Caries Res 1989;23:365–367

0008-6568/89/0235-0365 $ 2.75/0

Prevention of Rat Fissure Caries by Sodium Fluoride Varnish (Duraphat®) with Different Fluoride Concentrations

L. Seppä, H. Forss, P. Sormunen[1]

Faculty of Dentistry, University of Kuopio, Finland

Key Words. Fluoride concentration · Fluoride varnish · Rat caries

Abstract. 125 Osborne-Mendel rats were weaned at 22–23 days, inoculated with *Streptococcus sobrinus* and fed a cariogenic diet for 40 days. The control group received no treatment; the study groups received applications of Duraphat® containing 2.3, 1.1, or 0.6% fluoride or a placebo varnish on days 21–23. The placebo varnish had no effect on caries. Fissure caries was significantly reduced by the 2.3 and 1.1% fluoride varnishes, whereas the caries reduction found after treatment with the 0.6% fluoride varnish was not statistically significant. These results suggest that reducing the fluoride content of Duraphat by half does not significantly reduce its caries-preventive effect, but the progress of caries seems to be somewhat slower with the 2.3% fluoride varnish.

According to the modern concept, the caries-preventive effect of fluoride is based on the presence of small amounts of fluoride at the saliva-plaque-enamel interface rather than on the increase in the fluoride content of the enamel, which requires relatively high fluoride concentrations. The established efficacy of concentrated fluorides has been suggested to be mainly due to deposition of soluble calcium fluoride on the enamel and acting as a slowly dissolving fluoride reservoir [Fejerskov et al., 1981; Saxegaard and Rölla, 1988].

In several studies, the effect of a fluoride regimen in caries prevention or de- and remineralisation of enamel has not been directly proportional to the fluoride content of the preparation [ten Cate and Simons, 1986, De Bruyn et al., 1986, 1988; Goorhuis and Purdell-Lewis, 1986; Ripa et al., 1988]. Based on this, the fluoride concentrations of some professionally applied topical fluorides seem unnecessarily high. The sodium fluoride varnish Duraphat® commonly used for caries prevention in Europe has a fluoride concentration as high as 2.3%. Although application of Duraphat can be considered safe if varnish is applied sparsely [Ekstrand et al., 1980; Seppä and Hanhijärvi, 1983; Roberts and Longhurst, 1987], there is a potential for overdosage in younger children if the manufacturer's instructions are not followed. Therefore, it would seem reasonable to lower the fluoride concentration if the caries-preventive results were the same. In a previous in vitro study, no significant difference in remineralization of enamel was found between Duraphat containing 2.3 and 1.1% fluoride [Seppä, 1988]. The aim of this study was to determine whether reducing the amount of fluoride in Duraphat has any effect on caries prevention in rats.

[1] The excellent technical assistance of Mrs. Arja Korhonen is gratefully acknowledged.

Materials and Methods

The study was carried out on 125 Osborne-Mendel rats of both sexes with a male:female ratio of about 1:1. All animals were weaned 22–23 days after birth after which they were randomly distributed into five groups. On the 1st and 2nd days of the experiment, the mouths of all animals were inoculated with a fresh culture of *Streptococcus mutans* ATCC 27351 [K-1; Fitzgerald, 1968, now known as *S. sobrinus*]. After weaning, all rats were fed a cariogenic diet [modified formula 200 of Navia et al., 1969] containing 49% sucrose and 18% wheat fluor. On the 1st, 2nd and 3rd day the rats were treated as follows: group 1: control, no treatment; group 2: Duraphat placebo; group 3: 2.26% Duraphat (Woelm, FRG); group 4: 1.13% Duraphat; group 5: 0.56% Duraphat.

Using small forceps and a foam rubber swab, the varnish was applied on the molars of unanesthetized animals for about 15 s/an-

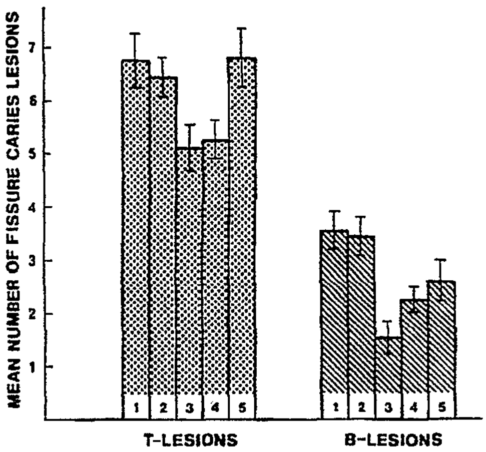

Fig. 1. Mean number of fissure caries lesions (+SE) in rats fed for 40 days. 1 = Control; 2 = placebo; 3 = Duraphat with 2.3% F; 4 = Duraphat with 1.1% F; 5 = Duraphat with 0.6% F.

imal. Immediately after the application the rats were returned to the cages, in which food and water were available.

