Effects of Fluoride and Chlorhexidine on the Microflora of Dental Root Surfaces and Progression of Root-surface Caries

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The effects of fluoride and chlorhexidine varnishes on the microflora of dental root surfaces and on the progression of root-surface caries were studied. Forty-four patients, surgically treated for advanced periodontal disease, were distributed at random among three groups. All patients received a standardized preventive treatment. Furthermore, the dentifrice of the patients in the two experimental groups was treated, at three-month intervals, with chlorhexidine and fluoride varnish, respectively. Patients in the control group received no additional treatment.

In the experimental groups, plaque samples were collected from selected sound and carious root surfaces at baseline and at three, six, and nine months after the onset of the study. The presence of root-surface caries was scored at baseline and after one year. In addition, the texture, depth, and color of the root-surface lesions were monitored.

Mutans streptococci on root surfaces were suppressed significantly (p<0.05) during the whole experimental period in the chlorhexidine varnish group, but not in the fluoride varnish group. A non-significant increase in the number of Actinomyces viscosus/naeslundii was noted after treatment with chlorhexidine and fluoride varnish.

The increase in the number of decayed and filled root surfaces after one year was significantly lower in the experimental groups than in the control group. After treatment with chlorhexidine varnish, significantly more initial root-surface lesions had hardened than in the other groups.


Introduction.

From epidemiological studies, it is known that root-surface caries frequently occurs in adults, the elderly, and patients with advanced periodontal disease (Katz et al., 1982; Banting et al., 1985; Keltjens et al., 1988; Locker et al., 1989). In several studies, a positive correlation has been reported between initial root-surface caries and subsequent caries development (Ravald and Hamp, 1981; Ravald et al., 1986; Hand et al., 1988; Leske and Ripa, 1989).

Fluoride can prevent or inhibit the development of root-surface caries (Burt et al., 1986; Ripa et al., 1987; Hunt et al., 1989). It has been shown that by meticulous toothbrushing with fluoride toothpaste, active root-surface lesions on buccal surfaces can be converted into inactive lesions (Nvyad and Fejerskov, 1986). However, rather than on buccal or lingual surfaces, root-caries lesions may develop on approximal surfaces that are difficult for the patient to clean (Katz et al., 1982; Billings et al., 1985; Ravald et al., 1986; Keltjens et al., 1988). For these surfaces, additional preventive measures may be needed.

The microbial etiology of root-surface caries (reviewed by Bowden, 1990) is associated with elevated levels of mutans streptococci (Ellen et al., 1985a; Brown et al., 1986; Fure et al., 1987; Keltjens et al., 1987). Also, Actinomyces spp. were found to be present in elevated numbers in plaque from carious root-surface lesions (Jordan and Hammond, 1972; Syed et al., 1975; Ellen et al., 1985b; Emilson et al., 1988).

Suppression of mutans streptococci by chlorhexidine gel can give additional caries reduction (Zickert et al., 1982; Gisselsson et al., 1988; Lindquist et al., 1989). Long-term suppression of mutans streptococci on tooth surfaces can also be achieved by application of highly concentrated chlorhexidine varnishes (Sandham et al., 1988; Schaeken and De Haan, 1989; Schaeken et al., 1989). The advantage of the use of chlorhexidine varnish over chlorhexidine gel or mouthrinse is that the effective agent can be applied specifically on caries predilection sites, thus diminishing side-effects involved in the use of mouthrinses or gel applications, such as bad taste, irritation of the oral mucosa, and staining of teeth and tongue.

The aim of the present study was to investigate the effect of locally applied fluoride and chlorhexidine varnishes on the plaque microflora on dental root surfaces and on root-surface caries in patients surgically treated for advanced periodontal disease.

Materials and methods.

Participants and treatments. — Forty-four patients with a mean age of 44.4 years participated in the study. All had undergone periodontal surgery more than two years previously on six teeth or more, and all patients had at least two decayed or filled root surfaces at the start of the study. The mean root-caries index (Katz et al., 1982) was 14.5%. All patients participated in a maintenance program and returned every three months to the Department of Periodontology of the university for the whole period of the study.

Prior to the first clinical examination, the subjects were distributed at random among the experimental groups. The exposed root surfaces of the subjects in the fluoride group were treated at three-month intervals with fluoride varnish (Duraphat®, Wolm, Eschwege, Germany) and in the chlorhexidine group with varnish containing 40% (w/w) chlorhexidine (Schaeken and De Haan, 1989). The other patients served as a control group and continued on the maintenance program.

