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Treatment of experimental periodontal disease with antimicrobial photodynamic therapy in nicotine-modified rats

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Abstract

Background: The aim of this study was to compare antimicrobial photodynamic therapy (aPDT) as an adjunctive treatment to scaling and root planing (SRP) for induced periodontitis in nicotine-modified rats.

Material & Methods: A total of 240 rats were evenly divided into two groups: C – saline solution treatment; N – nicotine treatment. Periodontal disease was induced in both groups at the first mandibular molar. After 7 days, the ligature was removed. All animals were submitted to SRP and were divided according to the following treatments: SRP – irrigation with saline solution; Toluidine Blue-O (TBO) – irrigation with phenothiazinium dye (100 μg/ml); LLLT – laser irradiation (660 nm; 0.03 W; 4 J); and aPDT – TBO and laser irradiation. Ten animals in each group/treatment were euthanized at 7, 15 and 30 days. The histometric and immunohistochemical values were statistically analysed.

Results: Intragroup analysis demonstrated that in both groups the aPDT treatment resulted in lower bone loss (BL) when compared to SRP in all experimental periods. Intergroup analysis demonstrated that aPDT treatment resulted in lower BL in Group N than in Group C treated with SRP in all experimental periods.

Conclusion: Antimicrobial photodynamic therapy was an effective adjunctive treatment to SRP for induced periodontitis in nicotine-modified rats.

Key words: alveolar bone loss; lasers; nicotine; osteoclasts; periodontitis; photochemotherapy

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Periodontal disease (PD) is a multifactorial pathological condition that involves both host systemic alterations and the presence of local pathogenic microbiota (Cullinan et al. 2009). The main signs that characterize PD are attachment loss caused by apical migration of the junctional epithelium and alveolar bone loss (BL) (Ebisu & Noiri 2007).

Conflict of interest and source of funding statement

The authors declare no conflicts of interest.

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The metabolism of alveolar bone is controlled by proteins such as RANK, RANKL and OPG, which mark cell activity. RANK is present on the membranes of osteoclasts and dendritic cells (Evans et al. 2006), and its dimerization allows for the formation of multinuclear TRAP-positive cells with clastic cell immunoreactivity (Iwamoto et al. 2004). RANKL, in turn, is produced by osteoblasts, bone stromal cells and activated T-lymphocytes, and promotes bone resorption when linked to RANK (Khosla 2001). By contrast, OPG inhibits osteoclast forma-

tion in the development stage, preventing the binding of RANKL to RANK (Shalhoub et al. 1999).

Tobacco is one of the most significant risk factors for the onset and progression of PD (Genco 1996). This association may be due to local vasoconstriction (which impairs periodontal tissue blood supply), increased concentrations of periodontal pathogens in the subgingival microbiota or alterations in their composition (Ramseier & Fundak 2009). Furthermore, nicotine in cigarettes may increase collagen fibre degradation by fibroblasts (Zhang et al. 2009), inhibit immune system responses (Breivik et al. 2009), promote decreases in both phagocytosis and polymorphonuclear leucocyte chemotaxis and reduce antibody production and the viability of T-lymphocytes (Holt & Keast 1977).

The treatment of PD is based on the elimination of pathogenic subgingival microbiota through scaling and root planing (SRP) (Kaldahl et al. 1993). However, mechanical therapy alone may be ineffective at eliminating pathogenic bacteria, which are frequently inserted into both soft and hard tissues and even in areas that are inaccessible to periodontal instruments (Adriaens et al. 1988).

Considering these limitations, adjunctive methods that promote the elimination of periodontal pathogens have been investigated, as with antimicrobial photodynamic therapy (aPDT). This therapy consists of the association of a photosensitizer with an intense light source to promote cell death (Bauer et al. 2001). The active mechanism of aPDT begins when the photosensitizer's electrons absorb energy from a light source with the same wavelength and are thus induced into an excited state (Dörtbudak et al. 2001). When the photosensitizer returns to its primary state, it can transfer the energy to a substrate when one is present, forming short lifespan species that are highly reactive to singlet oxygen, which may injure microorganisms due to the irreversible oxidation of cell components (Tomaselli et al. 2001). The lethal photosensitization of periodontopathogenic bacteria must involve changes in membranes and/or plasma membrane proteins and DNA damage mediated by singlet oxygen (Maisch 2007).

The major advantages of aPDT are that it is a specific therapy targeting cells that, it has no collateral effects, that its activity is initiated only upon exposure to light, and that its use will not lead to the selection of resistant bacterial species (Maisch 2007).

Some studies in animals have also shown satisfactory results with the use of aPDT for the treatment of experimental periodontal disease (Kömerik et al. 2003, Almeida et al. 2008a,b, Fernandes et al. 2009). Recent articles from the present research group have demonstrated the effectiveness of aPDT as an adjunct to conventional periodontal treatment in animals with diabetes mellitus (Almeida et al. 2008a) and inhibited with immunosuppressive drugs (Fernandes et al. 2009).

