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Efficacy of different strategies to treat root dentin eroded by liquid or gaseous hydrochloric acid associated with brushing abrasion

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ABSTRACT

Objective: This study aims to evaluate how casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) associated with Nd:YAG or Diode laser affects dentin exposed to hydrochloric acid (HCl) with or without tooth brushing.

Design: One hundred and sixty human root dentin blocks were selected after they were initially eroded with liquid HCl (pH 1.2) 3x for one day. The blocks were divided into the following groups: G1– liquid HCl (HCl-l), G2– HCl-l + brushing, G3– gaseous HCl (HCl-g), and G4– HCl-g + brushing. Each group was randomly assigned to the following treatments (n = 10): A) Control (no treatment), B) CPP-ACP, C) CPP-ACP associated with Nd:YAG laser (λ = 1064 nm) (40 mJ, 10 Hz, 0.4 W, 15 s), and D) CPP-ACP associated with Diode laser (λ = 980 nm) (0.5 W, 200 µs, 15 s). The treatment with CPP-ACP (G2, G3 and G4) was applied on the dentine surface for 5 min. Erosion (6x/day/20 s) and erosion (6x/day/20 s) with abrasion (2x/10 s) were performed for five days. Dentin volume loss was determined by 3D confocal laser microscopy. Data were analyzed with two-way ANOVA and Tukey's tests.

Results: G1 - CPP-ACP (10.77 ± 1.66) and CPP-ACP associated with Diode laser (9.98 ± 0.89) showed lower volume loss in relation Control group (12.86 ± 0.63) ($p < 0.05$). G2 - CPP-ACP associated with Diode laser (12.41 ± 1.08) elicited lower volume loss as compared to the Control (14.42 ± 1.24) ($p < 0.05$). As for G3 and G4, all treatments showed similar volume loss.

Conclusion: CPP-ACP and CPP-ACP associated with Diode laser could control dental tissue loss in dentin eroded by liquid HCl. Moreover, CPP-ACP associated with Diode laser could effectively decrease dental tissue loss in dentin exposed to liquid HCl and brushing.

1. Introduction

Dental erosion is the most common factor causing tooth surface loss (Shellis & Addy, 2014). Acids dissolve minerals during erosion; dentin can be exposed in advanced stages of erosion (Lee, Aminian, & Brunton, 2017; Milosevic, 2017). Moreover, endogenous acid (gastric acid) promotes more severe erosion than exogenous acids (Bartlett & Coward, 2001; Cheung, Zid, Hunt, & McIntyre, 2005).

Gastric acid, which is present in gastric juice, reaches the mouth cavity by reflux (Moazzez & Bartlett, 2014). Individuals with gastroesophageal reflux disease (GERD) are more susceptible to developing dental erosion (Lee et al., 2017; Milosevic, 2017) caused by stomach acid (Moazzez & Bartlett, 2014; Lee et al., 2017).

Individuals with GERD have greater frequency and duration of mixed reflux (liquid and gas reflux) as compared to healthy individuals (Sifrim et al., 2001). However, gastric acid gas (acid reflux) more often underlies severe dental erosion as compared to liquid gastric acid (liquid reflux) (Higo et al., 2009).

According to the literature, several treatments have been evaluated for the erosion prevention. In general, these treatments use different fluoride formulations (acidulated phosphate fluoride, sodium fluoride, amine fluoride, titanium tetrafluoride) (Lussi & Hellwig, 2014). Casein phosphopeptide-amorphous calcium phosphate is a promising material to prevent tooth surface erosion (Somani, Jaidka, Singh, & Arora, 2014) without the presence of fluoride. In addition, it inhibits demineralization, promotes re-mineralization (Neuhaus & Lussi, 2009; Rahiotis & Vougiouklakis, 2007), and reduces wear in eroded dental tissue (Alexandria et al., 2017; Milosevic, 2017; Ranjitkar, Kaidonis, Richards, & Townsend, 2009; Ranjitkar et al., 2009).

Nd:YAG laser (λ = 1064 nm) (neodymium-doped: yttrium

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aluminum garnet) has gained popularity (Acar, Tuncer, Yuzugullu, & Celik, 2014; Marimoto et al., 2013), and Diode lasers (λ = 980 nm) can elicit morphological changes such as signs of melting and recrystallization on the tooth surface (Naylor, Aranha, Eduardo, Arana-Chavez, & Sobral, 2006; Umana et al., 2013) and dentin tubule lumen occlusion (Gholami, Fekrazad, Esmaiel-Nejad, & Kalhori, 2011; Naylor et al., 2006; Umana et al., 2013).

Unfortunately, the toothbrush exerts an abrasive effect that accelerates dental loss during erosion (Dehghan et al., 2017; Shellis & Addy, 2014), especially if the patient brushes the teeth after reflux (Grippo, Simring, & Coleman, 2012). Dentin is more susceptible to wear by toothbrush abrasion than enamel (Ranjitkar, Kaidonis et al., 2009; Ranjitkar et al., 2009).

Although patients with GERD present high level of dental erosion, knowledge of the erosion and erosion/abrasion process and treatments is lacking.

This study aims to evaluate whether casein phosphopeptide-amorphous calcium phosphate associated or not with Nd:YAG or Diode laser diminishes dentin volume loss after exposure to liquid hydrochloric acid or hydrochloric acid gas with or without toothbrushing.

2. Materials and methods

This study was submitted to the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, and was only initiated after being approved (protocol approval number: 2011.1.1370.58.0).

