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Potential of CO_2 lasers (10.6 μm) associated with fluorides in inhibiting human enamel erosion

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Abstract: This *in vitro* study aimed to investigate the potential of CO_2 lasers associated with different fluoride agents in inhibiting enamel erosion. Human enamel samples were randomly divided into 9 groups (n = 12): G1-eroded enamel; G2-APF gel; G3-AmF/NaF gel; G4-AmF/SnF_2 solution; G5-CO_2 laser (λ = 10.6 μm)+APF gel; G6-CO_2 laser+AmF/NaF gel; G7-CO_2laser+AmF/SnF_2solution; G8-CO_2 laser; and G9-sound enamel. The CO_2 laser parameters were: 0.45 J/cm²; 6 μs; and 128 Hz. After surface treatment, the samples (except from G9) were immersed in 1% citric acid (pH 4.0, 3 min). Surface microhardness was measured at baseline and after surface softening. The data were statistically analyzed by one-way ANOVA and Tukey's tests ($p < 0.05$). G2 (407.6 ± 37.3) presented the highest mean SMH after softening, followed by G3 (407.5 ± 29.8) and G5 (399.7 ± 32.9). Within the fluoride-treated groups, G4 (309.0 ± 24.4) had a significantly lower mean SMH than G3 and G2, which were statistically similar to each other. AmF/NaF and APF application showed potential to protect and control erosion progression in dental enamel, and CO_2 laser irradiation at 0.45J/cm² did not influence its efficacy. CO_2 laser irradiation alone under the same conditions could also significantly decrease enamel erosive mineral loss, although at lower levels.

Keywords: Dental Enamel; Fluorides; Lasers; Hardness; Tooth Erosion.

Introduction

Dental erosion is a chemical process characterized by surface dissolution of dental hard tissues, without the involvement of microorganisms.[1] In earlier stages, the erosive process involves enamel demineralization, which is characterized by initial softening and increased roughness of the surface.[2] This process is of particular clinical importance because studies[3,4,5,6,7] have shown that softened enamel can be significantly protected and remineralized by exposure to fluorides.

During a caries challenge, calcium fluoride forms a protective coating on the enamel surface, after exposure to fluoride agents results in their incorporation into the enamel as fluorapatite. Regarding the mechanisms of action of amine and sodium fluoride compounds in erosion prevention, it can be speculated that deposition of CaF_2-like precipitates occurs on the enamel surface, preventing loss of enamel and providing some additional mineral to be dissolved in acidic solutions before the underlying enamel is attacked.[3]

Considering the limited effectiveness of fluoride in preventing dental hard tissue erosion and the effects of high-power lasers on dental hard tissues, previous experiments have shown that CO_2 laser irradiation could be an alternative method to modify the enamel surface and protect it against demineralization.[8,9,10] Furthermore, some authors have concluded that laser irradiation associated with fluoride agents[11,12] could increase the fluoride uptake into the enamel, making the enamel more acid resistant.[11,13] However, until now, there have been only a few studies[12,14,15,16] evaluating the use of CO_2 lasers to prevent dental erosion, and these studies have been inconclusive.

The effects of different fluoride agents (TiF_4, SnF_2, NaF, AmF, ZnF_2, $SnCl_2$) on erosive tissue loss in the enamel[5,6,7,17,18] have also been tested, but the results have not been conclusive. Different product presentations (gel/solution), fluoride concentrations, and recommended times of exposure to enamel can possibly lead to different levels of incorporation of fluoride ions into the dental structure and, consequently, can differently influence their potential to prevent dental erosion.

This *in vitro* study aimed to evaluate the potential of a pulsed CO_2 laser, associated or not with different fluoride agents, in inhibiting human enamel softening. The hypotheses considered were that pulsed CO_2 laser (λ = 10.6 µm) irradiation associated with fluoride agents would increase the reduction of mineral loss compared to other treatments and that different fluoride agents would have different protective effects on human enamel erosion.