In this context, the aim of the present study was to histologically, histometrically and immunohistochemically evaluate the efficacy of aPDT as an adjunct to conventional mechanical therapy (scaling and root planing) for alveolar BL in both normal and systemically nicotine-modified rats with experimentally induced periodontitis.

Animals

This study was conducted on 240 adult male Wistar rats (120–140 g). The animals were kept in plastic cages with access to food and water ad libitum. Prior to the surgical procedures, all animals were allowed to acclimatize to the laboratory environment for a period of 5 days. All protocols described below were approved by the Institutional Review Board of Araçatuba Dental School, São Paulo State University, Araçatuba, SP, Brazil (no. 1372/08).

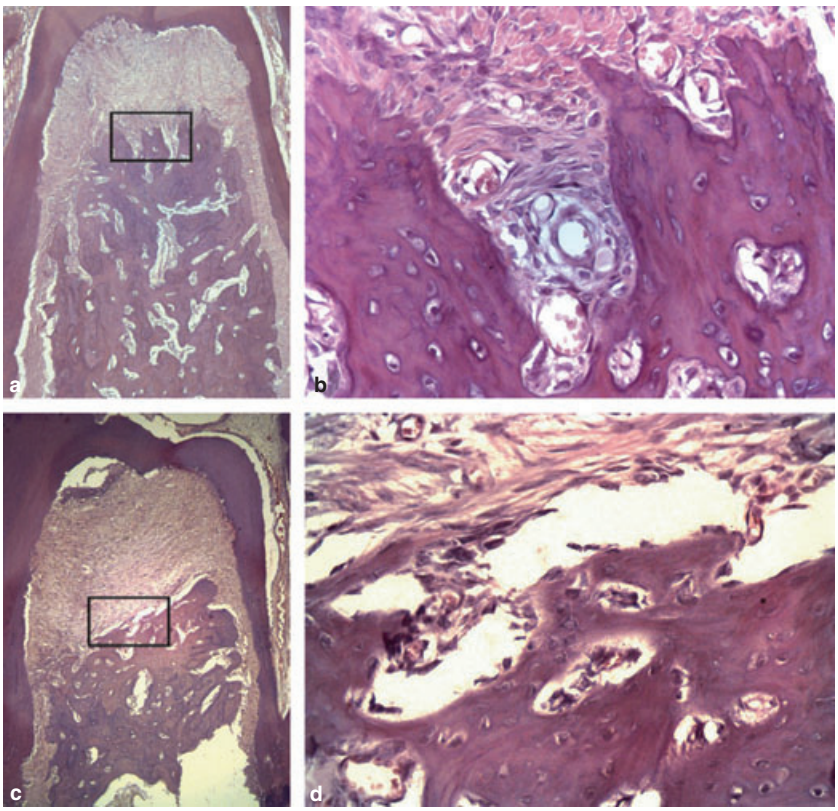

Fig. 1. Photomicrograph illustrating the areas of bone loss (BL) in the furcation region of the mandibular left first molar with induced periodontal disease in the different groups (C and N): (a) C Group, scaling and root planing (SRP) treatment for 30 days (HE, original magnification 5×); (b) C Group, SRP treatment for 30 days (HE, original magnification 40×); (c) N Group, SRP treatment for 30 days (HE, original magnification 5×) and (d) N Group, SRP treatment for 30 days (HE, original magnification 40×).

Experimental design

Drug administration protocol

The animals were numbered and randomly divided into two groups: C (n = 120) – Control – injected with saline solution at 3 mg/kg of body weight; N (n = 120) – Nicotine – subcutaneously injected with nicotine hemisulphate preparation at 3 mg/kg of body weight (Pinto et al. 2002). Nicotine hemisulphate preparation (Sigma Corporation, São Paulo, SP, Brazil) was obtained by diluting it in saline solution at a concentration of 5 mg/ml (Okamoto et al. 1994).

All injections were divided into two applications, one in the morning and the other in the evening, always with a 12-h interval between them. The injections were initiated 30 days before PD induction and performed daily up to the end of the respective euthanasia period. The administration site was the back of the animal, next to the cephalic region. The animals were weighed weekly with regard to dose maintenance throughout the experimental period.

