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Increased expression of genes after periodontal treatment with photodynamic therapy

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Summary
Background: The current study was devised with the objective of using a split-mouth, controlled clinical trial to compare conventional mechanical debridement (scaling and root planing) treatment (T1) with conventional mechanical treatment followed by photodynamic therapy (PDT) (T2) in patients with severe periodontitis.
Methods: Four PDT sessions were completed, and clinical parameters such as bleeding upon probing (BOP positive), plaque index (PI), probing pocket depth (PPD) and clinical attachment loss (CAL) were evaluated before and after the treatment series. In addition, gingival biopsies were collected at the start and finish of treatment, and were used for qPCR gene expression analysis of *TNFA*, *IL1B*, *IL8*, *IL10*, *IL17*, *MMP13*, *FGF2*, *RANK*, *RANKL* and *OPG*.
Results: The clinical results showed a significant improvement in BOP with treatment T2 ($p = 0.03$). The molecular data showed an up-regulation of *FGF2*, *RANK* and *OPG* gene expression after T2. The expression levels of the other genes were not significantly different between T1 and T2. PDT increased the expression of *RANK* and *OPG*, which could indicate a reduction in osteoclastogenesis. Furthermore, the use of PDT in conjunction with conventional treatment significantly increased the expression of *FGF2*, which has an important role in the periodontal repair process.

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Conclusions: PDT technology could be a means to improve conventional periodontitis treatment. Our results suggest that PDT acts in part by controlling bone resorption and increasing the expression of genes important for tissue repair.

Introduction

Periodontal disease is an inflammatory disease of multifactorial etiology that develops in response to the presence of specific bacteria. It has the potential to compromise protective structures and the retention of teeth, resulting in attachment loss, irreversible bone loss, and eventually, tooth loss [1,2]. The prevalence of periodontal disease is high; it is considered one of the most common infections observed in all populations and represents a significant cause for concern in public health worldwide [3—8].

Conventional periodontal treatments typically involve radicular mechanical treatments that aim to remove the causative disease agents through the use of manual and ultrasonic techniques. More recently, photodynamic therapy (PDT) has begun to be incorporated as a periodontal treatment with specific antimicrobial characteristics.

However, neither the effectiveness nor the mode of action of PDT at human periodontal sites is as yet completely clear, especially with regard to the possible molecular alterations resulting from this treatment.

Considering the etiopathogenesis of periodontal disease and the importance of the host as a main factor [9—14], the genes selected for this study are related to immunoinflammatory processes (*TNFA*, *IL1B*, *IL8*, *IL10*, *IL17* and *MMP13*), bone loss and periodontitis (*RANK*, *RANKL* and *OPG*), and to the repair process (*FGF2*). The expression levels of these genes were tested in the gingival tissues from periodontitis patients before and after scaling treatment followed by PDT.

Furthermore, clinical periodontal parameters such as bleeding upon probing (BOP positive), plaque index (PI), probing pocket depth (PPD) and clinical attachment loss (CAL) were evaluated before and after treatment in order to observe the clinical efficiency of PDT in the treatment of severe periodontitis.

Materials and methods

Patient selection

For patient selection, a database of 628 patients from the dental clinic at the Catholic University of Brasília (UCB) was used. Fifteen patients were selected based on the following inclusion criteria: aged 35—44 years, diagnosis of severe chronic periodontitis, presence of at least 20 teeth with at least one posterior tooth in each quadrant, and periodontal pockets $\geq$5 mm on at least seven teeth. Patients subjected to periodontal treatment within the previous six months, patients with systemic diseases that could influence the therapy, smokers, and patients taking antibiotics, corticoids, immunosuppressants or anti-inflammatories within the previous six months were excluded. All patients received orientation regarding the research and signed an informed consent form. This research was approved by the UCB ethics committee (#52/2010).

Clinical evaluation

This study was designed using the split mouth system in which the two quadrants of one side of the mouth were treated with conventional mechanical debridement (deep scaling and root planing) (treatment 1/T1), and the other two quadrants were treated with deep scaling and root planing followed by PDT (treatment 2/T2). All patients received orientation during the first treatment session regarding oral hygiene techniques according to individual necessity. This included information about the use of interdental brushes, soft brushes, and dental floss. In addition, motivational techniques were employed throughout the treatment. It is important to note that all patients were treated by the same therapist.

Side 1 (T1)
Scaling and root planing was performed using Gracey-type (Hu-Friedy®-Chicago, IL, USA) periodontal curettes (5/6; 7/8, 11/12 and 13/14), which were new and properly sharpened. Subgingival scaling was performed with 2% lidocaine anesthetic, with an adrenaline vasoconstrictor (1:100,000).

Side 2 (T2)
Scaling and root planing was performed with Gracey-type periodontal curettes (Hu-Friedy®-Chicago, IL, USA) (5/6; 7/8, 11/12 and 13/14), which were new and properly sharpened. Sub-gingival scaling was performed with 2% lidocaine anesthetic, with an adrenaline vasoconstrictor (1:100,000). After conventional scraping, PDT was performed using a laser diode (MMoptics-São Carlos-SP, Brazil) at a wavelength of 660 nm, potency of 60 mW/cm^2 and energy density of 5.4 J/cm^2. Methylene blue (0.01%) was applied to the subgingival sites using a sterile disposable syringe as a photosensitizing agent, and was left in place for 5 min (the pre-irradiation period). Periodontal pockets were then exposed to laser diode light using a 0.4 mm optic fiber (MMoptics-São Carlos-SP, Brazil). PDT was applied to six sites per tooth (mesiobuccal; buccal; distobuccal; mesiolingual; lingual and distolingual) for 15 s each, for a total of 90 s per tooth. Four sessions were performed, with intervals of seven days between the sessions.

All clinical parameters were registered on periograms containing the following clinical data: bleeding upon probing (BOP positive), plaque index (PI), probing pocket depth (PPD) and clinical attachment loss (CAL). These parameters were recorded before treatment and 90 days after treatment. For the measurement of the clinical parameters,