The fluoride or chlorhexidine treatments took place immediately after the periodontal check-up and the professional tooth cleaning, which were included in the maintenance treatment. The varnishes were applied onto the dried root surfaces with a small firm brush (as used for acid etching) and with a blunt dental instrument (Ash #6). With these instruments, the application of the varnishes was easy and took approximately 15 min. After the treatment, the subjects were allowed to rinse with tap water. Then, excess varnish on the mucosa was removed with the blunt dental instrument.

Bacteriological samples. — Prior to periodontal control and dental prophylaxis, plaque samples were taken from selected sound and carious root surfaces in the subjects in the varnish groups. The selected surfaces had a well-described outline and

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could be sampled easily. The samples were collected at baseline and after three, six, and nine months. Before plaque was sampled, adherent saliva on the surfaces was removed with water spray. Subsequently, the teeth were isolated with cotton rolls and dried by air. Plaque samples were collected with a small spoon excavator (Ash #153/154). Each plaque sample was transferred into a vial with 1 mL of reduced transport fluid (Loesche et al., 1972) and processed within 30 min.

The plaque samples were homogenized by ultrasonic dispersion for 20 s at 0°C in a Kontes sonifier. The suspensions were serially diluted 10-fold and plated onto blood agar, TSY20B agar (Schaecken et al., 1986), and CNAC-20 agar (Ellen and Balcerzak-Raczkowski, 1975). All plates were incubated at 37°C in an atmosphere of 91% N₂, 5% CO₂, and 4% H₂ for five days.

Total cultivable flora, Streptococcus sanguis, Streptococcus oralis, and Streptococcus gordonii were counted on blood agar. S. sanguis, S. oralis, and S. gordonii (Kilian et al., 1989) could not be distinguished by colonial morphology and were counted together. TSY20B served to enumerate mutans streptococci. On CNAC-20, Actinomyces viscosus/naeslundii were counted. On CNAC-20, the two Actinomyces species could not be distinguished by colonial morphology and were counted together.

All species were counted on the basis of colonial morphology. Representative colonies were regularly isolated on blood agar for identification. All isolates were first checked for cell morphological properties by a Gram stain. Isolates from blood agar with a S. sanguis/oralis/gordonii type of colony and isolates from TSY20B were identified with the API Strept system (API system, SA, Montalieu-Vercieu, France). The other isolates were identified with the API 20A system (anaerobic system).

Clinical examination.—After the bacteriological sampling, the clinical examination was performed. The exposed root surfaces per tooth were recorded, and decayed and filled root surfaces were scored. The criteria for epidemiological studies, as suggested by Katz et al. (1982), were used to diagnose caries lesions of the root surface. When more than half a lesion was located on the root surface, it was considered as originating from the root surface. Lesions due to erosion adjacent to restorations of the crowns or restorations were excluded.

The texture and depth of the lesions were scored in the central part of the lesion. When the lesion was easy to penetrate and carious dentin could be removed with moderate pressure of a small spoon excavator (diameter = 1.0 mm), the lesion was considered soft; otherwise, it was considered hard. Lesions were differentiated into initial lesions (depth <0.5 mm) and advanced lesions (depth >0.5 mm).

The color of the lesion was scored by comparison with a medium-brownish porcelain color sample. The color sample was made by mixing Vitadur® (Vita Zahnfabrik, Bad Säckingen, Germany) colors 272 (three parts) and 273 (one part).

After the periodontal control and tooth cleaning, the clinical examination was repeated and the varnishes applied. The study was carried out double-blind. One of the authors applied the varnishes, and another did the clinical examination and took the samples.

Statistical procedures.—The bacteriological counts were log₁₀ transformed prior to statistical analyses. The transformed data were subjected to a three-way ANOVA with interaction. The dependent variable was the change in the bacteriological counts, as compared with baseline. The independent variables were patient group (two levels), caries status (two levels), and interaction between group and caries status.

Differences in the numbers of carious surfaces among groups were tested with a Chi-square test. Changes in texture or color of the lesions were evaluated with the McNemar test (Siegel, 1956).

Results.

ANOVA revealed that during the experimental period there were no significant differences in the numbers of total counts (6.5 ± 0.2, mean ± SEM, log units) or in the numbers of S. sanguis/oralis/gordonii (5.2 ± 0.3) in the plaque samples.

The mutans streptococci levels in plaque samples from carious root surfaces at baseline (4.6 ± 0.3) were significantly higher than in the samples from sound root surfaces (3.0 ± 0.3; p<0.05). At baseline, in most of the samples from sound root surfaces, mutans streptococci were not present or were present only in low numbers. The level of A. viscosus/naeslundii at baseline was significantly higher in plaque samples from sound root surfaces (5.2 ± 0.2) than in plaque from carious root surfaces (4.7 ± 0.2; p<0.01).