Methodology

Ethical Aspects

This study was approved by the Research Ethics Committee of the School of Dentistry of the *Universidade de São Paulo* (Protocol#40/11) and by the National Committee for Ethics in Research (Protocol #453/2011).

Study Design

Enamel samples were randomly divided in 9 groups (n = 12): G1-eroded enamel (no surface treatment); G2-APF gel; G3-AmF/NaF gel; G4-AmF/SnF_2 solution; G5-CO_2 laser (λ = 10.6 µm); G6-CO_2 laser+APF gel; G7-CO_2 laser+AmF/NaF gel; G8-CO_2 laser+AmF/SnF_2 solution; and G9-sound enamel. The samples were submitted to an erosive challenge, and the effects of surface treatments were quantitatively analyzed for Knoop surface microhardness.

Sample Preparation

One hundred eight enamel blocks (5 x 5 x 2 mm), obtained from freshly extracted human third molars, were embedded in epoxy resin (Buehler, Lake Bluff, USA). They were ground flat and were serially polished using silicon carbide papers (#1200, #2400, #4000 grit) and 6µm diamond abrasive paste (Buehler, Lake Bluff, USA) on a polishing machine (EXAKT, Norderstedt, Germany). An adhesive tape 2.5 mm in diameter was fixed in the center of the polished surface, and the samples were completely covered with an acid-resistant varnish and left to dry. Subsequently, the adhesive tape was carefully removed, and a round window 2.5 mm in diameter of enamel was exposed. All of the samples were stored in a 100% humidity environment before the beginning of the experiment. Samples from G9 (sound enamel) were submitted to microhardness tests and were not exposed to any of the experimental surface treatments or to erosive challenge.

CO_2 Laser Irradiation

A CO_2 laser (λ = 10.6 µm) (Rofin SCx30, Rofin-Sinar Laser GmbH, Hamburg, Germany), emitting a beam with a TEM_{00} profile, was used. To allow for adequate determination of the energy density, the beam diameter at $1/e^2$ of the intensity level was determined using the knife-edge method. The emitted energy was controlled using an energy/power meter, and the irradiations were performed at a distance of 19.8 cm (focused mode) to obtain a beam diameter at the sample surface of 2.5 mm[10,14] (coincident with the exposed enamel area). The CO_2 laser irradiation parameters were: 0.45 J/cm^2; 15 µs; 128 Hz; 22 mJ; 9 s of irradiation time; and no air-water spray.[10,14]

Fluoride Treatment

The products used in the groups treated with fluoride were the following: G2 and G5-APF gel (DFL® Gel, Nova DFL, Rio de Janeiro, Brazil, 1.23%NaF, pH 3.6-3.9) for 4 min; G3 and G6-AmF/NaF gel (Elmex®

Gel, GABA International, Basel, Switzerland, 1.25%F, pH 4.8-6.0) for 4 min; and G4 and G7-SnF_2solution (Meridol® Mouthrinse, (GABA International, Basel, Switzerland, 0.16%AmF+0.05%SnF_2, pH 4.2) for 3 min. All of the products were applied on the enamel surfaces according to the manufacturers' instructions. After fluoride treatment, the samples were dried with absorbent paper. In G5, G6 and G7, fluoride was applied immediately after laser irradiation.

Enamel Surface Softening

Following the surface treatments, the samples were immersed in 20 mL of 1% citric acid ($C_6H_8O_7.H_2O$; M = 210.14 g/mol; E. Merck, Darmstadt, Germany) (pH 4.0) at 30ºC under constant agitation in a shaking water bath for 3 min. Then, all of the samples were rinsed with distilled/deionized water for 30 s and were dried for 5 s with absorbent paper. After surface softening, they were stored in a supersaturated mineral solution (1.5 mmol/L $CaCl_2$, 1.0 mmol/L KH_2PO_4, 50 mmol/L NaCl, pH 7.0) for 24 h.[19] This *in vitro* experimental model was designed to simulate the clinical conditions present during the early stages of dental erosion.[14,19]

