Acta Odontologica Scandinavica, 2007; 65: 284–291

informa
healthcare

ORIGINAL ARTICLE

Effect of supplementary amine fluoride gel in caries-active adolescents. A clinical QLF study

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Abstract

Objective. To investigate whether supplementary daily use of amine fluoride toothpaste with weekly brushing with amine fluoride gel enhances the remineralization of white spot lesions on smooth surfaces and also to investigate the possible effect of this treatment on salivary bacterial counts and oral hygiene. ***Material and Methods.*** The participants comprised 135 caries-active adolescents living in suburban Stockholm. They were given an amine fluoride dentifrice (1250 ppm F) to be used twice a day, and either a test gel (4000 ppm F) or a placebo gel for brushing for 2 min once a week. During the 12-month study period, the subjects were recalled every 3rd month (baseline, 3, 6, 9, and 12 months). At each visit, ΔF (average change in fluorescence, in%) and lesion area (in mm^2) were measured using quantitative light-induced fluorescence (QLF), followed by dietary counseling, oral hygiene instruction, and professional tooth-cleaning. At baseline, 6, and 12 months, saliva was sampled for *Streptococcus mutans* and lactobacillus counts, and gingival bleeding index was registered. ***Results.*** QLF indicated no enhancement of remineralization of white spot lesions by additional weekly brushing with amine fluoride gel. No inter-group differences emerged with regard to salivary bacterial counts. However, the oral hygiene of both groups improved, with a strong significance over time. ***Conclusion.*** Adjunctive weekly brushing of amine fluoride gel achieved no significant enhancement of remineralization of white spot lesions monitored with the QLF method.

Key Words: *Optical method, oral hygiene, salivary bacterial counts, white spot lesion*

Introduction

The cariostatic effects of fluoride are attributable primarily to its effects on white spot lesions: enhancement of remineralization and inhibition of demineralization [1,2]. Although use of fluoride is not the sole explanation for the decline in caries prevalence, the consensus opinion of a panel of international oral health experts is that the widespread use of fluoride toothpaste is a major factor [3]. Two recent reviews support this view [4,5]. Despite the dramatic decline in dental caries, however, the disease persists, albeit with highly skewed distribution [6,7]. In industrialized countries, a major challenge in modern clinical dentistry is the management of caries-active individuals in whom the disease progresses despite daily brushing with fluoride toothpaste.

In patients with high caries activity or at high risk for caries, daily use of fluoride toothpaste needs to be supplemented by other measures to prevent progression from initial to manifest lesions. In this context, the application of a gel containing a high concentration of fluoride has been recommended as a supplement to daily brushing with fluoride toothpaste [8,9], and has proved to be effective in children and adolescents at moderate or high caries risk [10–15], but of questionable benefit in low caries risk individuals [16,17].

The formulations most commonly used in topical fluoride gels are acidulated phosphate fluoride and sodium, stannous or amine fluoride. The slightly acidic amine fluoride is a substantive agent with the ability to bind to oral surfaces, causing an elevated salivary fluoride level [18] which subsequently leads to delayed oral fluoride clearance [19]. This could be advantageous in caries prevention, and therefore further investigation of the clinical effect of this organic fluoride component in dentifrices and gels is warranted [20].

Up until recently, clinical studies on the efficacy of fluoride treatments have been expensive and time-

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(Received 13 April 2007; accepted 9 August 2007)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350701620644

consuming projects requiring large numbers of subjects and extensive study periods, thus increasing the likelihood of non-compliance and withdrawal of subjects. Longitudinal monitoring of lesion response to non-invasive intervention has been hampered by the lack of appropriate diagnostic techniques, i.e. techniques of high sensitivity and specificity, which reflect the slow lesion progression in industrialized countries. The recent development of clinically applicable methods for detecting and quantifying very early phases of mineral loss in caries lesions has allowed flexibility in selecting intervention appropriate for the individual patient; this before lesion progression to a stage requiring expensive invasive therapy and when conducting caries clinical trials with a reduction in the duration of experimental periods and number of subjects required.

