Acta Odontologica Scandinavica, 2013; 71: 464–468

ORIGINAL ARTICLE

Effect of fluoridated milk on enamel demineralization adjacent to fixed orthodontic appliances

KERSTIN SKÖLD-LARSSON[1], OLA SOLLENIUS[1], LENA KARLSSON[2], LARS G. PETERSSON[3] & SVANTE TWETMAN[3,4]

[1]*Department of Orthodontics, Public Dental Service, Halmstad, Sweden,* [2]*Department of Dental Medicine, Cariology and Endodontology, Karolinska Institutet, Huddinge, Sweden,* [3]*Maxillofacial Unit, Halland Hospital, Halmstad, Sweden, and* [4]*Department of Odontology, Section for Cariology, Endodontics, Pediatric Dentistry and Clinical Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

Abstract

Objective. To investigate the effect of daily intake of fluoridated milk on enamel demineralization adjacent to fixed orthodontic brackets assessed with quantitative light-induced fluorescence (QLF). ***Materials and methods.*** Sixty-four healthy adolescents (13–18 years) undergoing orthodontic treatment with fixed appliances were enrolled and randomly allocated to a randomized controlled trial with two parallel groups. The intervention group was instructed to drink one glass of milk (~ 200 ml) supplemented with fluoride (5 ppm) once daily and the subjects of the control group to drink the same amount of milk without fluoride. The intervention period was 12 weeks and the end-point was mineral gain or loss in enamel, assessed by QLF on two selected sites from each individual. The attrition rate was 12.5% and 112 sites were included in the final evaluation. ***Results.*** There was no statistically significant difference between the groups concerning fluorescence (ΔF) values and lesion area (A mm^2) at baseline. After 12 weeks, a significant decrease ($p < 0.05$) in ΔF was registered in the fluoridated milk group and a significant increase in the non-fluoride control group ($p < 0.05$). The mean reduction in the test group was somewhat lower (14%) than the increase in the control group (18%), but individual variations were evident. Only minor alterations of lesion area were recorded over the 12-week period and no statistically significant differences compared with baseline were found in any of the groups. ***Conclusion.*** Daily intake of fluoridated milk may aid remineralization of white spot lesions adjacent to fixed orthodontic appliances.

Key Words: *caries, dairy products, fluoride, light induced fluorescence*

Introduction

White spot lesion (WSL) development in association with orthodontic treatment with fixed appliances remains an unwanted clinical problem [1–3]. The presence of orthodontic bands and brackets creates an ecological environment that facilitates the establishment and growth of aciduric and cariogenic mutans streptococci strains [4]. As such lesions have limited ability to improve after appliance removal [5], preventive activities during treatment are crucial. However, systematic reviews of the literature have failed to present sufficient evidence for most preventive measures, with topical applications of fluoride-containing products as the only exception, and the need of new approaches and further well-designed clinical trials is emphasized [6–9].

Fluoridated milk has been suggested as a relatively inexpensive alternative to water fluoride for community caries prevention [10], but may also constitute a convenient and cost-efficient way of targeted fluoride supplementation. According to Yeung et al. [11], there are still insufficient studies of good quality examining the effects of fluoridated milk and a controlled trial on a group of patients with elevated caries risk could be helpful in that aspect. The use of quantitative light-induced fluorescence (QLF) offers a possibility to assess early changes in the enamel mineral content in orthodontic patients without removing the appliances [12,13]. The aim of the

Correspondence: Svante Twetman, Institute of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Nørre Allé 20, 2200 Copenhagen N, Denmark. Tel: +45 35326810. E-mail: stwe@sund.ku.dk

(Received 19 October 2011; revised 21 March 2012; accepted 20 May 2012)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2013 Informa Healthcare
DOI: 10.3109/00016357.2012.696687

present study was therefore to investigate the effect of a daily intake of fluoridated milk on enamel demineralization adjacent to fixed orthodontic brackets assessed with QLF. The null hypothesis was that the mineral content would not differ from baseline in either the active intervention group or the placebo group.

