Interdental caries incidence and progression in relation to mutans streptococci suppression after chlorhexidine-thymol varnish treatments in schoolchildren

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The aim of this study was to evaluate interdental caries incidence and progression in relation to the effect of antibacterial varnish treatments in schoolchildren anticipated at caries risk. After a screening procedure, 110 healthy children (8–10 years) with moderate and high counts of salivary mutans streptococci (MS) were invited to join a 2-year longitudinal study. At baseline, MS were enumerated at all mesial interdental sites of the first permanent molars with a chair-side technique. The children were then treated 3 times within 2 weeks by interdental spot applications with a 1% chlorhexidine/thymol-containing varnish (Cervitec®). Follow-up samples of saliva and plaque were collected 1, 3, 6, and 12 months after onset of treatment. Caries prevalence, incidence and progression of the selected approximal surfaces were scored from bitewing radiographs exposed at baseline and after 2 years. Sixty-three children of the same age formed a non-varnish-treated reference group. Reduction of caries incidence and progression was clearly dependent on the outcome of the antibacterial treatment. A significantly ($P < 0.01$) higher progression score was found among children who exhibited a less marked suppression of interdental MS levels compared with those with high suppression and the children of the reference group. The results suggest that a suppression of MS in interdental plaque may be an important event to prevent and arrest approximal caries development in schoolchildren at risk. Monitoring the effect of antibacterial agents in a site-specific way could therefore be advocated. □ *Antibacterial varnish; caries progression; chlorhexidine; mutans streptococci; thymol*

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The introduction of chlorhexidine (CHX)-containing dental varnishes has gained current interest in the literature as well as in clinical practice (1–6). Although a large number of papers have been presented demonstrating a significant suppression of mutans streptococci (MS) in plaque and in saliva, clinical data on the effect on caries development are sparse. A protective role of CHX varnishes has been suggested on root surfaces in situ (3, 7) and on bovine enamel and dentine in a demineralization model (8). A CHX-inhibiting effect in first permanent molars was demonstrated in a split-mouth study when CHX/thymol varnish-treated fissures were compared with untreated control teeth (9). On the other hand, no protective effect on initial white spot demineralizations adjacent to brackets were noted in children with fixed orthodontic appliances, in spite of significant reductions of the cariogenic microflora (10, 11). Furthermore, it was recently suggested that a CHX/fluoride varnish mixture had no additional preventive effect on approximal caries incidence compared with fluoride varnish treatments alone (12). It has been shown that daily use of a CHX-fluoride rinsing solution produced marked reductions of both caries and gingivitis in schoolchildren, and the reductions were stronger than those obtained with the sodium fluoride rinsing solution (13). The clinical findings were in accordance with the earlier findings by Luoma (14) on the effect of a CHX-fluoride combination on the acid production and electrolyte metabolism of a mutans streptococcus strain.

One explanation for the clinically weak support for a varnish-induced caries reduction might be a low and unevenly distributed caries incidence in the previous study populations, in spite of efforts to select children at risk. Another reason might be that the targeted treatment has shortcomings due to factors such as varnish retention, in situ CHX concentration, or inadequate sustained release. We have previously reported a relatively high frequency of non-suppressed interdental sites (15, 16) following antibacterial varnish applications. The aim of the present study was therefore to analyse interdental caries incidence and progression in relation to the degree of mutans streptococci suppression following CHX-thymol varnish treatments in schoolchildren with an anticipated caries risk.

Material and methods

Subjects

In order to obtain a suitable study group, 210 healthy

schoolchildren (8–10 years) who had not had any antibiotic treatment within a period of 1 month were screened in their classroom for the levels of salivary MS using a chair-side test method (see below). The children with moderate and high counts of MS were invited to participate in the study ($n = 111$) and, with one exception, the parents of the children consented after written and verbal information. Three families moved from the region during the course of the study, and thus 107 children were available at the final examination. All children had received regular comprehensive dental care at a Public Dental School Clinic at least once a year since the age of 3 years. The participants were inhabitants in an area with a low natural fluoride content (0.1 ppm) in the drinking water and used fluoridated dentifrice on a daily basis. An age-matched reference group consisting of 63 children was selected from a Public Dental Clinic in the same urban area.

Study outline

In the study group, interdental plaque samples were taken at baseline from the mesial interdental sites of all first permanent molars, 4 in each subject. The interdental sites were then treated 3 times within a period of 2 weeks with an antibacterial varnish (see below). Follow-up microbial samplings of saliva and interdental plaque were carried out at predetermined intervals, 1, 3, and 6, and 12 months after onset of treatment. The prevalence, incidence, and progression of caries on the mesial surfaces of the first molars were scored from bitewing radiographs at baseline and after 2 years. The children of the reference group received a regular preventive oriented dental care without bacterial samplings or any antibacterial or placebo varnish treatments. Caries data were collected from bitewing radiographs in the same way as described above.