Quantitative light-induced fluorescence (QLF) is a reliable tool in clinical longitudinal studies for detecting and quantifying white spot lesions, and for monitoring de- and remineralization of incipient smooth surface lesions [21–24]. The method is based on the principle that mineral loss, caused by caries destruction of tooth enamel, can be detected and measured as a change in fluorescence [25]. In studies of the clinical efficacy of topical fluoride applications, the QLF method offers several advantages: fewer participants are required and lesion changes can be measured at intervals of just 6 weeks [22]. The high sensitivity of the method has been confirmed in several studies [26,27]. QLF measurements of incipient artificial lesions show good correlation with transversal microradiography (TMR) ($r = 0.84$), for the Xenon lamp system [26], and the positive correlation coefficient ($r = 0.82$) has been confirmed *in vitro* with a gradual modification of this system [28]. Repeatability and reproducibility of the QLF method were tested *in vivo* by Tranæus et al. [23] with excellent results.

The primary aim of the present study was to investigate whether weekly brushing with amine fluoride gel (Elmex®, 4000 ppm F) to supplement daily use of amine fluoride toothpaste (Elmex®, 1250 ppm F) in caries-active adolescents enhances the remineralization of white spot lesions on smooth surfaces over a period of 1 year. The lesions were monitored by QLF, with two quantities obtained: ΔF (average change in fluorescence, in %) and lesion area (in mm^2). A secondary aim was to evaluate the possible influence of this treatment on salivary bacterial counts and oral hygiene. The study therefore tested the following hypotheses: that the group using the test gel showed an increased fluorescence and decreased lesion area after 12 months compared with baseline, as well as compared with the group using the placebo gel.

Subjects, materials, and methods

Subjects and study design

The study was conducted in a double-blind, randomized, controlled manner approved by the Ethics Committee at Huddinge University Hospital and in accordance with ICH/GCP regulations [29]. Sample size was calculated with 90% power and $\alpha = 0.05$, and with expected SD 2.25 and smallest clinically relevant effect with 1.28% units of average change of fluorescence, 65 individuals were needed in each group. To compensate for drop outs, 181 subjects were invited to participate in the study and informed proxy consent was obtained for consenting subjects from their parents or guardians. They were all healthy, regular, patients at four public dental clinics in the southwest greater Stockholm area and at the Department of Pediatric Dentistry of the Institute of Odontology, Karolinska Institutet, and an area with naturally occurring fluoride in the drinking water of 0.2 ppm F. The inclusion criteria were young adolescents, 13 to 17 years of age, with two or more white spot lesions on the buccal surfaces of the premolars or permanent molars; these detected by the patients' regular dentist at the annual dental examination and verified by one of the operators. The exclusion criteria were cavitation or discoloration of the lesions and planned orthodontic treatment. Since the QLF method requires lesions that are limited to one surface facing the camera lens, circumferential lesions were excluded. In all participating subjects, one active buccal incipient carious lesion on a premolar or molar tooth was selected for inclusion in the study. Lesion activity was defined as a rough, whitish to yellowish colored area located close to the gingival margin, and with pronounced opacity and a dull appearance when dried with a blast of air.

After a pre-baseline visit for stratification by gender and in terms of lesion depth, the subjects were randomly assigned to either a placebo group or a test group (randomization procedure in clusters of 12: random binary outcome with a dice; even or odd numbers). All participating investigators carefully followed written standard operating procedures throughout the entire study period.

Experimental design

The study period from baseline to closed file lasted 12 months, with subjects recalled every 3rd month (Figure 1). Four operators, highly experienced in the use of QLF, conducted the examination procedure on the same group of subjects throughout the study period.