Surface Microhardness Measurement (SMH)

All of the samples had their microhardness measured prior to the beginning of the experiment (SMH average: 355.4). SMH was performed with a Knoop diamond placed perpendicular to the polished surfaces (0.49 N, 20 s).The indentation lengths were measured using a microscope and a specific computer software (DM 4000 M, Leica, Wetzlar, German; and a4i Analysis, Aquinto, Enfield, USA). After the surface softening, SMH was measured for G1-G8, placed 100 µm to the right of the baseline measurements. Six indentations were made at a distance of 50 µm from each other at each measurement time.

Statistical Analysis

The data were analyzed using SPSS (SPSS Inc., Chicago, USA) software, version 17.0 for Windows. The results were analyzed by one-way ANOVA with subsequent pairwise comparisons using Tukey's test ($\alpha = 0.05$).

Results

The results of the microhardness evaluation are shown in Table 1. The softening model chosen for the study was shown to be effective because there was a statistically significant difference between the microhardness values of the eroded (G1) and non-eroded enamel (G9). G2 (407.6 ± 37.3) presented the highest mean SMH after softening, followed by G3 (407.5 ± 29.8) and G5 (399.7 ± 32.9). Among the fluoride-treated groups, G4 (309.0 ± 24.4) had a significantly lower mean SMH than G3 and G2, which were statistically similar to each other. Regarding treatment with CO_2 laser (G8), when used alone, the mean SMH (341.2 ± 23.2) revealed that irradiation of enamel with the parameters used was significantly better than in G1 and was not statistically significant different from G9, indicating a preventive effect. However, greater mineral loss was revealed, compared with APF gel application alone (G2) or in combination with CO_2 laser irradiation (G5). Regarding the association of laser and fluoride treatments, G6 (373.9 ± 40.2) showed no significant difference in SMH compared to G2 and G3, but it differed from G7 (328.9 ± 25.7).

Table 1. Mean SMH and SD (standard deviation) for each experimental group

Group	Surface Treatment	KHN ± SD
G1	Eroded enamel	297.0 ± 34.2^{E}
G2	APF gel	407.6 ± 37.3^{A}
G3	AmF/NaF gel	407.5 ± 29.8^{A}
G4	AmF/SnF_2 solution	309.0 ± 24.4^{DE}
G5	CO_2 laser + APF gel	399.7 ± 32.9^{A}
G6	CO_2 laser + AmF/NaF gel	373.9 ± 40.2^{AB}
G7	CO_2 laser + AmF/SnF_2 solution	328.9 ± 25.7^{CDE}
G8	CO_2 laser	341.2 ± 23.2^{BCD}
G9	Sound enamel	358.5 ± 9.0^{BC}

Different letters indicate statistically significant differences between rows, $p < 0.05$

Discussion

Although different therapies have been reported for tooth erosion, none of them has been able to inhibit completely the mineral loss caused by erosive challenges. Significant studies of initial erosion have used acidic challenges consisting of plain citric acid, soft drinks and fruit-based juices.[20] The focus of our study was to evaluate the efficacy of different therapies in very incipient erosive lesions, which were created by a single exposure to citric acid for 3 min.[14] Citric acid is commonly found in fruit-based drinks and beverages, and it can provide a strong erosive challenge under certain conditions. Thus, it is considered ideal when testing the potential for fluorides to prevent enamel erosion.[21] In the mouth, the period for which the pH remains low is usually no longer than 2 min;[22] therefore, the time for exposure to acids should be minimal for initial erosion processes in *in vitro* models. The CO_2 laser irradiation and fluorides agents were applied only once to simulate the standard clinical procedure with a single professional application.

Surface microhardness, surface profilometry, microradiography, chemical analysis and SEM have been considered the most established laboratory assessments for enamel erosion.[23] In the present study, SMH was selected because it was reported to have sufficient sensitivity for measuring the very initial stages of erosion, when enamel softening starts,[23] and no quantitative substance loss is suspected to occur.

