fluoride treatment (1000 ppm) on in vitro remineralisation of initial enamel lesions. *J Biol Buccale* 1983; 11: 355–62.
3. Bodde HE, Nelson DGA, Koops PG, Arends J. Influence of repeated APF applications on long term remineralisation of initial lesions in bovine enamel. *J Dent Res* 1985; 64: 12–8.
4. Fejerskov O, Thylstrup A, Larsen JM. Rational use of fluorides in caries prevention. A concept based on possible cariostatic mechanisms. *Acta Odontol Scand* 1981; 39: 241–9.
5. Seppä L, Tuutti H, Luoma H. Post-treatment effect of a fluoride varnish in children with a high prevalence of dental caries in a community with fluoridated water. *J Dent Res* 1984; 63: 1221–2.
6. Seppä L. Fluoride content of enamel during treatment and 2 years after discontinuation of treatment with fluoride varnishes. *Caries Res* 1984; 18: 278–81.
7. Sluiter JA, Purdell-Lewis DJ. Lower fluoride concentrations for topical applications. *Caries Res* 1984; 18: 56–62.
8. Ten Cate JM, Simons Y. The efficacy of toothpastes with different fluoride contents, a pH cycling study. In: Leach SA, ed. *Factors relating to demineralisation and remineralisation of the teeth.* Oxford: IRL Press, 1986; 35–44.
9. Goorhuis J, Purdell-Lewis DJ. 0.25% and 0.4% amine fluoride gel for weekly topical application. *Caries Res* 1986; 20: 458–64.
10. De Bruyn HE, van Rijn LJ, Purdell-Lewis J, Arends J. Influence on various fluoride varnishes on mineral loss under plaque. *Caries Res* 1988; 22: 76–83.
11. Ripa L, Leske G, Forte A, Varma A. Caries

inhibition from two mixed NaF-Na_2 PO_3 F dentrifices containing 1000 and 2500 ppm F. Final results after three years. *J Dent Res* 1987; 66: 165 (only).

12. Dijkman AG, Tak J, Arends J. Comparison of fluoride uptake by human enamel from acidulated phosphate fluoride gels with different fluoride concentrations. *Caries Res* 1982; 16: 197–200.
13. Brudevold F, Aasenden R, McCann HG III, McCann HG. Use of an enamel biopsy method for determination of in vivo uptake of fluoride from topical treatments. *Caries Res* 1969; 3: 119–33.
14. Retief DH, Cleaton-Jones PE. The quantitative analysis of sixteen elements in normal human enamel and dentine by neutron activation analysis and high-resolution gamma-spectrometry. *Arch Oral Biol* 1971; 16: 1257–67.
15. Manly RS, Hodge HC. Density and refractive index studies of dental hard tissues. *J Dent Res* 1939; 18: 133–41.
16. Arends J, Schuthof J, Jongebloed WG. Microhardness indentations on artificial white spot lesions. *Caries Res* 1979; 13: 290–7.
17. Arends J, Schuthof J, Jongebloed WG. Lesion depth and microhardness indentations on artificial white spot lesions. *Caries Res* 1980; 14: 190–5.
18. Koulourides T, Housch T. Hardness testing and microradiography of enamel in relation to intra-oral de- and remineralisation. In: Leach SA, Edgar WM, eds. *Demineralisation and remineralisation of the teeth.* Oxford: IRL Press, 1983; 255–72.
19. Ten Cate JM, Duijsters PPE. Alternating demineralisation and remineralisation of artificial enamel lesions. *Caries Res* 1982; 16: 201–10.
20. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S. Enhancement of remineralisation in vitro and in vivo. In: Leach SA, ed. *Factors relating to demineralisation and remineralisation of the teeth.* Oxford: IRL Press, 1986; 23–34.
21. Nelson DGA, Jongebloed WL, Arends J. Morphology of enamel surfaces treated with topical fluoride agents: SEM considerations. *J Dent Res* 1983; 62: 1201–8.
22. Ögaard B, Arends J, Schuthof J, Rölla G, Ekstrand J, Oliveby A. Action of fluoride on initiation of early enamel caries in vivo. A microradiographical investigation. *Caries Res* 1986; 20: 270–7.
23. Seppä L, Pöllänen L. Caries preventive effect of two fluoride varnishes and a fluoride mouthrinse. *Caries Res* 1987; 21: 375–9.