Materials and methods

Study group

Sixty-four healthy patients (29 boys and 35 girls) projected to be undergoing orthodontic treatment with fixed appliances for a period of at least 6 months at the Public Specialist Clinic in Halmstad, Sweden were invited to participate in the project. Both the subjects and their parents received verbal and written information and signed a consent form. The inclusion criteria were (i) a regular habit of drinking milk, (ii) ≥2 non-cavitated proximal lesions located within the enamel as assessed from bitewing radiographs not older than 1 year and (iii) the appliances *in situ* for at least 3 months. Exclusion criteria were (i) ongoing medication for a chronic disease and (ii) not assessed with high caries risk behavior (e.g. behavior management problems, irregular non-daily tooth brushing, problems with meals in school, frequent no shows). A power calculation based on the QLF data (percent average 'change in fluorescence') from previous trials [14,15] revealed that 25 subjects would be needed in each group to detect a 20% difference between baseline and follow-up with α- and β-values set at 0.05 and 0.2, respectively. All subjects lived in a community with a low fluoride level in the piped water supply (<0.2 ppm F). Eight subjects dropped-out during the study so the final material consisted of 56 adolescents of both sexes, 29 in the intervention group and 27 in the control group. The mean age was 15.2 years (range 13–18 years) in both groups. The reasons for the attrition were that some started to 'dislike' milk (n = 3), one violated the protocol and one became pregnant. Three patients gave no reason for dropping out.

Study design

The study had a randomized, double-blind, controlled design with two parallel groups. The protocol was approved by the regional ethical committee in Lund (Dnr 2010/27). The subjects were allocated to one of the two groups with aid of computer randomization. The end-point measure was quantitative light-induced fluorescence readings performed at baseline and after 12 weeks. The researchers responsible for the study and evaluating the end-points were not involved in the clinical work and were blinded for the group assignment.

Intervention

The adolescents in the intervention group were instructed to drink one glass (~ 200 ml) of milk supplemented with fluoride once each day over a time period of 12 weeks. They were provided with a supply of small containers containing skim-milk powder and fluoride to form a final fluoride concentration of 5 mg/L when added to and suspended in fresh milk. The subjects of the placebo group were given identical containers with skim milk powder without fluoride and instructed in the same manner as those in the intervention group. The containers were marked by a colour ('yellow' and 'blue', respectively) and the code was kept in a locker by an independent monitor at Umeå University, Sweden. Along with the delivery of the containers, the patients were given detailed written information on how to comply with the program. They were strongly encouraged to drink the milk at a fixed time each day, for example when coming home from school or after the after-school activities. All patients were instructed to maintain their normal tooth brushing habits with fluoride toothpaste during the entire study period.

Manufacturing of containers and check of compliance

The experimental supplements were prepared exclusively for the study by a laboratory technician at the Department of Odontology, Faculty of Medicine, Umeå University, Sweden. The fluoride content was checked regularly in random samples of the production with aid of an ion-selective electrode according to Ekstrand [16]. All participants were requested to fill in a pre-prepared logbook in which all intakes of milk were registered with an 'x'. The book was checked every 6th week at a scheduled a recall visit and handed in after termination of intervention.

QLF-measurements

The QLF measurements have previously been described in detail [14,17]. Two buccal sites (upper incisors or cuspids) were selected from each subject. After thorough cleaning with a rubber-cup and polishing paste (RD40, Ivoclar Vivadent, Schaan, Liechtenstein) followed by a water rinse and drying with compressed air for 15 s, the hand-piece of the QLF-device (Inspektor Research Systems BV, Amsterdam, The Netherlands) was applied at the selected sites and a subsequent image was exposed and stored on a hard disc. The procedure was repeated with the same position at the follow-up visit after 12 weeks. The arch wires were off when the examination was performed since all patients had their wires changed or adjusted every 6th week. A commercial repositioning program was used for capturing the images (Inspektor Pro, version 2.0.0.32) while and older version (version (1.97e) was used for the evaluations. The final evaluation with image subtraction and quantification of the mineral content of a fixed size and the lesion area was done afterwards by two of the investigators blinded for the group assignment but not for the sequence of the images. The sound reference enamel was chosen from the same buccal surface, in most cases closer to the cusp. The reason for using the older version of the QLF software when analyzing the captured images was the superior possibility to manually define the contour of the lesion area with a mouse-pointer. The patch was placed in a reference area, close to the WSL, and in surrounding intact hard tissue and with de-activating points adjacent to the bracket. A total number of 112 white spot lesions (105 incisors and seven cuspids) were analyzed at baseline and follow-up. An inter- and intra-examiner reliability test was performed by a re-evaluation of a random sample (15%) of the material within 1 month.

