The influence of fluoride on in vitro remineralization of bovine enamel

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SUMMARY

Remineralization experiments using bovine enamel were carried out with 2 ppm fluoride or no fluoride added to the remineralizing solutions.

The group without fluoride showed (quantitative microradiography) significantly more remineralization in the first 50 μm of the lesion than the fluoride group.

It is suggested that fluoride may inhibit remineralization.

KEY WORDS:

Bovine enamel - Microradiography - Fluoride - Remineralization - Demineralization

RÉSUMÉ

Une expérimentation portant sur la reminéralisation de l'émail a été menée sur de l'émail bovin, en utilisant des solutions de reminéralisation sans adjonction de fluorure et avec adjonction de 2 ppm de fluorure.

La microradiographie quantitative a montré, dans le groupe sans fluorure, une reminéralisation plus importante, dans les premiers 50 μm de la lésion, que dans le groupe avec fluorure.

Ces résultats suggèrent que les fluorures puissent inhiber la reminéralisation.

MOTS-CLÉS:

Email bovin - Microradiographie - Fluorure - Reminéralisation - Déminéralisation.

INTRODUCTION

In many studies the vitro remineralization of enamel with subsurface lesions has been reported (Ten Cate and Arends, 1977; Ten Cate et al., 1981; Silverstone, 1980; Silverstone et al., 1981; White, 1988). It has been found that fluoride in low concentrations increased the remineralization rate. In in vitro studies with softened enamel similar results have been obtained (Feagin, 1971; Koulourides et al., 1961). However, in the above mentioned in vitro studies microradiographic data are scarce.

In longitudinal epidemiological studies, fluoride appeared to inhibit complete lesion remineralization in vivo (Backer Dirks, 1966; Pot and Groeneveld, 1976; Groeneveld, 1986). This has been attributed to a deposition of fluorapatite (FA) in the surface layer

of the lesion. In this way the pores of the enamel may have been blocked and diffusion into the lesion restricted.

The aim of the present study was to investigate, by means of microradiography, whether fluoride inhibits or accelerates the remineralization of demineralized dental enamel in vitro.

MATERIAL AND METHODS

Specimen preparation and demineralization.

The outer 500 μm of the labial surface of sixteen bovine incisors were removed by abrasive paper grid 600. After covering with wax, except for a window ($\simeq$ 15 mm^2), the enamel was demineralized during 14 days at 37°C in a 50 mM acetate buffer, pH 5.0, containing 2.2 mM Ca, and 2.2 mM P (see also Ten Cate and Duijsters, 1982). Per mm^2 enamel 1 ml solution was used. This demineralizing solution was undersaturated with respect to hydroxyapatite (OHA; Dijk van et al., 1979). After demineralization the enamel blocks were washed in distilled water and wiped off with a paper tissue. Half of the window of each enamel block was covered with coldcuring polymethylmethacrylate/polybutylmethacrylate (pMMA/pBMA) in order to be able to determine the amount of demineralization of the enamel prior to the remineralization step within the same blocks.

Remineralization.

After demineralization the lesions were remineralized for ten days at 37°C under stirring in a solution initially containing 1.5 mM Ca, 0.9 mM P, 130 mM KCl and 20 mM buffer (cacodylic acid/Na-cacodylate), pH 7.0, without (non-fluoride group) or with 2 ppm fluoride added (fluoride group) during remineralization (Ten Cate and Duijsters, 1982). The solutions were refreshed every fifth day. Per mm^2 enamel 2 ml solution was used.

The remineralizing solution was supersatured with respect to OHA, and in the presence of 2 ppm fluoride supersaturated with respect to fluorapatite (FA) too (Ten Cate et al., 1985).

After remineralization the enamel blocks were washed in distilled water and the covering (pMMA/pBMA) layer was removed carefully.

Microradiography and densitometry.

From the middle of every window a slice with a thickness of approximately 125 μm was cut with an annular saw in such a way that every slice contained a demineralized and a demineralized-remineralized region.

