Acta Odontologica Scandinavica, 2007; 65: 357–361

informa
healthcare

ORIGINAL ARTICLE

Effect of olive oil and an olive-oil-containing fluoridated mouthrinse on enamel and dentin erosion *in vitro*

ANNETTE WIEGAND[1,2], MIRJA GUTSCHE[2] & THOMAS ATTIN[1,2]

[1]*Clinic for Preventive Dentistry, Periodontology and Cariology, University of Zürich, Zürich, Switzerland and* [2]*Department of Operative Dentistry, Preventive Dentistry and Periodontology, Georg-August-University of Göttingen, Göttingen, Germany*

Abstract

Objective. The study aimed to analyse the impact of olive oil and an olive-oil-containing fluoridated mouthrinse on enamel and dentin erosion. ***Material and Methods.*** Bovine enamel and dentin specimens were submitted to 10 alternating demineralization and remineralization cycles each consisting of 5 min pretreatment with the test solutions, i.e. distilled water as negative control, 100% olive oil, 2% olive oil emulsion, 2% olive-oil-containing mouthrinse (Xerostom®), acidic 13.2 mmol·l^{-1} (250 ppm) fluoride solution as positive control, storage in artificial saliva (30 min), demineralization in citric acid (3 min, pH 2.3) and again storage in artificial saliva (60 min). Each group contained 10 enamel and dentin samples. Enamel and dentin loss was analyzed by profilometry after 10 cycles. ***Results.*** Treatment with 100% olive oil was not effective in reducing enamel and dentin loss. Application of 2% olive oil or the olive-oil-containing mouthrinse also showed protection against erosion, but to a lesser degree compared to the positive control. ***Conclusion.*** Olive oil offered protection against enamel and dentin erosion when applied as 2% emulsion or 2% olive-oil-containing mouthrinse, but is not effective when applied as pure oil (100%). However, 2% olive oil emulsion is less effective in reducing erosion compared to the acidic 13.2 mol·l^{-1} fluoride solution.

Key Words: *Dentin, enamel, erosion, fluoride, olive oil*

Introduction

An important aim concerning enamel and dentin erosion is to identify agents that might protect the tooth surface from demineralization. This aspect is even more critical in dry mouth dentate patients due to the lack of saliva and therefore the impairment of natural oral buffer capacity to protect teeth [1,2]. It has been shown in numerous studies that topical fluoridation is effective in decreasing erosive demineralization and in increasing remineralization or rehardening of the acid-softened surface (for review see [3]). In particular, intensive fluoridation by a combined application of fluoridated toothpaste, fluoridated mouthrinse and fluoride gel, or the application of highly concentrated fluorides, might be effective in decreasing the progression of erosive lesions [4–6]. As erosion cannot be inhibited or prevented totally with the use of fluorides in typical daily dosages [5,7], there is an increasing demand for identifying chemical agents or components that might have anti-erosive potential or increase the anti-erosive potential of oral hygiene products, especially in patients suffering from dry mouth.

It has been shown in several studies in caries research that lipids might play a role in the process of carious demineralization. Featherstone & Rosenberg [8] reported that lipids provide a diffusion barrier within the organic protein–lipid–water matrix of enamel and, thus, might decelerate caries demineralization. Buchalla et al. [9] found externally provided olive oil emulsions to be slightly effective in reducing artificial dentin caries lesions. It was therefore assumed that a film of lipids at the enamel or dentin surface would act as a protective coating and increase the lipid content in the outermost layer of enamel or dentin, thus hampering the diffusion of acids and mineral during demineralization.