Experimental periodontal disease protocol

General anaesthesia was induced by administering ketamine (0.4 ml/kg) together with xylazine (0.2 ml/kg) via intramuscular injection. One mandibular left first molar from each animal in both the C and N groups was selected to receive a cotton ligature in a submarginal position to induce experimental periodontitis (Johnson 1975). After 7 days of experimental periodontal disease induction, the mandibular ligature was removed from the left first molar of all animals in the C and N groups. The left molars were subjected to SRP with manual #1–2 micro mini five curettes (Hu-Friedy Co. Inc., Chicago, IL, USA) through 10 distal–mesial traction movements in both the buccal and lingual aspects. The furcation and interproximal areas were scaled with the same curettes through cervical-occlusal traction movements. The entire SRP procedure was performed by the same experienced operator. The 120 animals of each group (C and N) were randomly allocated by the use of a computer-generated table to treatments of SRP, TBO, LLLT or aPDT. For better standardization, animal 1 was the first choice, followed by animals 2 and 3, respectively. Thus, all animals in each group (C and N) were randomly assigned to one of the four treatments (30 animals/treatment): SRP – mandibular left molar submitted to SRP and irrigation with 1 ml of saline solution; Toluidine Blue-O (TBO) – mandibular left molar submitted to SRP and irrigation with 1 ml of phenothiazinium dye (TBO solution, 100 μg/ml; Sigma Chemical Co., St. Louis, MO, USA); LLLT – SRP and irrigation with 1 ml of saline solution followed by low-level laser therapy (LLLT) after 1 min.; and aPDT – mandibular left molar submitted to SRP and irrigation with 1 ml of TBO solution (100 μg/ml) followed by the application of LLLT after 1 min.

LLLT treatment

The laser used in this study was gallium–aluminium–arsenide (GaAlAs) (Bio Wave; Kondortech Equipment Ltd, São Carlos, SP, Brazil), with a wavelength of 660 nm and a spot size of 0.07 cm^2. After 1 min. of saline solution application, LLLT was applied to three equidistant points at each buccal and lingual aspect of the mandibular first molar in contact with the tissue. The therapeutic laser was released with a power of 0.03 W for 133 s/point, a power density of 0.428 W/cm^2, and energy of 4 J/point (57.14 J/cm^2/point) (Fernandes et al. 2009).

aPDT treatment

After 1 min. of TBO application, LLLT was applied using the above parameters. The TBO solution was slowly poured into the periodontal pocket using a syringe (1 ml) and an insulin needle (13 mm × 0.45 mm) (Becton Dickinson Ind. Ltd, Curitiba, PR, Brazil) without a bevel.

Experimental periods

Ten animals from each group/treatment were euthanized at 7, 15 and 30 days after the periodontal disease treatment by the administration of a lethal dose of thiopental (150 mg/kg) (Cristália, Ltd, Itapira, SP, Brazil). Their jaws were removed and fixed in 10% neutral formalin for 48 h.

Table 1. Means and standard deviations (M ± SD) of the histometric data for bone loss (BL, mm^2) in the furcation region of the mandibular left first molar according to each group, treatment and period

C Group – M ± SD Periods Treatments	7 days	15 days	30 days
SRP	1.09 ± 0.13‡,§	1.02 ± 0.19‡,§	0.99 ± 0.28‡,§
TBO	0.79 ± 0.99*,†	0.73 ± 0.26*,†	0.66 ± 0.14*,†
LLLT	0.65 ± 0.12*,†	0.56 ± 1.07*,†	0.50 ± 0.45*
aPDT	0.41 ± 0.03*	0.40 ± 0.02*	0.38 ± 0.05*
N Group – M ± SD Periods Treatments	7 days	15 days	30 days
SRP	1.92 ± 0.34‡	1.91 ± 0.21‡	1.89 ± 0.22‡
TBO	0.89 ± 0.22*,†	0.82 ± 0.26*,†	0.76 ± 0.09*,†
LLLT	0.68 ± 0.28*,†	0.58 ± 2.03*	0.51 ± 3.54*
aPDT	0.44 ± 0.08*,§	0.42 ± 0.06*,§	0.40 ± 0.03*,§
N	80	80	80

aPDT, antimicrobial photodynamic therapy; LLLT, low-level laser therapy; SRP, scaling and root planing; TBO, Toluidine Blue-O.

*Significant difference with SRP treatment, in the same group and period (ANOVA and Tukey; $p < 0.05$).

†Significant difference with aPDT treatment, in the same group and period (ANOVA and Tukey; $p < 0.05$).

‡Significant difference between groups, in the same period and treatment (ANOVA and Tukey; $p < 0.05$).

§Significant difference between groups and treatments, in the same period (ANOVA and Tukey; $p < 0.05$).

Laboratory procedures

The specimens were demineralized in a solution consisting of equal parts of 50% formic acid and 20% sodium citrate for 15 days. Paraffin serial sections (4 μm) were obtained in the mesial–distal direction and dyed with haematoxylin and eosin (HE) and Masson's Trichrome. To perform immunohistochemical reactions, the sections were submitted to indirect immunoperoxidase using polyclonal primary antibodies for RANKL (1:200, SC 7628, Lot#20908, goat anti-RANKL; Santa Cruz Biotechnology, Santa Cruz, CA, USA), OPG (1:100, SC 8468, Lot#H2208, goat anti-OPG; Santa Cruz Biotechnology) and TRAP (1:100, SC 30833, goat anti-TRAP; Santa Cruz Biotechnology).