Chlorhexidine treatment resulted in a significant decrease in the number of mutans streptococci on carious root surfaces (Fig.; p<0.05), but not on sound surfaces. No suppression in mutans streptococcal counts was observed following fluoride varnish applications on carious (Fig.) or sound surfaces. The number of A. viscosus/naeslundii was increased after fluoride and chlorhexidine treatment, as compared with the baseline values on sound and carious surfaces; however, the differences were not statistically significant (Fig.).

The caries prevalence in control and experimental groups at baseline and after one year is presented in Table 1. It is noted that the experimental groups were not balanced with respect to the initial prevalence of root-surface caries. Whereas 8.7% of the root surfaces in the control group were decayed or filled at the start of the study, this number was 12.5% in the fluoride group and 16.6% in the chlorhexidine group. In the control group, 11 out of 13 patients (85%) developed one or more...
root-caries lesions; in the fluoride group, nine patients out of 15 (60%), and in the chlorhexidine group, eight patients out of 16 (50%).

The increase in decayed and filled root surfaces in the control group (1.53 lesion per patient) was significantly higher (p<0.01; Chi-square test) than in the fluoride group (0.67) or chlorhexidine group (0.75). At baseline, almost 60% of the lesions were located on buccal or lingual surfaces, and 40% on approximal surfaces. Of the new lesions, 45% developed on buccal or lingual surfaces, and 55% on approximal surfaces.

Changes in the texture of initial lesions after one year are given in Table 2. After chlorhexidine varnish treatment, significantly more soft lesions had hardened than after fluoride treatment or in the control group (p<0.05, McNemar test). Concomitantly, fewer lesions in the chlorhexidine group had become softer than in the other two groups.

In Table 3, the relationship is given between color and texture of the root-surface lesions present at baseline and after one year. At the start of the experiment, 67% of the lesions were hard, increasing to 77% at the end of the study. Most hard lesions were yellowish to light-brown. Soft lesions were almost equally distributed over light and dark lesions. In all groups, the number of light lesions increased; however, the increase was significant only in the chlorhexidine group (p<0.025, McNemar test).

Discussion.

Caries lesions of the root surface often develop in approximal areas that are difficult for the patient to clean. Additional preventive methods are useful, especially in patients with many exposed root surfaces. The aim of the present study was to compare the effects of fluoride varnish and chlorhexidine varnish on the composition of plaque covering the root surfaces, and on caries development of root surfaces.

Higher levels of mutans streptococci were found on carious root surfaces than on sound surfaces, which is in agreement with other studies (Billings et al., 1985; Ellen et al., 1985a; Brown et al., 1986; Keltjens et al., 1987; Fure et al., 1987). The level of Actinomyces viscosus/naeslundii was higher on sound surfaces than on carious surfaces, confirming other studies (Brown et al., 1986; Fure et al., 1987; Keltjens et al., 1987). However, higher levels of A. viscosus/naeslundii on carious than on sound root surfaces have also been reported (Ellen et al., 1985b; Emilson et al., 1988). After chlorhexidine and fluoride treatment, an increase (non-significant) was observed in A. viscosus/naeslundii counts on carious surfaces. A similar phenomenon was also observed by Billings et al. (1985) after daily treatment of root surfaces with NaF gel.

Mutans streptococci on the root surfaces were effectively suppressed by the three monthly applications of chlorhexidine varnish. The long-term suppression of mutans streptococci after chlorhexidine varnish application confirms earlier studies (Sandham et al., 1988; Schaeken and De Haan, 1989; Schaeken et al., 1989). It has been speculated (Schaeken et al., 1989) that the long-term and selective suppression of mutans streptococci results from its high sensitivity to chlorhexidine (Emilson, 1977), as well as from a competition between A. viscosus/naeslundii and mutans streptococci (Van Der Hoeven and Rogers, 1979). The clinical findings are paralleled by observations in chemostat cultures. In the latter experiments, it was found that pulses of chlorhexidine led to a selective long-term suppression of Streptococcus mutans. The reduction of S. mutans in the culture was accompanied by an increase in the number of Streptococcus sanguis (McDermid et al., 1987). The increased levels of A. viscosus/naeslundii and the undisturbed levels of S. sanguis/oralis/gordonii after chlorhexidine treatment might have interfered with re-growth of mutans streptococci, and thus have contributed to their long-term suppression.