Contact microradiographs were made from these sections together with an aluminium stepwedge using a vacuum (3 mmHg) X-ray camera, Cu - K(α) radiation, be-window, Ni-filter, V = 20 kV, I = 20 mA, object to target distance: 0.4 m. Contact microradiographs were made on Kodak High Resolution Plates, developed in Nivenool (Amaloco) for 7.5 minutes (Groeneveld, 1974; de Groot et al., 1986; Theuns et al., 1984, 1985).

The density of the microradiograms was measured by slit-scanning with a Leitz densitometer connected with a DEC 350 Computer. Using these density profiles and the mineral absorption coefficient described by Angmar et al. (1963), the volume percentage of mineral in the lesion was calculated and plotted against the depth. The relevant parameters of a lesion were determined by averaging the results of 2-4 slit scans in that lesion within each region. Figure 1 shows the relevant parameters of a schematic densitogram of a subsurface lesion (Theuns et al., 1984). Student's t-tests were done to detect statistical significances between the de- and remineralization and between the fluoride and the non-fluoride group (Table I and II) for some microradiographic parameters.

RESULTS

After demineralization, enamel with a relatively low content of the surface layer was obtained. During remineralization a significant ($p < 0.05$) increase of the mineral content of the surface layer (MSL) and a significant decrease in area above the profile ($\triangle$A) was obtained in both groups (Table I). To determine the effects of fluoride on the amount of remineralization, $\triangle$MSL (mineral content of the surface layer after demineralization minus mineral content of the surface layer after remineralization) and $\triangle\triangle$A (area above the profile after demineralization minus area above the profile after remineralization) were calculated. The non-fluoride group showed a significantly higher $\triangle$MSL than the fluoride group (-33 ± 6 versus -22 ± 12). However, the $\triangle\triangle$A (Table II) in both groups did not differ significantly. For obtaining more detailed information about this parameter, $\triangle\triangle$A was determined in successive layers of the lesion (Table II). The $\triangle\triangle$A of the fluoride group differed significantly from the non-fluoride group in the first 50 μm of the lesion (including the surface), indicating that in this area the presence of fluoride caused a decrease in the amount of remineralization.

TABLE I
Microradiographic parameters after de- and remineralization of bovine enamel (means ±SD).

Group	n	DSL	MSL	DBL	MBL	DTL	△
Fluoride (+F)							
remineralization	8	16±4	66[a]± 7	37 ± 8	47[a]± 8	146±22	2700[a]± 730
demineralization	8	16±4	43 ±10	33 ± 6	34 ±10	157±25	4000 ±1212
Non-fluoride (−F)							
remineralization	8	18±5	81[a]± 3	51[a]±16	71[a]± 3	146±13	1400[a]± 211
demineralization	8	14±2	48 ±18	27 ± 4	41 ± 9	149±15	3100 ±1093

a=the remineralization group differs significantly ($p<0.05$) from the corresponding demineralization group.
n=number of blocks
For other notations: See figure 1.

TABLE II
The △△A (vol %×μm) in the total lesion and at some depths for the fluoride and the non-fluoride group (means±SD).

Group	n	△△A total lesion	△△A depth (μm) 12-32	32-52	25-72	72-132
Fluoride	8	1300± 832	371[a]±172	243[a]±137	173±168	378±461
Non-fluoride	8	1700±1026	708 ±103	507 ±199	325±190	298±443

n=number of blocks
a=fluoride group differs significantly ($p<0.05$) from the non-fluoride group.
△△A=△A after demineralization − △A after remineralization.