At each visit, ΔF and lesion area were first measured by QLF. All subjects received similar counseling on dietary habits, oral hygiene instruction using the "Bass technique", and were instructed to

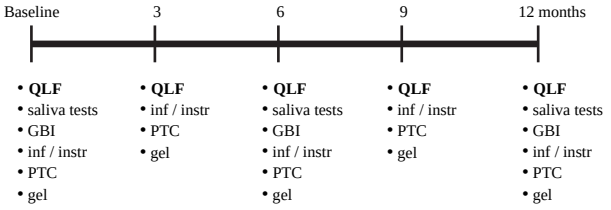

Figure 1. Study design. At each visit, ΔF and lesion area were monitored with the QLF method, and oral hygiene instruction (instr), dietary counselling (inf), and professional tooth cleaning (PTC) were carried out. A test gel or placebo gel was provided. At baseline, 6 months, and 12 months, gingival bleeding index (GBI) was registered and a saliva test was obtained to detect mutans streptococci and lactobacilli.

brush with an amine fluoride dentifrice (Elmex®; 1250 ppm F) for 2 min twice a day. The subjects were required to refrain from using any other supplementary fluorides. The teeth were professionally cleaned (PTC) using a rotating rubber cup and the same toothpaste as supplied to the subjects. If necessary, standard scaling instruments were used to remove calculus. At baseline, 6, and 12 months, paraffin-stimulated saliva was sampled for standardized chair-side tests to detect *Streptococcus mutans* (SM) and lactobacillus (Lb) (Dentocult® SM Strip Mutans and Dentocult LB®, respectively; Orion Diagnostica AB, Trosa, Sweden). Comparing the colonies on the test strip with a reference chart provided by the manufacturer assessed the density of SM in saliva, scored from 0 to 3, and growth of salivary Lb colonies on the dip-slide was compared with the densities on the corresponding reference chart. Gingival bleeding index (GBI), bleeding or no bleeding when inserting a probe (Hu-Friedy, P3-PCPUNCH15, USA) a few millimeters in and along the soft tissue pocket wall with light pressure in a circumferential direction, was recorded from the buccal, lingual, mesial and distal surfaces of all the 1st molar teeth (16, 26, 36, and 46) at each visit.

At each visit, the subjects were issued with two 25 g tubes of gel, test gel containing amine fluoride Elmex®, 4000 ppm F (33.28 mg organic Olaflur/Dectaflur, 3.32 mg NaF, aqua, propylene glycol, hydroxethylcellulose, saccharin, apple-aroma, pH 4.8) or placebo gel (without any active fluoride substance, colorant, saccharin, flavor, preservatives, and solubilizers), marked either A or B depending on the individual protocol. The subjects were instructed to brush with the gel for 2 min once a week (Sunday evening), to spit out the remaining foam, and to refrain from eating and drinking until Monday morning. They were also asked to report whether they had missed using the gel during the period, and to hand in the remaining gel from the previous period. At all visits, a panellist individual Case Report Form was signed. The toothpaste, test gel, and placebo gel used in the study were supplied by GABA International AG, Münchenstein, Switzerland.

QLF measurements and image analysis

The test surfaces were illuminated by blue light from a Xenon arc lamp with a blue filter (peak intensity of $\lambda = 370$ nm with a full width half measure of 80 nm). The lesions were recorded with a micro-CCD video camera (Panasonic; WV-KS 152, Tokyo, Japan) equipped with a yellow highpass filter (Hoya Y-50; Haya Corporation USA, Fremont, Calif., USA) to exclude scattered light and light with wavelengths <520 nm. A computer program (QLF 1.97e Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to display, store, browse, and analyse the images. All images were captured in a dental surgery under identical physical conditions. A roller blind in the window excluded direct daylight, which might have caused reflections at the enamel surface and thereby disturbed the image [30].

Dehydration results in a greater loss of lesion fluorescence [31,32]: before recording, the subjects therefore rinsed with water to standardize the hydration level. The actual area was then wiped once with a cotton roll, and the image was captured. Capturing each image took approximately 5 s, an interval brief enough to prevent dehydration. Corrections in distance deviations were made using contour points, a software facility that automatically compensates for changes in distance between camera and lesion during imaging. All images were analysed by one examiner. Two parameters were measured using a 5% threshold for technical noise: ΔF (average change in fluorescence, in%) and lesion area (in mm^2).