Histological analysis

The sections dyed with HE were analysed under light microscopy at a magnification of 40× to establish the BL level and characterize the periodontal ligamentation of the furcation region of the first molar (Almeida et al. 2008a, Fernandes et al. 2009).

Histometric analysis

Masson's Trichrome staining was used to assess inter-radicular bone levels at 12.5× magnification. The area of BL in the furcation region was determined histometrically using an image analysis system (Image Tool; University of Texas Health Science Center at San Antonio, San Antonio, TX, USA). After excluding the first and last sections where the furcation region was evident, five equidistant sections of each specimen block were selected and imaged by a digital camera coupled to a light microscope (César-Neto et al. 2006). The mean values were averaged and statistically compared.

Immunohistochemical analysis

The markers RANKL and OPG were analysed immunohistochemically in both the bone and periodontal ligament in the furcation area of the first molar with experimentally induced periodontal disease by percentages: no mark (0%), weak marking (<25% of cells); moderate marking (<50% of cells); and strong intensity (<75% of cells) (Kim et al. 2007).

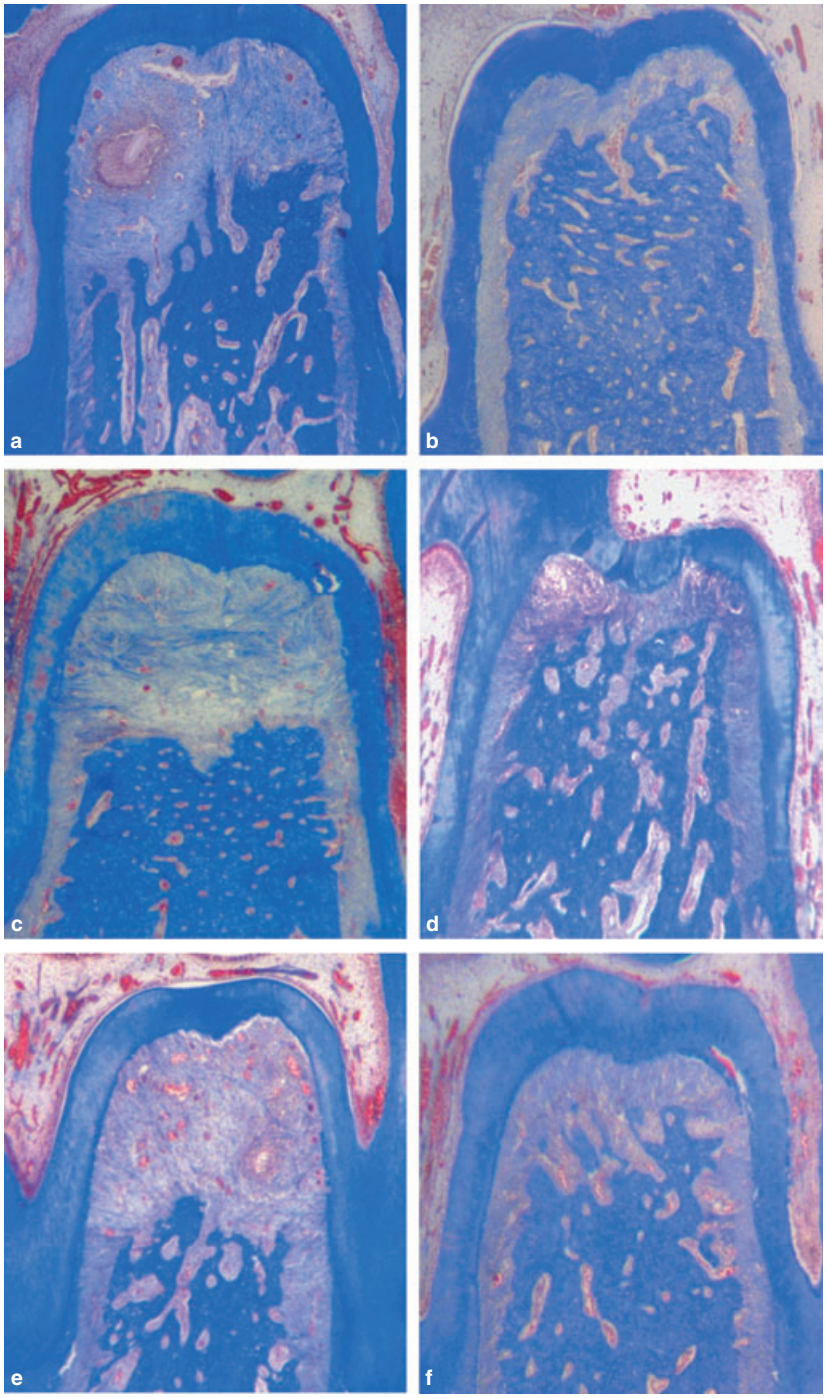

Fig. 2. Photomicrograph illustrating the areas of bone loss (BL) in the furcation region of the mandibular left first molar with induced periodontal disease in the different groups (C and N) and treatments: (a) C Group – scaling and root planing (SRP) treatment for 7 days; (b) N Group – antimicrobial photodynamic therapy (aPDT) treatment for 7 days; (c) C Group – SRP treatment for 15 days; (d) N Group – aPDT treatment for 15 days; (e) C Group – SRP treatment for 30 days and (f) N Group – aPDT treatment for 30 days (original magnification 12.5×; Masson's Trichrome).