2.1. Experimental design

This study consists of a randomized complete block factorial design with 10 experimental units per erosion/treatment. *Erosion* was evaluated in four levels: G1 – liquid HCl, G2 – liquid HCl with brushing, G3 – gaseous HCl and G4 – gaseous HCl gas with brushing; *Surface Treatment* was also analyzed in four levels: A) Control without treatment, B) CPP-ACP, C) CPP-ACP + Nd:YAG laser and D) CPP-ACP + Diode laser. The response variable was dentin volume loss measured by confocal laser scanning microscopy (CLSM).

2.2. Dentin sample preparation

Two hundred sound human molars were selected from the Tooth Bank of the School of Dentistry of Ribeirão Preto, University of São Paulo. After the molars were disinfected (Dominici, Eleazer, Clark, Staat, & Scheetz, 2001), the crowns and roots were separated by cutting 2 mm above the cementoenamel junction; each root was sectioned in two slabs (4 × 3 × 2 mm) to the cervical region with the aid of a water-cooled diamond saw in a precision cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA). The root dentin slabs were then individually fixed in a cylindrical resin matrix and adapted to a grinder-polishing (APL-4, Arotec S/A Ind. e Comercio, São Paulo, SP, Brazil) with #600 to #1200-grit silicon carbide paper (Norton Abrasivos Ltda, São Paulo, SP, Brazil) to remove the overlying cementum. Next, the root dentin slabs were coated with dental composite resin (Z350 FiltekTM, 3M ESPE, USA), except for an area of 2 × 3 mm on the buccal surface.

2.3. Initial erosive lesion

To simulate the patients' clinical situation in terms of dental erosion, first an erosion-like lesion was made by storing the specimens in artificial saliva (Amaechi, Higham, & Edgar, 1999) at 37 °C for 2 h, which was followed by individual immersion in 5 mL of 0.3% hydrochloric acid solution (pH = 1.2) inside containers placed on an orbital shaker (CT155, Cientec, Piracicaba, SP, Brazil) for 20 s (40 rpm). The teeth were immersed in the acid solution three times. Between each erosive challenge, the specimens were rinsed with deionized water and individually stored in 5 mL of artificial saliva at 37 °C for 1 h. At the end of the initial erosion cycle, the specimens were stored in artificial saliva at 37 °C for 12 h. Later, they were taken for morphological surface analysis with a 3D confocal laser microscope (LEXT OLS4000, Olympus, Tokyo, Japan). The specimens that presented cracks or defects on the surface were discarded. At the end of this procedure, 160 specimens were selected.

2.4. CPP-ACP application and laser irradiation

2.4.1. Casein phosphopeptide-amorphous calcium phosphate (CPP- ACP)

CPP-ACP paste (GC Tooth Mousse, Tokyo, Japan) was applied on the dentine surface for 5 min. After the first 3 min, excess paste was carefully removed. At the end of the 5- min treatment, the specimen was rinsed with deionized water.

2.4.2. CPP-ACP and Nd:YAG laser application

For irradiation with Nd:YAG laser, a unit of SmartFile (Deka, Florence, Italy) equipment operating at a wavelength of 1064 micrometers was employed. CPP-ACP with coal was applied and kept for a period of 5 min. After the first minute of this 5-min period, laser was applied for 15 s. Irradiation was carried out by scanning (long pulse, 40 mJ, 10 Hz, 0.4 W) on root dentin with the aid of a 0.3-mm quartz fiber positioned perpendicular to the specimen, at a distance of 1 mm. Next, the paste was carefully removed. At the end of the 5-min treatment, the specimen was rinsed with deionized water.

2.4.3. CPP-ACP and diode laser application

For irradiation with Diode laser, a unit of Einstein DL (CD International, Wellington, United States) equipment operating at a wavelength of 980 nm was used. CPP-ACP with coal was applied and kept for 5 min. After the first minute of this 5-min period, laser was applied for 15 s. Irradiation was conducted by scanning (0.5 W, 200 μs) on root dentin with the aid of a 0.4-mm acrylic fiber positioned perpendicular to the specimen at a distance of 1 mm. Then, the paste was carefully removed. At the end of the 5-min treatment, the specimen was rinsed with deionized water.

2.5. Erosive and erosive/abrasive challenges after surface treatment

The specimens were stored in artificial saliva at 37 °C for 2 h. Then, each group was exposed to erosive and erosive/abrasive challenges, as follows.

2.5.1. Erosion

- **Liquid hydrochloric acid (HCl-l):** The specimens were individually immersed in 5 mL of 0.3% HCl solution (pH = 1.2) and placed in an orbital shaker (CT155, Cientec, Piracicaba, SP, Brazil) for 20 s (40 rpm). The erosive process was performed for five days, six times per day. Between each erosive challenge, the specimens were rinsed with deionized water and stored in artificial saliva at 37 °C for 1 h. At the end of each erosive cycle, the specimens were stored in artificial saliva at 37 °C for 12 h.
- **Gaseous hydrochloric acid (HCl-g):** For this challenge, a designed apparatus where the specimens were exposed to gaseous HCl (pH = 1.2) for 20 s was employed. The gas was obtained by using 500 mL of 15.84% HCl solution (Derceli, 2014) in atmospheric ambient pressure. The erosive process was carried out for five days, six times per day. Between each erosive challenge, the specimens were rinsed with deionized water and stored in artificial saliva at 37 °C for 1 h. At the end of each erosive cycle, the specimens were stored in artificial saliva at 37 °C for 12 h.

2.5.2. Erosion/Abrasion

Erosion was conducted as described previously, and abrasion was