Statistical analyses/methods

The QLF data were exported to Excel and then to the statistics program STATISTICA, version 5 (StatSoft, Tulsa, Okla., USA). The data comprised three variables, which were repeated five times (five visits). As the QLF variables did not follow normal distribution, log transformation was performed. Besides the measured variables, there were additional group variables, such as thresholds, tooth numbers, etc. The data were also separated into placebo or test groups according to the supplementary treatment: Repeated measures ANOVA was then applied to determine differences between the two experimental groups and changes over time. Inter-group interactions and differences were determined and evaluated, and finally completed with multivariate statistical methods. Significance was set at the $p < 0.05$ levels by ANOVA.

Results

Sixteen subjects withdrew before baseline and a further 30 during the study. Among reasons for

withdrawal were extraction of the test tooth, fixed orthodontic appliance therapy and the subjects' own initiative. Seventeen subjects gave no explanation for withdrawal. One unexpected adverse event occurred during the study: one subject withdrew because of an undiagnosed oral yeast growth, although no association with the trial drug was confirmed. At closed file, 135 subjects had completed the study: 65 in the test group (31 M and 34 F, with a mean age of 14.31, range 11.6–17.2 years) and 70 in the placebo group (33 M and 37 F, with a mean age of 14.94, range 11.94–17.94 years). According to the patients' files, the numbers of new decayed teeth (DT) in the year before the start of the study were: test group mean 4.71 and placebo group mean 5.6.

There were no statistically significant differences in the baseline characteristics of the two experimental groups (lesion depth $p=0.12$; SM counts $p=0.39$; Lb counts $p=0.60$; GBI $p=0.64$). The baseline characteristics showed that 43 of 65 subjects in the test group and 39 of 70 in the placebo group had a heightened caries risk, i.e. they exhibited at least two or more of the following criteria: scores 2 or 3 of SM, Lb $>10^4$, or number of bleeding surfaces >4. The remaining subjects had one of these criteria. In 57% of subjects, the mandibular left 1st molar (tooth number 36) was selected as the test tooth.

Changes in fluorescence radiance

Small inter-group differences at baseline in regard to lesion area (test group 1.62 mm^2, placebo group 1.75 mm^2) and ΔF (test group 8.62%, placebo group 8.40%) were not statistically significant.

Figure 2A shows ΔF for all teeth during the study period. In the test group, ΔF values at the 6-month recall (8.09%) were significantly different from both the baseline values (7.63%) and those recorded at the 9-month recall (7.48%). When ΔFs at all visits were computed with ANOVA, a general $p=0.009$ was found. No corresponding significant differences were disclosed in the placebo group.

Figure 2B shows lesion area for all teeth during the study period. In the test group, the lesion areas recorded at the 3-month visit (1.58 mm^2) were significantly different from those at baseline (1.78 mm^2), after 9 months (1.75 mm^2), and after 12 months (1.73 mm^2). When ΔFs at all visits were computed with ANOVA, a general $p=0.001$ was found. There were no corresponding significant differences in the placebo group.

Figure 3A shows ΔF for the test tooth (no. 36) during the study period. In the test group, the values recorded at the 6-month visit (9.0%) were significantly different from those recorded at 9 months (8.40%). When ΔFs at all visits were computed with ANOVA, a general $p=0.012$ was found. There were no corresponding significant differences in the placebo group.