Statistical methods

All data were processed with the IBM-SPSS software (version 19.0, Chicago, IL) and checked for normality distribution. The follow-up fluorescence (F) and the lesion area (A) were compared with baseline within each group with aid of paired *t*-tests. A mean QLF-value of the two included buccal sites from each subject was calculated in order to get the patient as a unit. The reproducibility and repeatability (reliability) of the QLF analysis was calculated by intra-class correlation coefficient (ICC). A *p*-value less than 0.05 was considered as statistically significant.

Results

The results are summarized in Tables I and II. There was no statistically significant difference between the test and the control group concerning ΔF values and lesion area (A mm^2) at baseline. After 12 weeks, a significant decrease ($p < 0.05$) in fluorescence was registered in the fluoridated milk group and a significant increase in the non-fluoride control group

Table I. Change in fluorescence (ΔF, %) and lesion area (A, mm^2) at baseline and at the end of the intervention. Values denote mean (SD) and the number of units are based on individuals.

	Fluoridated milk (n = 29)		Standard milk (n = 27)	
Time	ΔF, %	A, mm^2	ΔF, %	A, mm^2
Baseline	9.28 (3.17)	1.12 (0.68)	8.46 (2.70)	0.74 (0.51)
12 weeks	8.68* (2.81)	0.99 (0.60)	9.66* (2.70)	0.88 (0.80)

*statistically significant difference compared with baseline ($p < 0.05$).

Table II. Average change in fluorescence and lesion area over time (follow-up vs baseline).

Group, variable	*M* (SD)	95% CI	*p*
Fluoride milk (n = 29)			
fluorescence, ΔF, %	1.09 (1.29)	1.59, 0.60	< 0.05
lesion area, A, mm^2	0.14 (0.44)	0.31, 0.03	NS
Standard milk (n = 27)			
fluorescence, ΔF, %	1.42 (2.15)	0.57, 2.27	< 0.05
lesion area, A, mm^2	0.14 (0.61)	0.10, 0.37	NS

NS, no statistically significant difference.

($p < 0.05$). The mean reduction in the test group, expressed as percent, was somewhat lower (14%) than the increase in the control group (18%), but considerable individual variations were evident. Reduced ΔF values were registered in 17 of the 29 subjects in the fluoride intervention group compared with seven in the placebo control group. Only minor alterations in lesion area were recorded over the 12-week period and those were not statistically significant in any of the groups. The intra-examiner ICC values for the two examiners were 0.92 and 0.95, respectively. The corresponding value for the inter-examiner test was 0.87.

No side- or adverse effects during intervention were reported among those that fulfilled the protocol. The returned self-reported logbooks displayed a good adherence to the study regime in most of the patients. However, five subjects failed to drink the assigned milk for more than 10 days, two in the fluoride group (17 and 17 days) and three in the control group (10, 12 and 15 days). The fluoride concentration in the experimental containers showed a variation of less than ± 10%.