Some studies[6,7] have shown the potential of fluoride treatments to prevent or reduce mineral loss during the initial stages of enamel erosion. However, recent studies have shown that its efficacy depends essentially on the nature of the fluoridated compound.[5] Based on this information, the current study investigated the effects of APF gel, AmF/NaF gel and SnF_2 solution on the inhibition of enamel demineralization during an erosive challenge, associating them with an innovative tool: laser.

There has been *in vitro* evidence that pre-treatment of enamel with stannous fluoride could provide protective effects by inhibiting or reducing the erosive effects of acids.[5,6,16] Furthermore, SEM images showed that SnF_2 treatment could protect the enamel surface, possibly forming a coating with very low dissolution rate.[5] Babcock[24] reported that Sn^{++} ions reacted with the pure hydroxyapatite on the surface of the enamel, reducing the enamel's solubility through the precipitation of Sn_2OHPO_4, $Sn_3F_3PO_4$, $Ca(SnF_3)_2$ or CaF_2 salts. In contrast with this literature, the present findings revealed that the treatment that has the least effect on inhibiting enamel erosion was stannous fluoride solution. Some hypotheses could be raised. One is related to the possible difference in efficacy of the gels compared to aqueous solution, as reported previously by Vieira *et al.*,[4] who evaluated the effects of TiF_4, AmF and fluoride varnish on bovine enamel erosion. Differences between the substrates used in both studies (human/bovine enamel) were not considered because the authors showed that bovine enamel was a possible substitute for human substrate in erosion models.[25] Although the enamel surface was cleaned after exposure to fluoride products, the gel products (APF, AmF/NaF) might have remained for a longer time on the enamel surface than the products in the solution (SnF_2). Another hypothesis concerns the fluoride concentration and the exposure time. The SnF_2 product had the lowest fluoride concentration (250 ppm F^-) and was applied only once to the enamel surface. It is believed that the higher the fluoride concentration is and the longer the exposure time is, the greater the acid resistance of enamel is to erosion.[26,27] The application time considered in this study (3 min) was possibly not sufficient to allow for the formation of the protective coating formed by some salts. Moreover, studies using different products, with different pH values and/or formulations (gels, solutions or varnishes), make the interpretation of the results difficult with regard to the role of the fluoride compound.[5]

AmF and NaF gels, independent of laser irradiation, were shown to be more effective than the other surface treatments. The anti-erosive effects of these fluoridated agents could be justified by the formation of a layer of spherical CaF precipitates on the enamel surface following topical application,[6,17] acting as a reservoir of fluoride ions to inducing the formation of fluorapatite or as a physical barrier isolating the enamel surface from further acid attacks, thus preventing demineralization.[12,27]

Regarding the use of high-power lasers, previous *in vitro* studies have shown promising results with CO_2 laser in enhancing the acid resistance of enamel during cariogenic challenges.[9,13] Featherstone *et al.*[8] reported that CO_2 laser produced radiation in the infrared region, which coincided closely with the phosphate absorption band. This finding indicated that CO_2 laser irradiation – at safe energy levels – could be used to modify carbonated hydroxyapatite thermally to form a purer hydroxyapatite phase that is more resistant to acid dissolution. In addition to chemical changes, it is also believed that temperature increases on the enamel surface, with consequent alteration in the composition of the mineral phase,[28] can lead to decreased permeability and solubility of the enamel. Recently, authors have shown that a short-pulsed CO_2 laser markedly inhibited enamel mineral loss compared to fluoride varnish alone over 12 months.[29] In the present study, the energy density and pulse duration (0.45 J/cm^2,15 µs) used for surface irradiation did not result in a greater reduction in enamel surface mineral loss, compared to APF or AmF/NaF gel application. However, results have shown that laser irradiation indeed had preventive effects because the enamel SMH was statistically significant greater than that of eroded enamel but was not different than that of sound enamel.