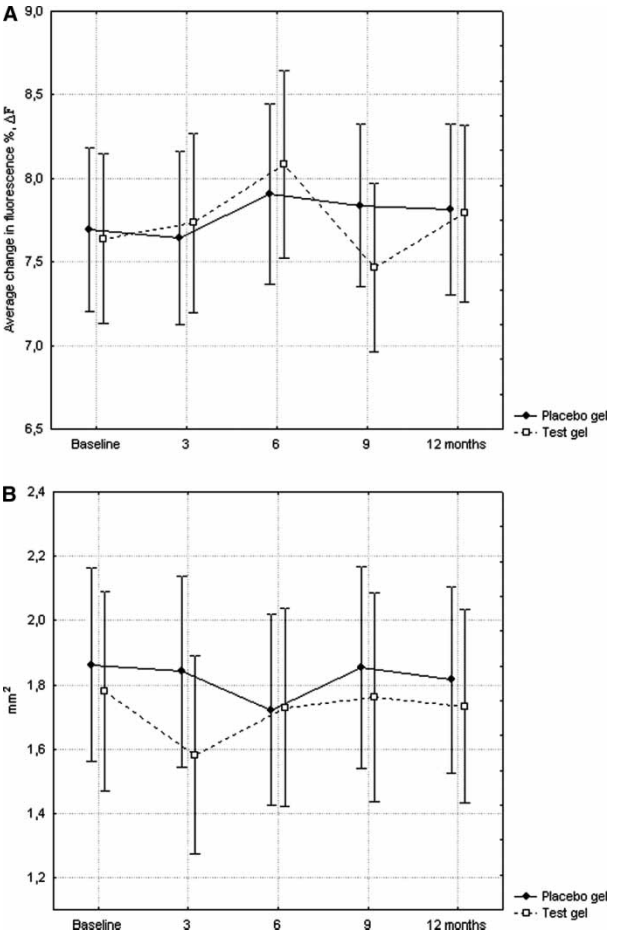

Figure 2. A. ΔF with −5% threshold for all teeth over time ($n=135$). Vertical bars denote 0.95 confidence intervals. B. Lesion area with −5% threshold for all teeth over time ($n=135$). Vertical bars denote 0.95 confidence intervals.

Figure 3B shows lesion areas recorded on tooth no. 36 during the study period. There were no general significant differences.

Microbiology

With respect to SM and Lb counts, there were no statistically significant differences between placebo and test groups after 12 months. However, SM counts increased significantly over time in the placebo group ($p<0.05$), while no such changes were observed in the test group. No significant changes occurred for Lb (log transformed data) over time in either group (Figure 4).

Gingival bleeding index

In the placebo group, GBI at baseline ranged from 0 to 11 bleeding sites per subject (mean 3.6). The corresponding values for the test group were 0–16 sites (mean 4.5). GBI decreased significantly over time in both groups, with $p=0.001$ for the placebo group and $p=0.0004$ for the test group (Figure 5).