The immunohistochemical marker TRAP was assessed by counting TRAP-positive cells located in the boundary of the most coronal por-

tion of the bone tissue of the furcation region in mandibular first molars with periodontal disease (Jin et al. 2007, Khojasteh et al. 2008, Liu et al. 2010). Mature osteoclasts containing three or more nuclei were considered positive TRAP cells (Hassumi et al. 2009). A blinded trained examiner selected the sections for the histological, histometric and immunohistochemical analyses. Another blinded calibrated examiner conducted the data analysis. The values for each specimen section were measured three times by the same examiner on different days to reduce variations in the data.

Intra-examiner reproducibility

Before the histometric and immunohistochemical analyses were performed, the examiner was trained by double measurements of 30 specimens with a 1-week interval. Paired *t*-test statistics was calculated, and no differences were observed in the mean values for comparison ($p > 0.05$). In addition, the calculation of Pearson's correlation coefficient revealed a very high correlation (0.95) between the two measurements for both the histometric and immunohistochemical analyses.

Statistical analysis

The hypothesis that neither BL rates nor the number of TRAP-positive cells in the furcation region differed among groups/treatments/periods was tested by BIOESTAT 3.0 software (Bioestat, Windows 1995; Sonopress Brazilian Industry, Manaus, AM, Brazil).

After normality testing, the histometric and immunohistochemical data were analysed by the Shapiro–Wilk test, and the intra-group and inter-group analyses were performed with a two-way analysis of variance (ANOVA; $p < 0.05$). When ANOVA detected a statistical difference, multiple comparisons were performed with the Tukey test ($p < 0.05$).

Results

Histological assessment

At 7, 15 and 30 days, disorganized connective tissue was visible in both groups submitted to SRP treatment with numerous neutrophils and a discrete number of fibroblasts. The bone tissue showed thin bone trabeculae (Fig. 1).

In both the C and N Groups, the animals that received the TBO and LLLT treatments showed poorly organized connective tissue in all experimental periods, with a predominantly chronic inflammatory process and a moderate number of fibroblasts. The bone tissue showed thin bone trabeculae.

At 7 and 15 days, animals from the C and N Groups, which were all treated with aPDT showed well developed connective tissues with a moderate number of fibroblasts, discrete chronic inflammatory infiltrate and moderately developed bone tissue. At 30 days, they showed complete and organized periodontal ligaments with parallel collagen fibres. The connective tissue was well developed, complete and showed mild inflammatory infiltrate.

Histometric assessment

Intra-group assessments demonstrated that in the C Group, animals treated with aPDT showed BL levels lower than the SRP or TBO animals at 7, 15 and 30 days and lower than the LLLT animals at 7 and 15 days ($p < 0.05$). In the N Group, the aPDT animals also presented BL levels lower than the SRP or TBO animals at 7, 15 and 30 days and lower than those in the LLLT animals at 7 days ($p < 0.05$) (Table 1).

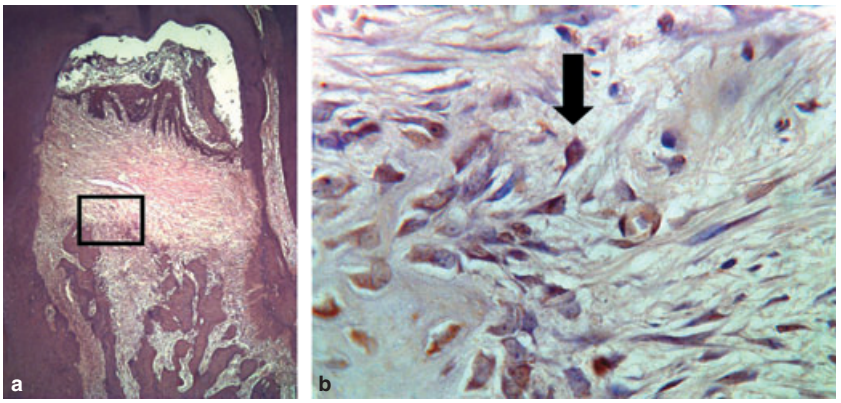

Fig. 3. Immunostaining for RANKL in the areas of bone loss (BL) in the furcation region of the mandibular left first molar with induced periodontal disease: (a) N Group, scaling and root planing (SRP) treatment for 7 days (HE, original magnification 5×) and (b) N Group, SRP treatment for 7 days (arrow) (original magnification 100×).