Clinical procedures

Baseline caries data from the study and the reference groups were obtained from the dental records. One bitewing radiograph was exposed on each side with special reference to the mesial surface of the first permanent molars using a plastic film holder. The prevalence of caries within the enamel and dentine was assessed and scored on the mesial surface of the first permanent molar according to Gröndahl et al. (17). One examiner assessed the radiographs with a Mattson binocular (18) under optimal light conditions, unaware of group and treatment outcome. Approximal caries was defined as a radiolucent area that could not be related to normal anatomy or hypoplasia. Sound surfaces (S), caries lesions in the outer (E_1) and inner (E_2) half of the enamel and outer dentine (D_1) as well as fillings (F) were recorded. Between the baseline and final radiographs, each transition was regarded as a progression step with the exception of $D_1 \rightarrow F$, which was scored as non-progression. A progression score was calculated for each individual. Repeated recordings were performed from 30 patients after a period of 4 weeks in order to check the intra-examiner reliability.

The antibacterial varnish was commercially available (Cervitec®, Vivacare, Schaan, Liechtenstein) and contained 1% w/w chlorhexidine diacetate and 1% w/w thymol as active ingredients. The applications were carried out at a Public Dental Clinic with modern facilities. Prior to the first application, all teeth were professionally cleaned with a rubber cup and pumice paste. The interdental areas of the permanent and primary molars were cleaned with an unwaxed dental floss. Quadrant by quadrant was then isolated with cotton rolls and dried with compressed air. Using a micro-brush, the varnish was spot-applied interdentally between the first permanent molars and the second primary molars and left to set for a few minutes. The subjects were instructed not to eat for 3 h and not to brush their teeth on the very same day. No further cleaning preceded the second and the third varnish application.

Microbiological procedures

The number of salivary mutans streptococci was determined with the chair-side Strip mutans method (Dentocult SM, Vivadent, Schaan, Liechtenstein) according to Jensen and Bratthall (19). The strips were cultivated for 48 h at 37°C and evaluated and scored with the aid of a special device and a stereomicroscope with 6–25× magnification (20). The number of colony-forming units (CFU) with the characteristic morphology was counted in a $1cm^2$ squared lattice and scored 0–3. Subjects with scores 2 and 3, corresponding to approximately $\geq 10^5$ CFU/ml saliva, were invited to the further study.

The interdental levels of mutans streptococci were determined with a site-specific modified Strip mutans technique (21). After isolation and drying of the approximal area, a small brush was slightly wetted in sterile saline and then inserted in between the first permanent molar and the second primary molar. The sample was taken from the buccal side in the lower jaw and from the palatal side in the upper jaw. The brush was immediately streaked once, straight across the strip, on the rough side. In that way all four first molar approximal sites of one subject were sampled on the same strip. After a 48 h cultivation period, the strips were evaluated as described above. A mean value of the interdental mutans streptococci colonization was calculated on each sampling occasion where 0–20 CFU was considered as low and >20 CFU as a high colonization level.

Outcome of treatment

For children with highly colonized interdental sites (>20 CFU) at baseline, the following cut-off values were used to define the outcome of the varnish treatments: a *highly suppressive* effect was considered either when the counts of interdental mutans streptococci were reduced by >90% compared with the baseline values at the 1-month follow-

Table 1. Distribution of mesial caries incidence/progression in first molars of 107 schoolchildren during the 2-year study period. Values in table denote number of surfaces. Four mesial surfaces were unreadable due to orthodontic bands. S = intact surface; E_1 = enamel caries $<\frac{1}{2}$ enamel width; E_2 = enamel caries $>\frac{1}{2}$ enamel width; D_1 = dentine caries $<\frac{1}{2}$ dentine width; F = filling

	Bitewing score after 2 years					
Baseline	S	E_1	E_2	D_1	F	*n*
S	265	35	1	–	–	301
E_1	–	59	15	10	1	85
E_2	–	–	15	4	3	22
D_1	–	–	–	4	8	12
F	–	–	–	–	4	4
Sum	265	94	31	18	16	424

Table 2. Mean levels of salivary and interdental mutans streptococci (MS) in schoolchildren at risk ($n = 107$) at baseline and during the first study year. Values denote number of colony-forming units (CFU)

	Saliva		Interdental sites	
Time	Mean	±SD	Mean	±SD
Baseline	482	381	46	32
1 month	298*	300	18*	21
3 months	413	356	33*	24
6 months	458	367	37	31
12 months	503	384	42	33

* Statistically significant difference ($P < 0.05$) compared with baseline.

up or when a >50% reduction during the first 3 months after the treatments was recorded. Children who exhibited less reduction were characterized as *less suppressed*. Those with low or non-detectable interdental mutans streptococci levels at baseline (0–20 CFU) were scored as *increased* when the mutans streptococci levels were >30 CFU at a minimum of 2 follow-up sampling occasions during the first study year and *unchanged* when the levels remained below 30 CFU at all 4 follow-up samplings.