Discussion

The present study was undertaken to evaluate the potential of fluoride milk to affect enamel demineralization adjacent to brackets in patients undergoing orthodontic treatment with fixed appliances. The incidence of WSL visible at debonding of fixed appliance ranges between 7–11% at the Public Orthodontic Clinic in Halmstad. In this study, only a few WSL (n = 10) were detected clinically and scored 1 according to the ordinal index by Gorelick et al. [18]. This means a 'slight white spot formation (thin rim)'. None of the proximal enamel lesions were filled or sealed at baseline or during the intervention. The obtained findings were clear-cut and the null hypothesis was therefore rejected. The results were not surprising in the light of current knowledge on the local mechanism of action concerning fluoride. Even in very low concentrations (~ 0.03 ppm) in the oral biofilm–enamel interface, fluoride can stabilize the balance between enamel de- and remineralization

[19]. It has long been known that milk is an excellent vehicle for local fluoride administration, as demonstrated in several *in vitro* and *in vivo* trials [20]. The consolidating insight, demonstrated here with the aid of the QLF-technique, was that both the de- and remineralization processes seemed to follow a continuum also during the relatively short intervention period. The observation that one additional fluoride exposure on top of regular tooth brushing with fluoride toothpaste could make a difference was also clinically informative. It should, however, be stressed that both the gain of mineral in the test group and the loss of mineral in the control group were of limited magnitude and not detectable by normal visible examination during the relatively short follow-up. It is, however, reasonable to believe that the obtained patterns would have been even more clear and of possible clinical importance with a prolonged fluoride intervention.

The disclosed individual differences in response to the milk intake were interesting. It is generally though that milk *per se* may have a caries protective effect [21] and that this effect is amplified by fluoride [20]. The fact that a total of 32 patients (20 in the control group and 12 in the fluoridated milk group) actually exhibited a net loss of mineral during the intervention underlines the great environmental stress and high cariogenic challenge exerted by the orthodontic appliances. We decided to use a 3-month period with appliances *in situ* as inclusion criteria of this study in order to allow a natural ecological selection of aciduric and acidogenic microbiota responsible for WSL development [22]. Furthermore, the rationale to use presence of proximal enamel lesions as inclusion criteria was based on a previous study at the clinic showing that enamel caries on bitewing radiographs was a valuable and useful predictor of WSL development during treatment with fixed alliances [23]. Obviously, the 5 ppm fluoride supplement in milk used here was not sufficient to counteract the deminereralization process in all the participants on top of regular use of fluoridated toothpaste.

The QLF-technique has previously been used to investigate de- and remineralization adjacent to orthodontic appliances and an actual correlation with mineral loss of ~ r = 0.8 has been reported [24]. The method is based on a visible light system with a wavelength of 370 nm that is applied on the enamel and produces an image that is comprised of green and red channels and the predominant color of the enamel is green (for a review, see Pretty [25]). Demineralization of enamel results in a reduction of autofluorescence and the loss (or gain) in minerals can be quantified with aid of an appropriate software. The main advantage with the QLF method is that it can be applied directly without removing the brackets and, in fact, the brackets facilitated the repositioning of the images. The possibility to manually define the analysis area prevented a false detection of an increased lesion area which would have been the case if a pre-set analyzing window size had been used. Some concerns have, however, been raised for its use within orthodontics, since the movement of teeth may jeopardize the provision of congruent images [26]. We did not experience this as a problem during our short-term study, but the possibility that tooth movements could have influenced and somewhat obscured the present results cannot be excluded. The variation of repeated measurements was found to be highly acceptable, but we were not able to study the variation caused by repeated images.

In conclusion, the findings suggest that daily intake of fluoridated milk may influence the balance between de- and remineralization of white spot lesions adjacent to fixed orthodontic appliances.

Acknowledgments

The authors would like to thank Associate Professor Sofia Tranæus, Swedish Council on Health Technology Assessment, Stockholm, Sweden, for technical support and performing QLF-analysis. Mrs Dana Ban (RDH), Public Orthodontic Specialist Clinic in Halmstad, Sweden and Mrs Inger Sjöström, Umeå University, Sweden are thanked for skilful clinical work and preparation of experimental products. The study was supported by a grant from the Borrow Foundation, UK.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2010;138:188–9.