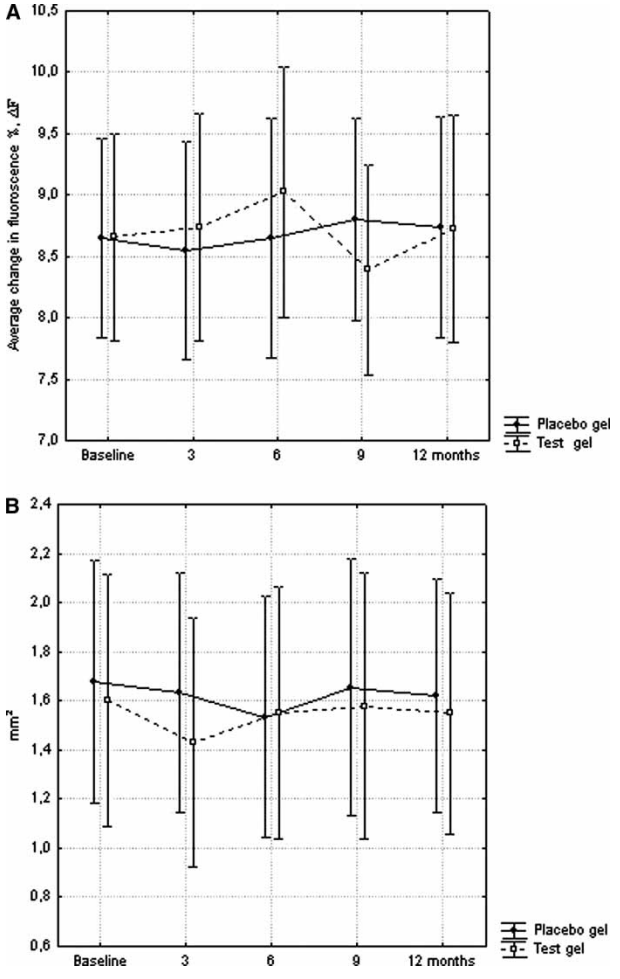

Figure 3. A. ΔF with 5% threshold for solitary tooth number 36 over time ($n=77$). Vertical bars denote 0.95 confidence intervals. B. Lesion area with −5% threshold for solitary tooth number 36 over time ($n=77$). Vertical bars denote 0.95 confidence intervals.

Discussion

General

Toothpaste is the most common vehicle for topical fluoride application, and daily brushing with fluoride toothpaste is considered to provide adequate caries prevention in children and adolescents. However, caries-active individuals require professional support to control disease progression, e.g. a program comprising intensified fluoride therapy, improved plaque control and dietary counseling until the situation is under control, and in this context supplementary fluoride is frequently applied in gel form.

Different fluoride compounds are available in gel form, and concentration, method, duration, and frequency of application vary widely. This is reflected in the literature, indicating a wide variety of study designs and range in the reported efficacy of fluoride gels. In an overview of 14 placebo-controlled trials, Marinho et al. [33] estimated the caries-inhibiting effect of fluoride gel in children and adolescents to be a 21% (95% CI, 14–28%) reduction in D(M)FS. These studies represented various forms of interventions, and basic caries preventive measures, such as daily use of fluoride toothpaste, were not always assumed. Because of the wide variations, it is difficult to compare the present results with those of earlier studies. Madléna et al. [14] conducted a study similar in design to the present one on adolescents with high caries risk. A combination of amine fluoride toothpaste and gel for 2 years resulted in a 38% reduction in DMFS; however, the fluoride concentrations of the products used in the study were not disclosed.

The subjects in our study were all regular patients attending four public dental clinics and the Department of Pediatric Dentistry of the Dental School in the southwest greater Stockholm area, which has a higher proportion of caries risk patients than the average in Sweden. Tests of the baseline characteristics showed that the panellists had a heightened caries risk (i.e. they exhibited at least two or more of the following criteria: scores 2 or 3 of SM, Lb $>10^4$, or number of bleeding surfaces >4). The preventive measures tested in this study were selected as appropriate for the target group. No clinical changes in the lesions were detected by visual inspection. QLF was thus the only way to detect and quantify the small changes that occurred during the study period.

Changes in fluorescence radiance

The changes in fluorescence radiance (lesion area in mm^2 and ΔF in %) that were observed over time as well as between the experimental groups were relatively minor, and although some were statistically significant the clinical relevance of these changes must be considered as low. Since the changes were minor, an attempt to increase the sensitivity of the analyses was made. As standard for QLF measurements, a −5% threshold for exclusion of the methodological error was used. The analyses were therefore also run at the −4% and −3% thresholds to increase sensitivity. After subjective evaluation of the images in respect of technical noise, the −4% and −3% thresholds were rejected, and the −5% threshold was chosen. An attempt to refine the data was made by excluding all other teeth than 36 (since 36 comprised 57% of the material). However, no more clinically relevant information was gained.

Changes in microbiology and gingival bleeding index

At the end of 12 months, there were no statistically significant differences between the experimental groups in either *Streptococcus mutans* or lactobacillus counts. The applied measures in this study design were apparently not strong enough to reduce the microbiological challenge. However, GBI showed a highly significant improvement over time in both groups. This might indicate improved compliance in respect of oral hygiene but not of dietary habits. It might also indicate that the combinations of PTC