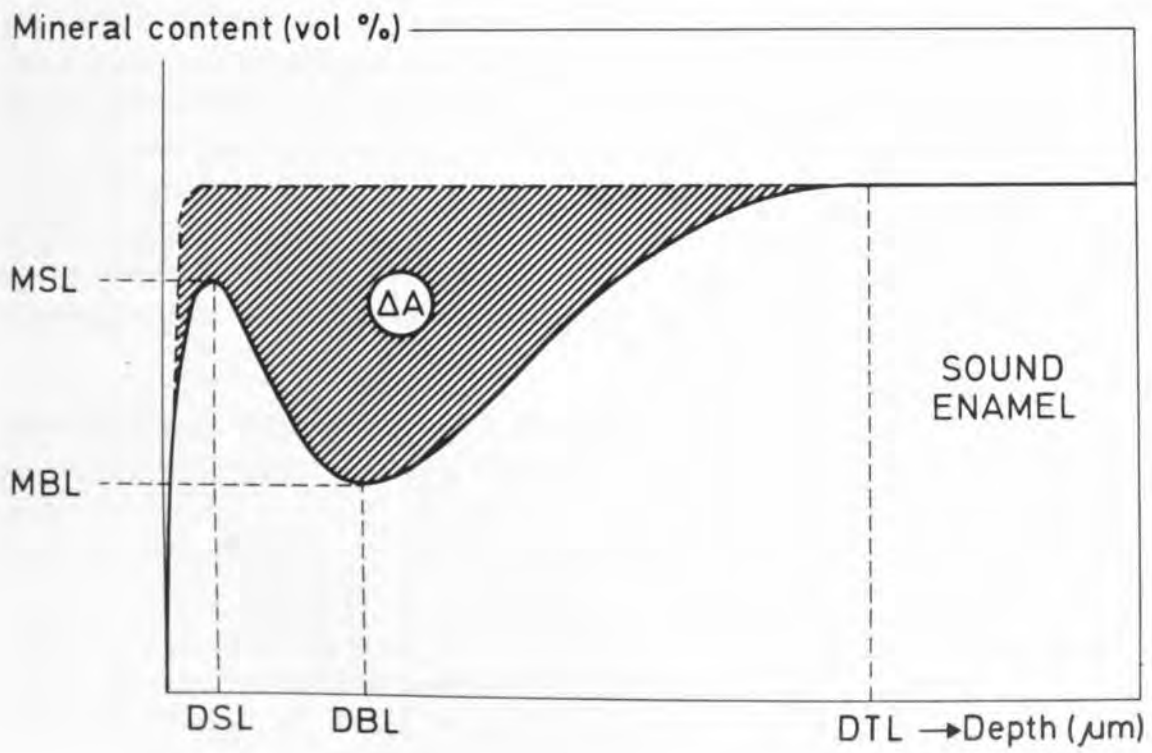

Fig. 1: Schematic densitogram for enamel with a subsurface lesion. DSL = depth of the surface layer. MSL = mineral content of the surface layer, DBL = depth of the body of the lesion, MBL = mineral content of the body of the lesion, DTL = depth of the total lesion, △A = area (vol % × μm) above the profile.
The upper profile (– –) represents sound enamel.

DISCUSSION

The results of the present study indicate that fluoride delays the in vitro remineralization in the first 50 μm of the lesion and that it has no significant influence on the remineralization of the rest of the lesion. This seems contradictory to studies in the literature (Introduction) which report a stimulating effect of fluoride on the remineralization. Experiments performed previously with hydroxyapatite (OHA) seeds, and enamel reveal some factors which may be of importance for the understanding of the mentioned differences.

Feagin (1971) found that the deposition rate of calcium and phosphate from solutions onto acid-softened enamel was stimulated by the presence of fluoride. However, Meyer and Nancollas (1972) reported that fluoride in small concentrations (2×10^{-6} to 1×10^{-4} M) inhibits the initial crystal growth of OHA. These studies indicate that fluoride may increase or decrease the deposition of mineral, depending on its concentration. Silverstone et al. (1981), pointed out that the degree of supersaturation of the remineralization fluid has a marked effect on the remineralization of caries lesions. When a low calcium concentration was used, remineralization occurred throughout the entire depth of a lesion. When higher calcium concentrations were used, remineralization occured, but was limited to the surface of the lesion. The lesion type too is important for the remineralization process because it determines the precipitation kinetics in the sense of a diffusion- or surface-controlled precipitation (Ten Cate, 1979; Arends and Ten Cate, 1981; Damato et al., 1988). These studies show that the remineralization of a caries lesion is a complex process, which depends strongly on the exact experimental conditions used.