However, the influence of externally applied lipids on enamel and dentin erosion has not yet been investigated. Therefore, this study aimed to analyze

Correspondence: Annette Wiegand, Clinic for Preventive Dentistry, Periodontology and Cariology, University of Zürich, Plattenstrasse 11, CH-8032 Zürich, Switzerland. Tel: +41 44 634 32 71. Fax: +41 44 634 43 08. E-mail: annette.wiegand@zzmk.unizh.ch

(Received 25 April 2007; accepted 26 October 2007)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350701771843

the anti-erosive potential of olive oil as well as of a newly developed olive-oil-containing fluoridated mouthrinse (Xerostom; Biocosmetics Laboratories, Madrid, Spain) on enamel and dentin erosion. To evaluate the effects an acidic 13.2 mmol·l^{-1}, fluoride solution was chosen, since solution with these characteristics has been shown to be effective in reducing erosive effects on dental hard tissues [10,11].

Material and methods

Sample preparation

Bovine permanent incisors were stored in 0.5% thymol solution at room temperature until used for sample preparation. The teeth were sectioned at the cementum–enamel junction using a water-cooled diamond bandsaw (Exakt; Norderstedt, Germany), and crowns and roots were embedded in acrylic resin cylinders (Paladur; Heraeus Kulzer, Wehrheim, Germany). The cementum layer of the roots was completely removed by grinding (grinding machine: DAP-U, Struers, Denmark) with 280-grit and 800-grit disks (LECO, St. Joseph, USA). Enamel and dentin surfaces were then polished with water-cooled carborundum paper (2400-grit and 4000-grit; Stuers, Erkrat, Germany) resulting in a plane enamel or dentin surface, which is necessary for profilometric measurement. Thereby, the thickness of the removed outermost enamel or dentin layer amounted to approximately 200 μm and was controlled with a micrometer (Digimatic®; Micrometer, Mitutoyo, Tokyo, Japan). Surface microhardness of enamel samples was determined as a criterion for stratified allocation of enamel and dentin specimens of the same tooth among 8 groups (each 10 enamel and 10 dentin samples). On each enamel or dentin sample, respectively, the surface was covered with tape (Tesa, Beiersdorf, Hamburg, Germany) on two sides, leaving an exposed window of 3 mm width. The tape was applied to the surface to ensure the maintenance of reference surfaces for profilometric measurement. After preparation, the samples were stored in distilled water until used for the experiment; this in order to avoid dehydration.

Profilometric measurement

Enamel and dentin loss was quantified using profilometric measurements (Mahr Perthometer, Göttingen, Germany). Prior to the experiment, baseline measurements were performed in order to evaluate reference surfaces for calculation of enamel and dentin loss. Six profilometric traces were performed in the center of each specimen at intervals of 1000 μm. After the experiment, the tapes were removed and the samples were again analyzed. The average depth of the eroded surface relative to the basic surface profiles at the respective site was calculated by the corresponding software (Mahr Perthometer Concept 7.0; Mahr, Göttingen, Germany).

Study design

Enamel and dentin specimens were submitted to 10 alternating demineralization and remineralization cycles, each including 5 min pretreatment with one of the test agents: distilled water (negative control), 100% olive oil (Tip, Goldhand Vertriebsgesellschaft mbH, Düsseldorf, Germany), 2% emulsion of olive oil and distilled water, 2% olive oil containing mouthrinse (pH: 6.9), 13.2 mmol·l^{-1} (250 ppm) fluoride solution (pH 3.88) as positive control. The compositions of the olive-oil-containing mouthrinse and the acidic fluoride solution are given in Table I. The 2% olive oil emulsion was prepared using a high-speed household mixer and remixed prior to each pretreatment, resulting in a finely dispersed emulsion.

After pretreatment, samples were rinsed in tap water and transferred to artificial saliva (formulation accordingly to Klimek et al. [12]) for 30 min. Erosive demineralization was then performed by storing the specimens in 1% citric acid (pH 2.3) for 3 min. After demineralization, the enamel and dentin samples were rinsed in tap water and again transferred to artificial saliva for 60 min. This cycle (5 min pretreatment, 30 min artificial saliva, 3 min erosion, 60 min artificial saliva) was repeated 10 times.