Vehkalahti and Paunio (1988) found, in their epidemiological study, that dental health behavior influenced root caries occurrence and that caries on root surfaces was associated with poor dental-health habits, such as low toothbrushing frequency and high sucrose intake. Locker et al. (1989) reported similar findings. Despite excellent oral hygiene, a considerable number of patients in our study still developed caries lesions. The caries increment in the control group, expressed as number of lesions as well as softening, i.e., progression of initial lesions, indicated the high risk for root-surface caries in these periodontal patients, compared with other populations (Hand et al., 1988; Hunt et al., 1989; Leske and Ripa, 1989). Ravald et al. (1986) also reported that of 31 periodontally treated patients

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TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Decayed (D) Surfaces</th>
<th>Filled (F) Surfaces</th>
<th>Missing (M) Surfaces</th>
<th>Increase in DMF Surfaces</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>B</td>
<td>1 year</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>13 (11)</td>
<td>32</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Fluoride</td>
<td>15 (9)</td>
<td>66</td>
<td>70</td>
<td>23</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>16 (6)</td>
<td>93</td>
<td>95</td>
<td>28</td>
</tr>
</tbody>
</table>

B = Baseline; n = number of patients; and numbers in brackets, patients that developed caries.

*One tooth extracted; § = p<0.01, compared with control; Chi-square test.

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TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 29)</th>
<th>Fluoride (n = 49)</th>
<th>Chlorhexidine (n = 62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged</td>
<td>76%</td>
<td>81%</td>
<td>80%</td>
</tr>
<tr>
<td>Hardened</td>
<td>3%</td>
<td>11%</td>
<td>15%*</td>
</tr>
<tr>
<td>Softened</td>
<td>10%</td>
<td>6%</td>
<td>2%*</td>
</tr>
<tr>
<td>Filled</td>
<td>10%</td>
<td>2%</td>
<td>3%</td>
</tr>
</tbody>
</table>

* = p<0.05, McNemar test.

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TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 181)</th>
<th>1 year (n = 210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light and soft</td>
<td>17%</td>
<td>13%</td>
</tr>
<tr>
<td>Light and hard</td>
<td>51%</td>
<td>67%</td>
</tr>
<tr>
<td>Total light</td>
<td>68%</td>
<td>80%*</td>
</tr>
<tr>
<td>Dark and soft</td>
<td>16%</td>
<td>10%</td>
</tr>
<tr>
<td>Dark and hard</td>
<td>16%</td>
<td>10%</td>
</tr>
<tr>
<td>Total dark</td>
<td>32%</td>
<td>20%*</td>
</tr>
</tbody>
</table>

* = p<0.025, McNemar test.
who were followed longitudinally, 19 developed new root-caries lesions.

Compared with the control group, caries progression after fluoride and chlorhexidine treatments was decreased. The increase in decayed and filled root surfaces after fluoride and chlorhexidine varnish treatment was approximately the same. The increase in numbers of lesions in the treatment groups was low, and a shift was observed from soft lesions at baseline toward hard lesions at the end of the experiment, indicating arrest of caries. Hardening could also be due to removal of the softened surface during the periodontal check-ups or by caries diagnosis during the study; however, in the fluoride and chlorhexidine group, more lesions hardened than in the control group. The color of the lesion was not reliable for differentiating arrested (hard) from progressive (soft) caries lesions. The effect of fluoride on root-surface caries has been studied in clinical trials. Ripa et al. (1987) determined the effect of daily rinsing with a 0.05%-NaF mouthrinse on coronal and root-surface caries. They found that the root-caries increment on approximal surfaces was less in the fluoride group than in the placebo group. The effect of excavating and reconstructing root-caries lesions in combination with application of concentrated fluoride preparations was studied by Billings et al. (1985) in six patients and by Markitziu et al. (1988) in 12 patients. Nyvad and Fejerskov (1986) studied the effect of meticulous toothbrushing with fluoride toothpaste in ten subjects. These three groups of researchers reported a high percentage of arrested root-caries lesions, viz., respectively, 70%, 97%, and 100%. However, since in none of these three studies was a control group included, the effect of fluoride is difficult to assess. The effect of chlorhexidine gel application and suppression of mutants streptococci on enamel caries in schoolchildren has been demonstrated in several studies (Zickert et al., 1982; Gisselsson et al., 1988; Lindquist et al., 1989). The present study suggests that the preventive effect of chlorhexidine varnish treatment was due to suppression of mutants streptococci.

In the present study, subjects were randomly assigned to the experimental groups. This resulted in unequal distribution of root caries over the groups. Thus, in the chlorhexidine group, more root-surface lesions occurred at baseline than in the other groups. This may well indicate a higher caries susceptibility of the individuals in the chlorhexidine group (Raval and Hamp, 1981; Raval et al., 1986; Leske and Ripa, 1989). Other factors that have been used in the prediction of root caries are the salivary level of mutants streptococci and lactobacilli and the root-caries incidence at baseline (Raval and Hamp, 1981; Ellen et al., 1985b; Raval et al., 1986). In view of the above shortcomings, additional studies are required to evaluate the caries-inhibiting properties of chlorhexidine varnish treatment.

REFERENCES