Statistical methods

Bacterial alterations were evaluated with ANOVA for repeated measures. Data on the progression score in the various treatment outcome groups were processed with Student's *t* test.

Results

The caries prevalence (mean DMFS ± SD) at baseline was 1.2 ± 1.6 in the study group and 0.8 ± 1.2 in the reference group. The corresponding values for the primary teeth (dmfs) were 3.8 ± 4.3 and 2.9 ± 3.3, respectively. The differences between the groups were not statistically significant.

In 24 children (22%) of the study group, new enamel caries lesions developed at the mesial surfaces of the first permanent molars, and in 19 subjects (18%) a progression of already existing lesions occurred. The values were similar in the reference group; 20% and 19%, respectively. Only one individual of the study group exhibited both new lesions and progressions. The caries incidence and progression during the study period are detailed as transitions in Table 1. The mean salivary and interdental mutans streptococci levels at baseline and at the follow-ups are given in Table 2. A statistically significant reduction ($P < 0.05$) of the bacterial colonization levels was found at the 1-month follow-up in saliva and after 1 and 3 months in interdental plaque. The incidence and progression of caries at the mesial surfaces of the first permanent molars in relation to the outcome of the antibacterial varnish treatment is shown in Fig. 1. Fifty-seven children (54%) displayed high mutans streptococci counts at baseline and 27 fulfilled the criteria for a significant suppression. Nine of them developed caries compared with 18 children of those with a less favorable treatment outcome. The mean values of the progression score for the various outcome groups and the reference group are presented in Table 3. The caries progression score was significantly higher ($P < 0.01$) in the "less suppressed" group compared with the other 3 outcome groups as well as the reference group. Twelve approximal fillings were performed during the course of the study in the study group, 1 in the highly and 11 in the less suppressed treatment outcome group.

The intra-examiner reliability test showed an 89% agreement between the two radiographic evaluations.

Discussion

The present study was an attempt to evaluate whether suppression of mutans streptococci, induced by CHX-thymol varnish treatments, was followed by a subsequent reduction of approximal caries incidence and progression. For this purpose, children with high counts of salivary mutans streptococci and with an anticipated caries risk were recruited. The antibacterial varnish regimen represented a preventive strategy implemented for children at risk at our clinic and a reference group consisting of children of the same age was therefore selected from a neighboring clinic to represent a conventional treatment regimen. Since not selected according to risk, the reference group exhibited slightly less caries at baseline, but the difference was not significant.

It has been stated that the best cariostatic effect of antibacterial regimens is achieved when the outcome of the treatment is verified with follow-up samples (22), and the prospective study design enabled us to monitor the outcome of the varnish applications regularly during the

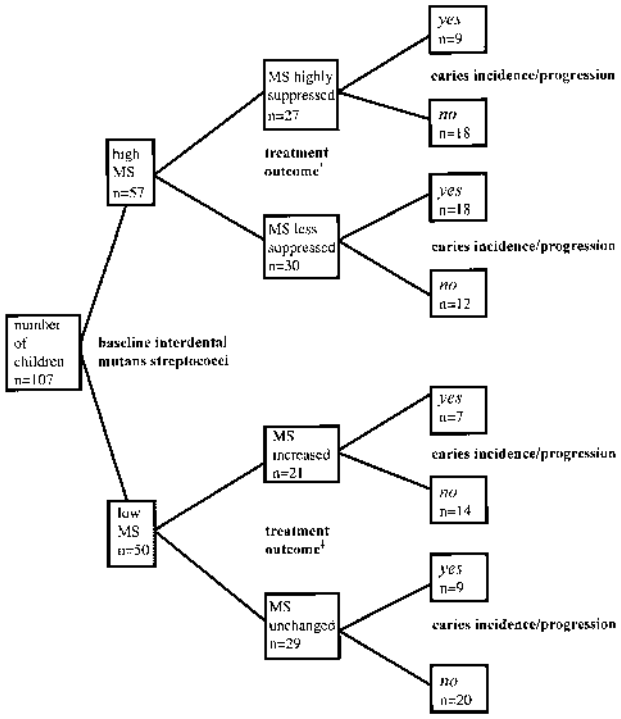

Fig. 1. Flow chart based on mean values of mutans streptococci at the mesial surfaces of the first permanent molar in 107 schoolchildren. Two-year caries progression is given for the variables "high" (>20 CFU) and "low" (0–20 CFU) levels of mutans streptococci (MS) colonization at baseline and for microbial alterations following antibacterial varnish treatments. †MS highly suppressed: >90% mutans streptococci reduction. MS less suppressed: <90% mutans streptococci reduction. ‡MS increased: at least 2 follow-up samplings with >30 CFU. MS unchanged: less than <30 CFU at all follow-ups.