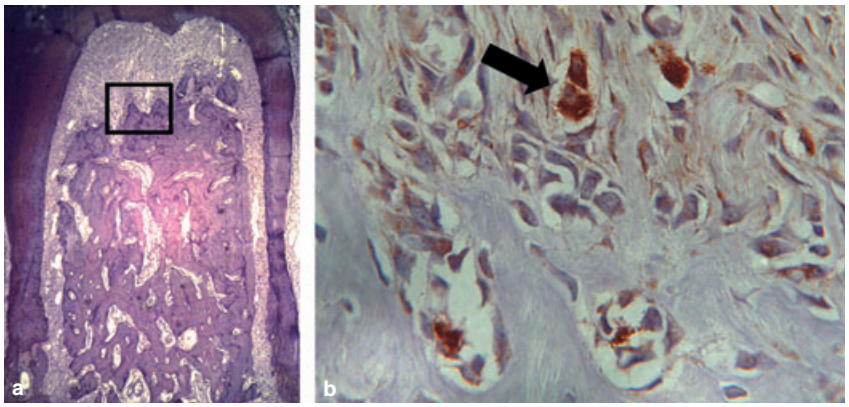

Fig. 4. Immunostaining for OPG in the areas of bone loss (BL) in the furcation region of the mandibular left first molar with induced periodontal disease: (a) N Group, antimicrobial photodynamic therapy (aPDT) treatment for 15 days (HE, original magnification 5×) and (b) N Group, aPDT treatment for 15 days (arrow) (original magnification 100×).

With regard to inter-group assessments, the SRP treatment caused a significantly higher BL level in the N Group animals than in the C Group animals at 7, 15 and 30 days ($p < 0.05$). Furthermore, this study showed that in the N group, aPDT caused a lower BL level than was observed with the C group animals, which were treated with SRP at 7, 15 and 30 days ($p < 0.05$) (Table 1 and Fig. 2).

Immunohistochemical assessment

An immunohistochemical analysis of the patterns of the RANKL and OPG antigenic markers revealed the specificity of the employed antibodies. The tissue structures that showed immunoreactivity to RANKL and OPG were similar. The signal was restricted to the cytosol of cells similar to osteoblasts, fibroblasts and mononuclear macrophages. RANKL immunoreactivity was most evident at 7 and 15 days, whereas OPG immunoreactivity was significant at 15 days. An analysis of these markers between the experimental groups revealed strong RANKL immunoreactivity in animals treated with SRP at 7 days (Fig. 3) and weak OPG immunoreactivity at 15 days. The animals treated with aPDT showed weak RANKL immunoreactivity at 7 days and strong OPG immunoreactivity at 15 days (Fig. 4).

Immunohistochemical assessment to identify the number of TRAP-positive cells corroborated the histometric results. The intra-group assessment showed that the animals from the C Group that were treated with aPDT presented significantly fewer TRAP-positive cells than did the SRP and TBO animals at 7, 15 and 30 days, while also displaying fewer cells than did the LLLT animals at 7 and 15 days ($p < 0.05$; Table 2). In the N Group, aPDT also resulted in fewer TRAP-positive cells than did the SRP and TBO animals at 7, 15 and 30 days, while also displaying fewer cells than did the LLLT animals at 7 days ($p < 0.05$; Table 2).

With regard to inter-group assessments, SRP treatment resulted in more TRAP-positive cells in the N Group animals than in the C Group animals at 7, 15 and 30 days (Fig. 5) ($p < 0.05$; Table 2). Furthermore, this study showed that the N group animals treated with aPDT presented fewer TRAP-positive cells than the C Group animals treated with SRP at 7, 15 and 30 days ($p < 0.05$; Table 2).

Discussion

The aim of this study was to assess aPDT as an adjunct to SRP treatment for induced periodontitis in nicotine-modified rats. In the inter-group assessment, the SRP treatment caused significantly higher BL levels and more TRAP-positive cells in the N Group animals than levels seen in the C Group animals for all time points. The immunohistochemical data are in agreement with these results, and they are also consistent with recent studies (Bosco et al. 2007, Breivik et al. 2009). This shows that the application of nicotine in rats significantly increases periodontal BL. This increase is likely due to both local and systemic effects of nicotine (Ramseier & Fundak 2009). Nicotine causes gingival vasoconstriction, thereby compromising blood supply and defences. It also changes the composition of the subgingival microbiota, thus enabling greater accumulation of periodontal pathogens. Furthermore, this substance may increase collagen fibre deterioration by fibroblasts (Zhang et al. 2009) and inhibit immune system responses (Breivik et al. 2009) because it decreases the phagocytosis and chemotaxis of polymorphonuclear leucocytes, reduces the production of antibodies and reduces T-lymphocyte viability (Holt & Keast 1977).