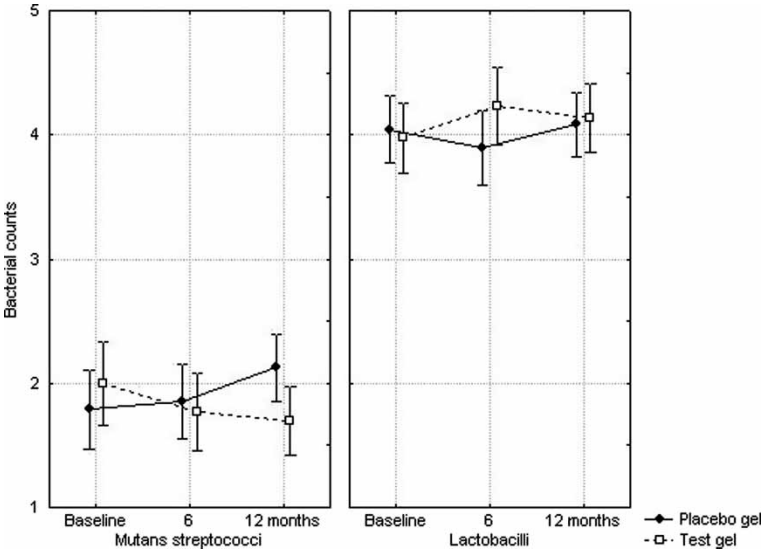

Figure 4. Bacterial counts over time and between the experimental groups. Vertical bars denote 0.95 confidence intervals.

every 3rd month, dietary counseling and oral hygiene instruction had a beneficial effect on gingival bleeding.

Participation in the study and exposure to the QLF method *per se* probably boosted motivation and cooperation. Able to follow the measurements on the computer screen, the subjects were interested in the appearance of their own lesions as well as in the modern camera and computer technique. Some increase of fluorescence in both trial groups might therefore be attributable to improved oral hygiene, and the possible potential supplementary benefit of the gel was likely to be confounded by this improved regime. As the degree of interest of each of the trial groups seemed to be similar, this factor probably had an even influence on the results.

Confounding factors

A well-known predictor for progression of the caries disease is past caries experience [34]. All recruited subjects had been assessed as highly caries active, and tests of the baseline characteristics showed that the panellists had a heightened caries risk. We attempted to assess general lesion activity in each subject, as well as the individual activity of each lesion included in the study. Although we appraised the chosen lesion in the context of the subject's general caries activity (presence of plaque, surface texture, color, etc.), the activity of the specific lesion could be misinterpreted, and while white spots newly generated under orthodontic bands have been reported to remineralize readily, older lesions may not respond to fluoride [24]. We also considered the possibility that progression of some of the included lesions had reached a "plateau": continued progression was unlikely, irrespective of additional brushing with the amine fluoride gel. An attempt to investigate the longitudinal development of the individual caries activity of the participants, before and after this study, was made with reference to dental records maintained by the clinicians. A measure of the individual caries increment over the previous 4 years was obtained by collecting the data registered at the regular annual dental examinations by traditional diagnostic methods, such as mirror and probe and bitewing X-rays. Based on data from 2 years and 1 year before the study, at study start, and 1 year after the end of the study, a measure of the lesion development within each individual was obtained. During the 4-year period, all five clinics had changed from analog to digital radiographic technique. Moreover, two or more dentists had examined most of the subjects. The data in the patient files were therefore considered not trustworthy and no additional relevant information was gained. There is a possibility that the time-span applied in this study (1 year) was too short. However, QLF is a sensitive tool and it has previously been shown that remineralization of white spot lesions can be monitored at intervals of

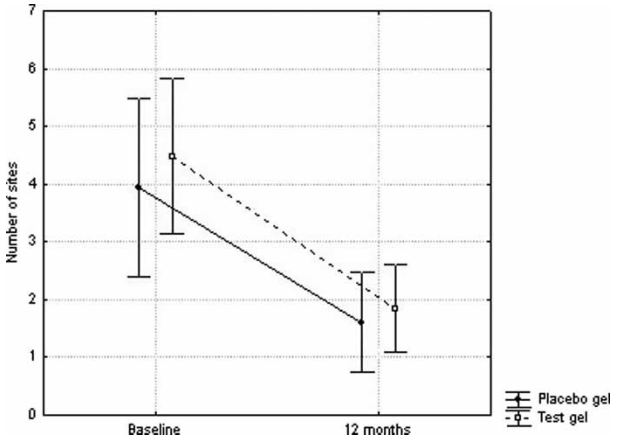

Figure 5. Gingival bleeding index over time and between the experimental groups. Vertical bars denote 0.95 confidence intervals.