first study year. The obtained MS reduction following treatment was of the same magnitude and in agreement with previous published results with the same varnish and mode of application (15). As shown earlier (15, 16), a marked inter-individual variation in mutans streptococci suppression was found within the study group, allowing a relatively clear-cut subgrouping of the children into treatment outcome groups. The cut-off levels were selected according to earlier experience (15, 16) and less than 50% of the highly colonized children was considered as highly suppressed after the varnish treatments. It can be argued that the predetermined criterion of a 90% bacterial reduction was rigorous and this can always be disputed. However, a moderate lowering of the criteria in the present material did not alter the obtained differences in caries progression score in a significant way. The variation in susceptibility to the combination of the two antimicrobial agents might be explained by the fact that dental plaque bacteria grow in biofilms on the tooth surfaces. Such biofilms are individually composed of several bacterial species and can be modified by local host factors

Table 3. Calculated progression score in relation to interdental mutans streptococci suppression after treatments with an antibacterial varnish in 107 schoolchildren and in a reference group. For definition of progression score and treatment outcome groups, see "Material and methods"

	Progression score			
Baseline MS/ outcome group	*n*	Mean	SD	Range
High/highly suppressed	27	0.41	±0.64	0–2
High/less suppressed	30	1.43*	±1.61	0–6
Low/increased	21	0.43	±0.75	0–3
Low/unchanged	29	0.45	±0.83	0–3
Reference group	63	0.62	±0.90	0–4

* $P < 0.01$ as compared to the other groups.

that may require very high concentrations of a drug to be affected compared with pure batch cultures (23, 24).

The main finding of this study was that a suppression of mutans streptococci caused by the antibacterial varnish treatments seemed to be accompanied by a reduced caries incidence and progression rate in most cases and, interestingly, the progression score was of the same level as in the reference group. Since many previous intervention studies with antibacterial varnishes were focused on either microbial or clinical evaluation, the present findings can be regarded as a complement to previous knowledge of the two parameters (3). It is stressed, however, that the results must be interpreted with some caution, especially in the light of the fact that bacterial mean values were calculated reflecting the subject rather then the single surface. The low incidence of new enamel lesions and the slow rate of progression, although the participants were classified at caries risk, should also be considered. For example, 82% of the surfaces in the study group and 79% in the reference group showed the same caries score at baseline and after 2 years. Thus, no difference in caries development was obtained when the complete study group was compared with the reference group.

It might be of interest to speculate over the mechanisms behind the possible caries protective role of the antibacterial agent. The CHX-thymol varnish could induce a decrease in the proportion of streptococci in the interdental plaque that would lead to a locally diminished acidogenic challenge. Thereby, the mineral precipitation through the presence of fluoride is promoted (25, 26). In this respect it seems conceivable that also a short-term microbial shift might be sufficient to institute a long-lasting remineralization.

The finding of an even distribution between incidence of new lesions and progressive events was expected considering the young age of the participants. However, we were unable to demonstrate any differences regarding the protective effect of the varnish on demineralized lesions compared with clinically intact enamel. Such an effect might also have been obscured by the exfoliation of second primary molars that occurred in some children

during the second year of the study. Furthermore, it should be underlined that all cavities were evaluated from bitewing radiographs, so the question whether the surfaces were cavitated clinically or not remains opens. In addition, the finding that a substantial number of non- or low-colonized mesial sites became colonized or increased their level of colonization, despite the antibacterial varnish treatments during the first year of the study, merits further attention.

In conclusion, the present study demonstrated a reduced interdental caries incidence and progression rate in subjects exhibiting varnish-induced mutans streptococci suppression compared with those with a less successful treatment outcome. Bacterial suppression may therefore be an important event in preventing and arresting caries development in schoolchildren with high counts of mutans streptococci in their plaque and saliva. In this respect, antibacterial varnishes can be useful because of the sustained release of antibacterial agents and the independence of patient co-operation and compliance. To monitor the levels of interdental bacteria, the use of a site-specific method is encouraged. Further clinical studies are needed, however, to verify the specific caries protective role of antibacterial varnishes on interdental enamel surfaces.

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