Statistical analysis

Mean and standard deviation (SD) for enamel and dentin loss in each group was calculated and

Table I. Ingredients of the 2% olive-oil-containing mouthrinse and the acidic fluoride solution according to the manufacturer's information

Group	Solution	Ingredients
2% olive-oil-containing solution	Xerostom (Biocosmetics Laboratories, Madrid, Spain)	2% extra virgin olive oil, 2% betaine, xylitol, 0.5% pro-vitamin B5, 0.5% pro-vitamin E, 0.24% lemon aroma, 0.2% allantoin, 0.07% potassium fluoride, aqua, PEG-40 hydrogenated castor oil, parsley seed oil, benzoic acid, sodium saccharin, sodium benzoate
Acidic 13.2 mol·l^{-1} (250 ppm) fluoride solution	Meridol (GABA, Lörrach, Germany)	0.025% fluoride as SnF_2/Olaflur, aqua, xylitol, PVP, PEG-40 hydrogented castor oil, aroma, sodium saccharin, Cl 42051

statistically analyzed by *t*-test following Bonferroni correction due to multiple comparisons (Statistica 6.0; Statsoft, Tulsa, USA).

Results

Mean enamel and dentin loss in the experimental groups is presented in Table II. The application of pure olive oil (100%) was not effective in reducing erosion of enamel and dentin; 2% olive oil offered significant protection against enamel loss compared to the negative control, but to a significantly less degree than the acidic fluoridated mouthrinse. Moreover, while 2% olive oil was not effective in inhibiting dentin erosion, the olive-oil-containing mouthrinse showed a significant reduction of both enamel and dentin loss. The positive control group exhibited the highest protective effect against erosion in both enamel and dentin specimens.

Discussion

The present study should provide a proof of principle as to whether olive oil or an olive oil emulsion is effective in protecting enamel or dentin erosion. Therefore, severe erosive conditions (3 min demineralization with citric acid at pH 2.3 each cycle) were applied to focus entirely on the protective effects of the lipid or the test solutions rather than on remineralization by artificial saliva. However, the remineralization time of 60 min was chosen according to Attin et al. [13,14], who found that 30–60 min saliva exposure time was sufficient to increase abrasion resistance of eroded dental hard tissues, indicating that a partial remineralization of the demineralized surfaces was achieved. Bovine teeth were used as substitute for human teeth, as done in other investigations [15,16]. Erosion treatment was performed by incubation in citric acid to mimic the situation during the consumption of acidic soft drinks, mostly showing pH levels between 2 and 3 [17].

The results of the present study indicate that the application of a 2% olive oil emulsion or a 2% olive-oil-containing mouthrinse prior to an erosive attack might decrease enamel or dentin demineralization, while pure olive oil (100%) is not effective in reducing dental erosion.

As the 2% olive oil emulsion and the 2% olive-oil-containing mouthrinse perform similarly with regard to inhibition of enamel erosion, the protective effect of the Xerostom® mouthrinse might be attributed to its olive oil content. However, also other compounds of Xerostom®, such as potassium fluoride or xylitol, might influence enamel and dentin erosion. Thereby, the compounds might act synergistically, additively or antagonistically with regard to inhibition of demineralization.

Chemically, olive oil belongs to the group of glycerolipids, where three fatty acids (mostly oleic, palmatic, linoleic or linolenic) are attached to a glycerol backbone (triglyceride). Owing to its low polarity, the adhesion of pure olive oil on enamel or dentin surfaces might be reduced. Especially under *in situ* conditions, when dental hard tissues are covered with an acquired salivary pellicle, the adhesion of externally applied lipids might be reduced as this depends on the interaction with protein and glycoprotein constituents of the pellicle. It is assumed that the adhesion properties of olive oil might be increased when applied as emulsion leading to a protective coating of the enamel or dentin surface, which might act as a diffusion barrier during acid contact. However, it has to be proved whether changes in the concentration of olive oil emulsions or the application of more polar lipids, such as phospholipids, might be more effective in reducing dental erosion. Moreover, the physical properties and the quality of the acquired lipid layer have to be investigated in detail. It is known from studies in caries research that individual compositional differences among lipids might affect the pellicle attachment on dental hard tissues [18]. However, the lipid content of the acquired salivary pellicle contributes significantly to its ability to retard the acid diffusion [18–20].