[2] Shungin D, Olsson AI, Persson M. Orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up, including bonding material assessment. Am J Orthod Dentofacial Orthop. 2010;138:136e1–8.

[3] Richter AE, Arruda AO, Peters MC, Sohn W. Incidence of caries lesions among patients treated with comprehensive orthodontics. Am J Orthod Dentofacial Orthop 2011;139: 657–64.

[4] Ahn SJ, Lim BS, Lee SJ. Prevalence of cariogenic streptococci on incisor brackets detected by polymerase chain reaction. Am J Orthod Dentofacial Orthop 2007;131:736–41.

[5] Mattousch TJ, van der Veen MH, Zentner A. Caries lesions after orthodontic treatment followed by quantitative light-induced fluorescence: a 2-year follow-up. Eur J Orthod 2007;29:294–8.

[6] Benson PE, Parkin N, Millett DT, Dyer FE, Vine S, Shah A. Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database Syst Rev 2004; CD003809.

[7] Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures

during orthodontic treatment with fixed appliances. A systematic review. Caries Res 2004;38:413–20.

[8] Chadwick BL, Roy J, Knox J, Treasure ET. The effect of topical fluorides on decalcification in patients with fixed orthodontic appliances: a systematic review. Am J Orthod Dentofacial Orthop 2005;128:601–6.

[9] Bergstrand F, Twetman S. A review on prevention and treatment of post-orthodontic white spot lesions - evidence-based methods and emerging technologies. Open Dent J 2011;5:158–62.

[10] Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dent Oral Epidemiol 2004;32:319–21.

[11] Yeung A, Hitchings JL, Macfarlane TV, Threlfall A, Tickle M, Glenny AM. Fluoridated milk for preventing dental caries. Cochrane Database of Systematic Reviews 2005;3: CD003876.

[12] Benson PE, Pender N, Higham SM. Quantifying enamel demineralization from teeth with orthodontic brackets–a comparison of two methods. Part 2: validity. Eur J Orthod 2003; 25:159–65.

[13] Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Res 2005;39:41–7.

[14] Tranaeus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci 2001;109:71–5.

[15] Bröchner A, Christensen C, Kristensen B, Tranæus S, Karlsson L, Sonnesen L, et al. Treatment of white spot lesions with casein-phosphopeptide-stabilized amorphous calcium phosphate. Clin Oral Invest 2011;15:369–73.

[16] Ekstrand J. A micromethod for the determination of fluoride in blood plasma and saliva. Calcif Tissue Res 2011;15:369–73.

[17] Karlsson L, Lindgren LE, Trollsås K, Angmar-Månsson B, Tranaeus S. Effect of supplementary amine fluoride gel in caries active adolescents. A clinical QLF study. Acta Odontol Scand 2007;65:284–21.

[18] Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod 1982;81:93–8.

[19] ten Cate JM. Fluorides in caries prevention and control: empiricism or science. Caries Res 2004;38:254–7.

[20] Bánóczy J, Rugg-Gunn AJ. Caries prevention through the fluoridation of milk. A review. Fogorv Sz 2007;100:185–92; 177–84.

[21] Merritt J, Qi F, Shi W. Milk helps build strong teeth and promotes oral health. J Calif Dent Assoc 2006;34:361–6.

[22] Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. Dent Clin North Am 2010;54:441–54.

[23] Fornell AC, Twetman S. Prediction of enamel demineralisation (white spots) during orthodontic treatment with fixed appliances. Orthodontics 2004;1:121–5.

[24] Tranæus S, Shi X-Q, Lindgren L, Trollsås K, Angmar-Månsson B. *In vivo* repeatability and reproducibility of Quantitative Light-induced Fluorescence. Caries Res 2002;36:3–9.

[25] Pretty IA. Caries detection and diagnosis: novel technologies. J Dent 2006;34:727–39.

[26] Kronenberg O, Lussi A, Ruf S. Preventive effect of Ozone on the development of white spot lesions during multibracket appliance therapy. Angle Orthod 2009;79:64–9.