Three and 11 days after the last fluoride varnish application, bacterial cultivations were made in 5 rats/group. One upper and lower molar segment was swabbed 10 s each with a sterile cotton swab which was cut off into 1 ml KH_2PO_4 buffer. 10^{-1} and 10^{-2} dilutions were made and 50-µl aliquots were plated in MSB media [Gold et al., 1973]. Plates were incubated anaerobically at 37 °C for 72 h. Colonies of *S. mutans* were counted.

The rats were kept in plastic cages with wire bottoms made of stainless steel, 4 rats per cage (room temperature 22-24 °C, relative humidity 55-60%). Food and distilled water were available ad libitum. After the 40-day feeding period, the rats were sacrificed and the jaws were removed. The lower jaw and one half of the upper jaw were bisectioned sagittally and stained with Schiff's reagent. Caries was scored in three grades (A = enamel lesions, T = lesions reaching dentinoenamel junction, B = advanced dentin lesions) and cumulated [König, 1966]. Sixteen fissures/animal were scored.

Data were analyzed using one-way analysis of variance for detecting significant differences. To avoid an inflated rate of type I error, Newman-Keuls multiple comparison tests were used for pairwise comparisons.

Results

The numbers of rats in groups 1–5 were 26, 25, 25, 25, and 24, respectively. The mean weight gains for the respective groups were 81.5 g (SE 3.9), 93.7 g (4.8), 91.7 g (5.7), 89.9 g (4.4), and 87.0 g (4.7). The mean weight gains of the groups did not differ significantly.

Great variations were found in *S. mutans* counts between the animals irrespective of the group. In all groups, the counts of the first cultivation tended to be larger than those of the second one. However, no significant differences were found either between the control and test groups or between the test groups.

Analysis of variance revealed a significant overall difference between the groups in the mean number of all types of fissure caries lesions (A and T lesions: $p<0.05$, B lesions: $p<0.001$). There was no significant difference between the control group and the placebo varnish group, therefore the control group was used in the comparisons. The mean numbers of fissure A lesions were 11.8 (SE 0.4), 11.9 (0.4), 10.4 (0.4), 10.8 (0.3), and 11.6 (0.4) in groups 1–5, respectively. In spite of the significant overall difference, pairwise comparisons (Newman-Keuls test) did not reveal significant differences.

The mean amounts of fissure T and B lesions are shown in figure 1. The varnishes with 2.3 and 1.1% fluoride reduced the amount of B lesions significantly ($p<0.05$). There was a tendency toward larger reduction by the 2.3% varnish compared to the 1.1% varnish, but the difference did not reach statistical significance. T lesions were reduced significantly only by the 2.3% varnish ($p<0.05$). The varnish with 0.6% fluoride did not reduce caries significantly.

Discussion

Since there was no reason to believe that the pattern of eating would differ between the groups, the amount of food intake was monitored only by following the weight gains of the rats. The differences in the mean weight gains were not significant.

Concentrated fluorides may affect bacterial metabolism, and in this case, delayed establishment of the inoculated *S. sobrinus* might have accounted for the results. However, in the study of Zickert and Emilson [1982], Duraphat had no significant effect on plaque and salivary levels of *S. mutans* in children. Bacterial cultivations made in the present study revealed no differences in the recovery of *S. mutans* between the groups, which suggests that caries prevention is not explained by inhibition of bacterial growth.

The 2.3% sodium fluoride varnish significantly reduced fissure caries in rats, which agrees with our previous findings [Seppä et al., 1982, 1984]. The greatest reduction was obtained in the occurrence of advanced dentinal lesions (B lesions). Considering these lesions, the varnish with 1.1% fluoride was less effective than the 2.3% varnish, although the difference was not significant statistically. Since T and A lesions are cumulated values, the difference found for less advanced caries mainly originated from the difference in the amount of B lesions. The results suggest that the varnishes were equally effective in preventing caries, but the progress of caries was slower with the 2.3% varnish.