Considering the limited efficacy of fluoride in inhibiting enamel mineral loss during erosive challenges and that fluoride alone might not be effective against dental erosion,[19] this study evaluated the additional effects of CO_2 laser irradiation. Some authors have studied the effects of laser irradiation combined with fluorides on enamel demineralization[11,12] and have concluded that there was some significant synergism between treatments. Although it was expected that CO_2 laser irradiation would increase the efficacy of fluoride, as shown in this previous studies performing cariogenic challenges, in the present report, CO_2 laser irradiation did not significantly increase the effects of either fluoride agents (AmF and NaF) on erosion. Corroborating our results, Wiegand *et al.*[12] reported that AmF was able to decrease enamel erosion, but CO_2 laser irradiation did not improve its efficacy. Due to the variety of parameters and methodologies employed in the literature, it is difficult to make comparisons with previous studies. Further studies conducting chemical analyses of the enamel surface and the erosive cycling solutions and microscopic evaluations following laser and fluoride treatment should be performed to clarify the mechanisms by which different fluoride products and high-power lasers act on erosion inhibition. In addition, other laser parameters should be tested because other irradiation conditions have already been shown to cause greater protective effects against erosion, and the increase in the resistance of enamel to acid demineralization is highly dependent on laser parameters such as pulse duration, energy density and the number of overlapped pulses.

Conclusion

Within the limits of the present *in vitro* study, it was concluded that AmF/NaF and APF treatment showed the potential to protect and control erosion progression in human dental enamel and that CO_2 laser irradiation at 0.45 J/cm^2 (15 µs, 128 Hz) did not influence its efficacy. CO_2 laser irradiation alone, under the same conditions, could also significantly decrease enamel erosive mineral loss, although at lower levels.

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References

1. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental Erosion – An overview with emphasis on chemical and histopathological aspects. Caries Res. 2011 May;45(suppl 1):2-12.
2. Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res. 2011 May;45(suppl 1):13-23.
3. Ganss C, Klimek J, Brune V, Schürmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res. 2004 Nov-Dec;38(6):561-6.
4. Vieira A, Ruben JL, Huysmans MCDNJM. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res. 2005 Sep-Oct;39(5):371-9.
5. Ganss C, Schlueter N, Hardt M, Schattenberg P, Klimek J. Effect of fluoride compounds on enamel erosion in vitro: a comparison of amine, sodium and stannous fluoride. Caries Res. 2008;42(1):2-7.
6. Schlueter N, Duran A, Klimek J, Ganss C. Investigation of the effect of various fluoride compounds and preparations thereof on erosive tissue loss in enamel in vitro. Caries Res. 2009 Jan;43(1):10-6.