Table I. Participant flow.

Included	181		
Drop-outs before baseline		16	
Baseline	165		
Drop-outs after baseline		30	
No show			17
Drop-out by own initiative			5
Technically depressed images			3
Tooth extracted			1
Fixed orthodontic appliances			1
Wrong gel distributed			1
Wrong toothpaste and no gel			1
Moved			1
Closed file	135		
Placebo group	70		
Test group	65		

only 6 weeks [22]. Another possible influencing factor could be a micro abrasion of the initial lesions. Årtun & Thylstrup [35] studied the surface changes of incipient caries lesions after debonding and found time-related changes with gradual removal of the external microsurfaces and of the outermost partly dissolved enamel lesions. These findings are supported by Kielbassa et al. [36], who investigated the abrasive effects of toothbrushing with an amine fluoride gel (Elmex Gelée, 12 500 ppm F) on demineralized bovine enamel. Results from this study showed considerable wear in artificial subsurface lesions two to three times deeper than in sound bovine enamel, and correlations between abrasion and microhardness and between abrasion and mineral content, respectively. We found an initially significant decrease in lesion area (Figure 2b), and in previous studies it has been speculated whether the clinically observed disappearance of smooth surface white spot lesions is due to remineralization, abrasion, or both [35,37]. A partial loss of surface area might render the lesion more vulnerable, thus leading to increased caries susceptibility and subsequently to increased lesion area again. Both these aspects could have influenced the result in the present study with an initially increased ΔF and an initially decreased lesion area; this due to a combination of an acidic gel, improved toothbrushing and PTC every 3rd month.

Another confounding factor could be compliance of the panellists. It is not known to what extent the subjects really followed the instructions during the study period. At all visits, they were asked whether they had carried out the home treatment, and were encouraged to be honest in their responses. Although only a few lapses were reported by subjects over the entire study period, their response had to be interpreted with caution. At all visits, the panellists returned the used tubes of test gel or placebo gel to the investigator. These were then labelled with subject number and returned to the sponsor for recording of consumption.

There were also some confounding technical factors. The lamp bulbs (light source) in the QLF devices had to be changed several times during the study. Differences in light intensity could have occurred, which probably resulted in some technically depressed images. The light ignition in one of the devices had to be changed, resulting in an inconsistency in that the same QLF device was not used for each subject throughout the individual study period. These problems might have had an impact on the study outcome. However, the changes in lesion area after 9 months could indicate that the compliance of the panellists decreased (increasing lesion area). This visit was not the same time-point for all the panellists, but occurred over a period of 18 months. Therefore, the change in lesion area after 9 months was probably not caused by technical noise. This indicates that the technical noise might not have had a major impact on the outcome.

Smooth surface lesions in caries-active adolescents were monitored by QLF (decrease ΔF and lesion area) over a 12-month period. The conclusion drawn from the results is that supplementary daily brushing with amine fluoride dentifrice (1250 ppm) by weekly brushing with amine fluoride gel (4000 ppm F) achieved no significant enhancement of remineralization of smooth surface lesions.

Acknowledgments

We thank Dr Joan Bevenius for invaluable language revision assistance, Dr Göran Granath for statistical assistance, the Public Dental Clinics in the southwest greater Stockholm area (Södertälje Fjärilen, Tumba sjukhem, Alby, Hallunda), and the Department of Pediatric Dentistry at the Department of Odontology, Karolinska Institutet. We also express our appreciation to Professor Jan Ekstrand for valuable advice and CRA/Monitor Charlotta Björk for introducing us to the world of Good Clinical Practice. The study was supported by GABA International AG, Emil Frey-Strasse 100, CH-4142 Münchenstein, Switzerland.

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