Comparison of the experimental set-up of our study with those in the literature dealing with remineralization of subsurface lesions, reveal differences in the experimental conditions which will have their influence on the results. Silverstone (1980) reported a stimulating influence of 1 ppm fluoride on the remineralization during ten days. In that study a supersaturation different from that in our study, and acidified gel lesions were used. Ten Cate and Arends (1977) reported a twofold increase in remineralization rate in the presence of 1 ppm fluoride. However, a remineralization time of 8 h, a HEC gel for preparation of the lesions, and a different supersaturation of the calcified fluid were used in comparison with our study. Ten Cate et al. (1981), found that fluoride stimulated the calcium deposition. In this study a remineralization time of 5 days, HEC gel for preparation of the lesions, and 10 ppm fluoride were used. Ten Cate and Duijsters (1982) used the same kind of enamel and the same de- and remineralization conditions as used in the present study. The amount of remineralization in that study was determined by using a cumulative plot of calcium gain from the solution for the fluoride and non-fluoride group. A slightly greater calcium gain for the non-fluoride group than for the fluoride group was found after ten days. This implies the absence of a stimulating effect of fluoride on the remineralization. This result is in agreement with the findings of the present study: the difference between the amount of remineralization of the total lesion (Table II, $\triangle\triangle$A total lesion) of the fluoride and non-fluoride group was too small to be significant. More recently, another study too reported no stimulating influence of fluoride on remineralization (Ten Cate and Timmer, 1986). The presence of 0.25-1.0 ppm fluoride did not increase the amount of remineralization in a series of pH-cycling experiments. It was found that fluoride inhibited the amount of remineralization for all concentrations used. However, it is not clear from the results of that study to which amount this inhibition was caused by the slower solubilization of the enamel mineral during the demineralization periods of the pH-cycles in the presence of fluoride.

In the study of ten Cate and Duijsters (1982) a fluoride uptake of 0.043 μmol fluoride per mm^2 of enamel was found during the total remineralization period of ten days. By extrapolation of this result to the present study (same experimental conditions) it was estimated that the decrease of fluoride in our remineralization solutions will not be more than 10% during the total remineralization period. Similarly, the calcium loss can be calculated to be not more than 7% and 6% in the non-fluoride group and fluoride group respectively. This means that the absence of a stimulating effect of fluoride on the remineralization in our experiments is not caused by a more rapid exhaustion of the calcifying solutions of the fluoride group compared with those of the non-fluoride group.

The above mentioned study of Meyer and Nancollas (1972) suggests that a range of fluoride concentrations of 2×10^{-6} to 1×10^{-4} M may have an inhibiting effect on the remineralization. In our experiments fluoride levels of this magnitude are probably present in the outer layers (50 μm) of the enamel, and responsible for the inhibition of remineralization observed in this part of the lesion (Table II). In the deeper parts the

calcium, phosphate, and fluoride concentrations will be lowered by adsorption and precipitation in the surface layer during remineralization. In the deeper layers the fluoride concentration may become so low that the fluoride has no or only little influence on the remineralization rate. This is in accordance with the results presented in Table II, which indicate that the fluoride and the non-fluoride group do not differ significantly in the remineralization rate of the deeper parts.

In principle the inhibiting influence of fluoride on remineralization could also be explained by the formation of a blocking layer in the very outer part of the lesion by the presence of fluoride. The existence of such a blocking layer was proposed previously to explain the clinical observation that in areas with drinking water fluoridation, the amount of remineralized repaired white spots is decreased (Backer Dircks, 1966; Pot and Groeneveld, 1976; Groeneveld, 1986), and to explain the results of alternating de- and remineralization of artificial enamel lesions (Ten Cate and Duijsters, 1982; Ten Cate et al., 1988). However, in the present study no microradiographic indications were obtained for the existence of such a layer. In addition, it is expected that a blocking layer retards the remineralization of the total lesion, and not only of the outer 50 μm of a lesion as was observed.

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