Generally, the protective effect of 2% olive oil was less evident on dentin samples; however, it was significant for the 2% olive-oil-containing mouthrinse. This could be the result of the higher hydrophilicity of dentin, which might decrease the adhesion of lipids. Moreover, other compounds of the 2% olive-oil-containing mouthrinse, such as potassium fluoride, betaine or xylitol, might influence the physical and adhesion properties of the solution and possibly also affect the demineralization process.

Compared to the acidic fluoridated solution, the protective effect of the 2% olive oil emulsion or the 2% olive-oil-containing mouthrinse is significantly less on both enamel and dentin erosion. In regard to both fluoride-containing solutions, the acidic fluoridated solution might be more effective in reducing enamel erosion owing to its lower pH value. Under

Table II. Mean (SD) enamel and dentin loss in the different groups. Within each column, groups marked with same capital letter are not significantly different

Group	Mean (SD) enamel loss (μm)	Mean (SD) dentin loss (μm)
Distilled water	26.7 (1.4)^{A}	9.6 (1.0)^{A}
100% olive oil	28.6 (1.3)^{A}	9.2 (1.4)$^{A, B}$
2% olive oil	14.0 (1.7)^{B}	9.7 (1.9)$^{A, B}$
2% olive oil containing mouthrinse	21.2 (1.3)^{C}	7.5 (0.8)^{B}
13.2 mol·l^{-1} fluoride solution	2.8 (0.4)^{D}	3.9 (0.7)^{C}

acidic conditions, more fluoride is presented in its dissociated form, resulting in a higher amount of fluoride ions available compared to neutral fluoride solutions (pH value of Xerostom: 6.9).

While the effect of pretreating enamel or dentin surfaces with an acidic 13.2 $mmol \cdot l^{-1}$ fluoride solution has not yet been investigated, its remineralizing effect on erosively demineralized dental hard tissues has been discussed controversially. Gedalia et al. [10] and Attin et al. [11] showed that the application of an acidic 13.2 $mmol \cdot l^{-1}$ (250 ppm) fluoride solution increased microhardness or abrasion resistance of demineralized enamel or dentin, while other authors found no remineralizing effect on enamel and dentin erosion [7,21]. However, it has to be taken into consideration that the acidic mouthrinse might induce an increase of mineral loss in predemineralized dentin and is assumed not to be suitable for patients suffering from hyposalivation and exhibiting initial lesions [22]. As the 2% olive-oil-containing mouthrinse (neutral pH value) is primarily designed as saliva substitute in patients with xerostomia, it might be interesting to analyze whether the protective properties of olive oil might be increased by the addition of other fluoride compounds, such as amine or stannous fluoride, or whether these fluorides might interfere with the lipid effects. The results of Yu et al. [23] and Zero et al. [24] suggest that the presence of essential oils in fluoridated mouthrinses does not adversely affect their ability to promote fluoride uptake and enamel remineralization.

It might be concluded from the results of the present study that olive oil offers protection against enamel and dentin erosion when applied as 2% emulsion or 2% olive-oil-containing mouthrinse. Due to the fact that the protective effect of the 2% olive oil emulsion or the 2% olive oil-containing mouthrinse is still visible under the severe erosive conditions of the present study, it could be speculated that the protective effect might be more enhanced under less severe erosive conditions.

Acknowledgment

We thank Dr. Carola Werner and Mr. Klaus Becker for statistical advice. The study was supported by Biocosmetics Laboratories, Madrid, Spain.