The amount of B lesions seemed to be slightly reduced also by the 0.6% fluoride varnish, but this varnish was clearly less effective than the more concentrated ones. This is somewhat surprising, since a silane fluoride varnish (Fluor Protector®) with approximately the same fluoride concentration (0.7%) has been found effective in caries prevention in some clinical studies [Salem et al., 1979; Clark et al., 1985], and even much lower concentrations have inhibited enamel dissolution in vitro and in vivo [De Bruyn et al., 1986, 1988]. The difference may be explained by different physicochemical properties of the two types of varnishes [De Bruyn and Arends, 1987, a review]. For instance, Fluor Protector containing 0.7% fluoride has been shown to deposit more acquired fluoride in the enamel and soluble fluoride on the enamel than 2.3% Duraphat does [Dijkman et al., 1982].

At present, we do not know the duration of increased fluoride activity at the plaque-enamel interface after Duraphat application. When considering the results, we must take into account that with all used varnishes, the duration may have exceeded the duration of the experiment. The results might have been different if the experiment had lasted longer. For this reason among others, the results must be interpreted with caution. However, reducing the fluoride content of Duraphat by half seems to be worth studying in a clinical trial. Even though there are no reports of side effects due to the high fluoride concentration of Duraphat, reducing the concentration would still increase the safety of application in children.

References

Clark DC, Stamm JW, Robert G et al: Results of a 32-month fluoride varnish study in Sherbrooke and Lac-Megantic, Canada. J Am Dent Assoc 1985;111:949-953.

De Bruyn H, Arends J: Fluoride varnishes: A review. J Biol Buccale 1987;15:71-82.

De Bruyn H, Buskes JAKM, Arends J: The inhibition of demineralization of human enamel after fluoride varnish application as a function of the fluoride content. An in vitro study under constant composition demineralizing conditions. J Biol Buccale 1986;14:133-138.

De Bruyn H, Van Rijn LJ, Purdell-Lewis DJ, et al: Influence of various fluoride varnishes on mineral loss under plaque. Caries Res 1988;2:76-83.

ten Cate JM, Simons Y: The efficacy of toothpastes with different fluoride contents, a pH-cycling study; in Factors relating to demineralisation of the teeth. Oxford, IRL Press Ltd, 1986,35-44.

Dijkman AG, Tak J, Arends J: Fluoride deposited by topical applications in enamel. KOH-soluble and acquired fluoride. Caries Res 1982;16:147-155.

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;14:185-189.

Fejerskov O, Thylstrup A, Joost Larsen M: Rational use of fluorides in caries prevention. A concept based on possible cariostatic mechanisms. Acta Odontol Scand 1981;39:241-249.

Fitzgerald RJ: Dental caries research in gnotobiotic animals. Caries Res 1968;2:139-146.

Gold OG, Jordan HV, Van Houte J: A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357-1364.

Goorhuis J, Purdell-Lewis DJ: 0.25% and 0.4% amine fluoride gel for weekly topical application. Caries Res 1986;20:458-464.

König KG: Möglichkeiten der Kariesprophylaxe beim Menschen und ihre Untersuchung im kurzfristigen Rattenexperiment. Bern, Huber, 1966.

Navia J, Lopez H, Harris RS: Purified diet for caries research with rats. J Nutr 1969;97:133-140.

Ripa LW, Leske GS, Forte F, et al: Caries inhibition of mixed NaF-Na_2PO_3F dentifrices containing 1000 and 2500 ppm F: 3-year results. J Am Dent Assoc 1988;116:69-73.

Roberts JF, Longhurst P: A clinical estimation of the fluoride used during application of a fluoride varnish. Br Dent J 1987; 162:463-466.

Salem LV, Raschio AJ, Reategui TJ, et al: Klinische Untersuchung über die karieshemmende Langzeitwirkung des Fluor-Protector-Lackes. Kariesprophylaxe 1979;1:145-148.

Saxegaard E, Rölla G: Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res 1988;96:523-535.

Seppä L: Effects of a sodium fluoride solution and varnish with different fluoride concentrations on enamel remineralization in vitro. Scand J Dent Res 1988;96:304-309.

Seppä L, Hanhijärvi H: Fluoride concentration in whole and parotid saliva after application of fluoride varnishes. Caries Res 1983;17:476-480.

Seppä L, Koskinen M, Luoma H: Caries prevention by two fluoride varnishes in Osborne-Mendel rats. Caries Res 1982;16: 453-459.

Seppä L, Luoma H, Koskinen M, et al: Caries prevention in rats by fluoride varnish treatment combined with addition of fluoride to dietary sugar. J Dent Res 1984;63:1193-1196.

Zickert I, Emilson CG: Effect of a fluoride-containing varnish on *Streptococcus mutans* in plaque and saliva. Scand J Dent Res 1982;90:423-428.

Liisa Seppä
Faculty of Dentistry
University of Kuopio
POB 6
SF-70211 Kuopio (Finland)