The isolated use of TBO (100 μg) in both groups promoted lower BL levels and fewer TRAP-positive cells than did SRP treatment alone. This finding differs from the results of Kömerik et al. (2003), when TBO was used alone at concentrations of 0.01, 0.1 and 1 mg/ml (10, 100 and 1000 μg/ml) wherein morphometric analysis showed no significant differences in BL levels. However, in the microbiological analysis, a reduction in *Porphyromonas gingivallis* was observed at the concentration of 1 mg/ml (1000 μg/ml) after 4 and 8 min. of photosensitizing drug use.

The use of local antimicrobial agents such as aPDT has been recommended as an adjuvant periodon-

Table 2. Means and standard deviations (M ± SD) of the number of TRAP-positive cells in the furcation region of the mandibular left first molar according to each group, treatment and period

C Group – M ± SD Periods Treatments	7 days	15 days	30 days
SRP	5.80 ± 1.48‡,§	5.20 ± 2.16‡,§	4.80 ± 0.83‡,§
TBO	4.60 ± 1.14*,†	4.54 ± 1.34†	4.40 ± 1.14†
LLLT	3.76 ± 0.22*,†	3.07 ± 1.50*,†	2.87 ± 1.23*
aPDT	2.01 ± 0.34*	2.00 ± 0.70*	1.80 ± 0.83*
N Group – M ± SD **Periods** **Treatments**	**7 days**	**15 days**	**30 days**
SRP	8.80 ± 0.90‡	8.20 ± 0.86‡	7.80 ± 0.83‡
TBO	5.93 ± 1.23*,†	5.60 ± 1.14*,†	5.00 ± 1.12*,†
LLLT	4.05 ± 0.56*,†	3.89 ± 1.99*	3.19 ± 2.09*
aPDT	2.47 ± 1.34*,§	2.24 ± 1.74*,§	2.16 ± 0.54*,§
N	80	80	80

aPDT, antimicrobial photodynamic therapy; LLLT, low-level laser therapy; SRP, scaling and root planing; TBO, Toluidine Blue-O.

*Significant difference with SRP treatment, in the same group and period (ANOVA and Tukey; $p < 0.05$).

†Significant difference with aPDT treatment, in the same group and period (ANOVA and Tukey; $p < 0.05$).

‡Significant difference between groups, in the same period and treatment (ANOVA and Tukey; $p < 0.05$).

§Significant difference between groups and treatments, in the same period (ANOVA and Tukey; $p < 0.05$).