References

[1] Bardow A, Nyvad B, Nauntofte B. Relationships between medication intake, complaints of dry mouth, salivary flow rate and composition, and the rate of tooth demineralisation in situ. Arch Oral Biol 2001;46:413–23.

[2] Leung KC, McMillan AS, Leung WK, Wong MC, Lau CS, Mok TM. Oral health condition and saliva flow in southern Chinese with Sjögren's syndrome. Int Dent J 2004;54:159–65.

[3] Wiegand A, Attin T. Influence of fluoride applications on dental erosions – a review. Oral Health Prev Dent 2003;1: 245–53.

[4] Ganss C, Klimek J, Brune V, Schurmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561–6.

[5] Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 2006;40:148–53.

[6] Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371–9.

[7] Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a casein/calcium phosphate containing tooth cream and fluoride on enamel erosion in vitro. Caries Res 2006;40:154–7.

[8] Featherstone JD, Rosenberg H. Lipid effect on the progress of artificial carious lesions in dental enamel. Caries Res 1984;18:52–5.

[9] Buchalla W, Attin T, Roth P, Hellwig E. Influence of olive oil emulsions on dentin demineralization in vitro. Caries Res 2003;37:100–7.

[10] Gedalia I, Braustein E, Lewinstein I, Shapira L, Ever-Hadani P, Sela M. Fluoride and hard cheese exposure on etched enamel in neck-irradiated patients in situ. J Dent 1996;24:365–8.

[11] Attin T, Zirkel C, Hellwig E. Brushing abrasion of eroded dentin after application of sodium fluoride solutions. Caries Res 1998;32:344–50.

[12] Klimek J, Hellwig E, Ahrens G. Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. Caries Res 1982;16:156–61.

[13] Attin T, Knöfel S, Buchalla W, Tütüncü R. In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res 2001;35:216–22.

[14] Attin T, Siegel S, Buchalla W, Lennon AM, Hannig C, Becker K. Brushing abrasion of softened and remineralised dentin: an in situ study. Caries Res 2004;38:62–6.

[15] Wiegand A, Stock A, Attin R, Werner C, Attin T. Impact of the acid flow rate on dentin erosion. J Dent 2006;17:21–7.

[16] Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. Arch Oral Biol 2005;50:541–52.

[17] Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Res 1999;33:81–7.

[18] Slomiany BL, Murty VLN, Mandel ID, Sengupta S, Slomiany A. Effect of lipids on the lactic acid retardation capacity of tooth enamel and cementum pellicles formed in vitro from saliva of caries-resistant and caries-susceptible human adults. Arch Oral Biol 1990;35:175–80.

[19] Slomiany BL, Murty VLN, Zdebska E, Slomiany A, Gwozdzinzki K, Mandel ID. Tooth surface-pellicle lipids and their role in the protection of dental enamel against lactic-acid diffusion in man. Arch Oral Biol 1986;31:187–91.

[20] Featherstone JDB, Behrman JM, Bell JE. Effect of whole saliva components on enamel demineralisation in vitro. Crit Rev Oral Biol Med 1993;4:357–62.

[21] Lussi A, Jaeggi T, Gerber C, Megert B. Effect of amine/sodium fluoride rinsing on toothbrush abrasion of softened enamel in situ. Caries Res 2004;38:567–71.

[22] Meyer-Lueckel H, Schulte-Mönting J, Kielbassa AM. The effect of commercially available saliva substitutes on predemineralized bovine dentin in vitro. Oral Dis 2002;8:192–8.

[23] Yu D, Sipos T, Wu MM, Bilbault T, Lynch MC, Naleway C. Effect of fluoride/essential oil-containing mouthrinse on the microhardness of demineralized bovine enamel. Am J Dent 2004;17:216–8.

[24] Zero DT, Zhang JZ, Harper DS, Wu M, Kelly S, Waskow J, et al. The remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. J Am Dent Assoc 2004;135:231–7.