7. Yu H, Attin T, Wiegand A, Buchalla W. Effects of various fluoride solutions on enamel erosion in vitro. Caries Res. 2010 Aug;44(4):390-401.
8. Featherstone JD, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res. 1998 Jun;77(6):1397-403.
9. Hsu CYS, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res. 2000 Sep;79(9):1725-30.
10. Esteves-Oliveira M, Zezell DM, Meister J, Franzen R, Stanzel S, Lampert F et al. CO_2 laser (10.6µm) parameters for caries prevention in dental enamel. Caries Res. 2009 May;43(4):261-8.
11. Tepper SA, Zehnder M, Pajarola GF, Schmidlin PR. Increased fluoride uptake and acid resistance by CO_2 laser-irradiation through topically applied fluoride on human enamel in vitro. J Dent. 2004 Nov;32(8):635-41.
12. Wiegand A, Magalhães AC, Navarro RS, Schmidlin PR, Rios D, Buzalaf MA, et al. Effect of titanium tetrafluoride and amine fluoride treatment combined with carbon dioxide laser irradiation on enamel and dentin erosion. Photomed Laser Surg. 2010 Apr;28(2):219-26.
13. Steiner-Oliveira C, Rodrigues LKA, Soares LES, Martin AA, Zezell DM, Nobre-dos-Santos M. Chemical, morphological and thermal effects of 10.6µm CO_2 laser on the inhibition of enamel demineralization. Dent Mater J. 2006 Sep;25(3):455-62.
14. Esteves-Oliveira M, Pasaporti C, Heussen N, Eduardo CP, Lampert F, Apel C. Rehardening of acid-softened enamel and prevention of enamel softening through CO_2 laser irradiation. J Dent. 2011 Jun;39(6):414-21.
15. Ramalho KM, Eduardo CP, Heussen N, Rocha RG, Lampert F, Apel C, et al. Protective effect of CO_2 laser (10.6 µm) and fluoride on enamel erosion in vitro. Lasers Med Sci. 2013 Jan;28(1):71-8.
16. Esteves-Oliveira M, Yu H, Eduardo CP, Meister J, Lampert F, Attin T, et al. Screening of CO_2 laser (10.6µm) parameters for prevention of enamel erosion. Photomed Laser Surg. 2012 Jun;30(6):331-8.
17. Schlueter N, Hardt M, Lussi A, Engelmann F, Klimek J, Ganss C. Tin-containing fluoride solutions as anti-erosive agents in enamel: an in vitro tin-uptake, tissue loss and scanning electron micrograph study. Eur J Oral Sci. 2009 Aug;117(4):427-34.
18. Hjortsjö C, Jonski G, Young A, Saxegaard E. Effect of acidic fluoride treatments on early enamel erosion lesions – a comparison of calcium and profilometric analyses. Arch Oral Biol. 2010 Mar;55(3):229-34.
19. Lussi A, Megert B, Eggenberger D, Jaeggi T. Impact of different toothpastes on the prevention of erosion. Caries Res. 2008 Dec;42(1):62-7.
20. Young A, Tenuta LMA. Initial Erosion Models. Caries Res. 2011 May;45(suppl 1):33-42.
21. Hjortsjö C, Jonski G, Thrane PS, Saxegaard E, Young A. The effects of acidic fluoride solutions on early enamel erosion in vivo. Caries Res. 2009 Mar;43(2):126-31.
22. Millward A, Shaw L, Harrington E, Smith AJ. Continuous monitoring of salivary flow rate and pH at the surface of the dentition following consumption of acidic beverages. Caries Res. 1997;31(1):44-9.
23. Barbour ME, Rees JS. The laboratory assessment of enamel erosion: a review. J Dent. 2004 Nov;32(8):591-602.
24. Babcock FD, King JC, Jordan TH. The reaction of stannous fluoride and hydroxyapatite. J Dent Res. 1978 Sep-Oct;57(9-10):933-8.
25. Turssi CP, Messias DF, Corona SM, Serra MC. Viability of using enamel and dentin from bovine origin as a substitute for human counterparts in an intraoral erosion model. Braz Dent J. 2010;21(4):332-6.
26. Austin RS, Rodriguez JM, Dunne S, Moazzez R, Bartlett DW. The effect of increasing sodium fluoride concentrations on erosion and attrition of enamel and dentine in vitro. J Dent. 2010 Oct;38(10):782-7.
27. Ren YF, Liu X, Fadel N, Malmstrom H, Barnes V, Xu T. Preventive effects of dentrifice containing 5000 ppm fluoride against dental erosion in situ. J Dent. 2011 Oct;39(10):672-8.
28. Nelson DGA, Wefel JS, Jongebloed WL, Featherstone JD. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. Caries Res. 1987;21(5):411-26.
29. Rechmann P, Charland DA, Rechmann BM, Le CQ, Featherstone JD. In-vivo occlusal caries prevention by pulsed CO_2 -laser and fluoride varnish treatment--a clinical pilot study. Lasers Surg Med. 2013 Jul;45(5):302-10. DOI: 10.1002/lsm.22141. Epub 2013 Jun 4.