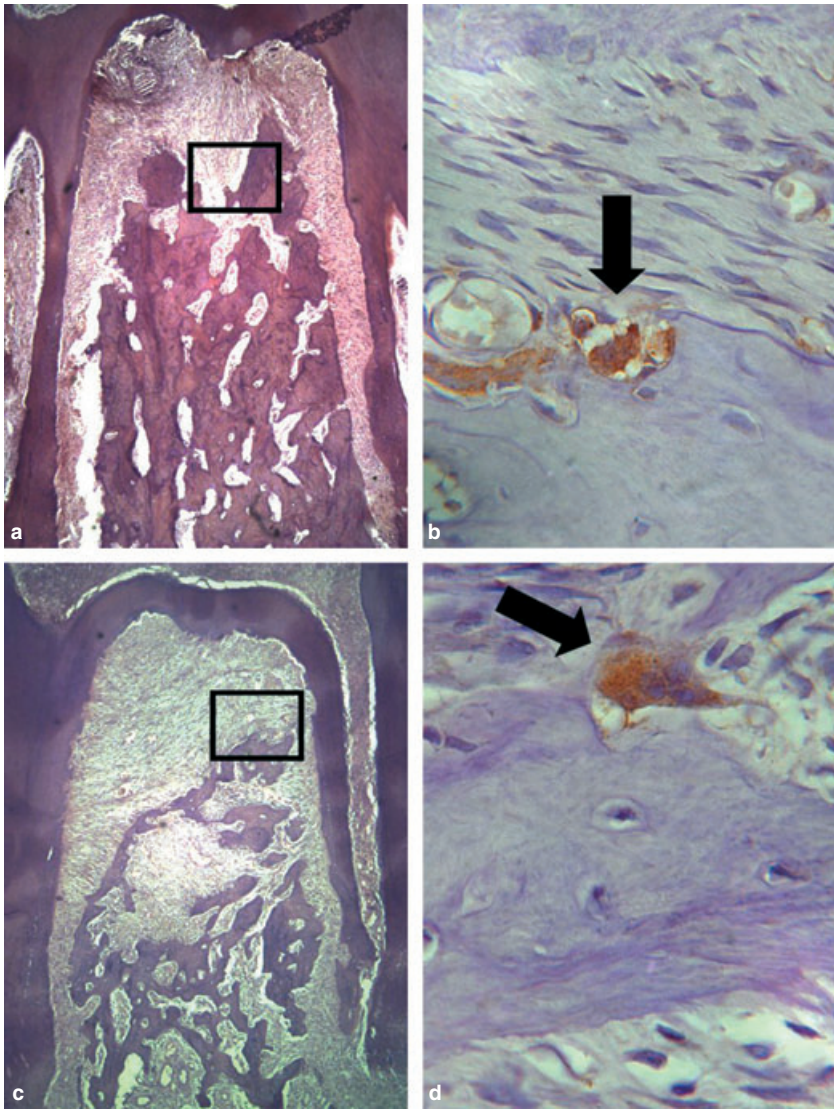

Fig. 5. Immunostaining for TRAP in the areas of bone loss (BL) in the furcation region of mandibular left first molars with induced periodontal disease in the different groups (C and N): (a) C Group, scaling and root planing (SRP) treatment for 7 days (arrow) (original magnification 100×); (b) C Group, SRP treatment for 7 days (arrow) (original magnification 100×); (c) N Group, SRP treatment for 7 days (HE, original magnification 5×) and (d) N Group, SRP treatment for 7 days (arrow) (original magnification 100×).

tal therapy to SRP (Almeida et al. 2008b, Fernandes et al. 2009). In the present study, the groups locally treated with aPDT showed lower BL levels and fewer TRAP-positive cells than did the TBO- or SRP-treated animals across all time points. Furthermore, lower BL levels and fewer TRAP-positive cells were observed in the N Group animals receiving aPDT treatment than in the C Group animals receiving SRP treatment alone. The beneficial property of aPDT as an adjunctive technique to mechanical treatment for periodontal disease probably lies in its photo-destructive effects on the various bacterial species that are present in the areas of induced periodontal disease. The photodynamic activity of the photosensitizer is based on photo-oxidant reactions that provoke both biochemical and morphological alterations in the target cells (Prates et al. 2007). When a molecule of the photosensitizer drug absorbs photons from a light source resonant to the absorbance band of the photosensitizer, it transforms from a basic state into a simple excited state. Depending on the structure of the molecule and its environment, it may lose its energy through either an electronic or physical process and return to its basic state, or else transition to a triple excited state (triplet state). In this state, the molecule may again undergo electronic decay and return its basic state through redox reactions with its environment (Type-I reaction). Otherwise its excitation energy may be transferred to an oxygen molecule, allowing the formation of singlet oxygen (Type-II reaction). These reactive oxygen species are responsible for irreversible damage to the bacterial cytoplasmic membrane (Wainwright 1998).

In the present study, the groups that were treated locally by LLLT showed lower BL levels and fewer TRAP-positive cells than groups receiving SRP treatment at all experimental periods. This fact is probably related to the ability of LLLT to promote angiogenesis and cell proliferation control inflammation and accelerate events involved in tissue repair (Houreld & Abrahamse 2007). However, the animals treated with aPDT showed lower BL than did the LLLT animals at 7 and 15 days in the C Group and at 7 days in the N Group.

Studies have reported that the use of LLLT favours cellular chemotaxis (Al-Watban & Zhang 1997, Houreld & Abrahamse 2007), providing vasodilatation and local angiogenesis that increase oxygen diffusion through the tissue, which favours the repair process because collagen secretion by fibroblasts in the extracellular space occurs only in the presence of high levels of oxygen pressure.

Stabilization of the bone tissue depends on balanced dynamics between the activity of osteoblasts (bone formation cells) and osteoclasts (bone resorption cells) (Takayanagi 2005). During bone resorption, pro-inflammatory cytokines such as interleukin 1 α, interleukin 1 β and tumour necrosis factor α (TNF-α) recruit and differentiate precursors of osteoclasts in peri-alveolar bone areas (Wang et al. 1997). This entire system is influenced by multiple factors. For example, LLLT may tip this balance

towards osteoblasts, accelerating their formation and stimulating their activity (Freitas et al. 2000), while it inhibits the production of inflammatory mediators by periodontal ligament cells (Saito et al. 1991).

In our study, RANKL immunoreactivity was most evident at 7 and 15 days, whereas OPG immunoreactivity was significant at 15 days. This finding likely occurred because RANKL plays an important role in osteoclast differentiation, acting as a survival factor for osteoclast precursors and promoting pre-resorption, whereas OPG acts as a protective agent by inhibiting clastic differentiation (Lacey et al. 1998). Analysis of these markers between the experimental groups revealed weak RANKL immunoreactivity in animals treated with aPDT at 7 days and strong OPG immunoreactivity at 15 days.

Within the limits of this study, it can be concluded that aPDT was an effective adjunct to conventional non-surgical SRP treatment that reduced BL in systemically nicotine-modified rats with induced experimental periodontitis.

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Clinical Relevance

Scientific rationale for the study: Smoking is a risk factor for periodontal disease. In these cases, SRP alone is inadequate for case resolution. aPDT has shown satisfactory results as an adjunctive periodontal treatment, although articles regarding its application in smoking patients are scarce in the literature.

Main results: aPDT was efficient as an adjunctive treatment to SRP; it reduced BL in systemically nicotine-modified rats with experimentally induced periodontitis.

Practical implications: aPDT may be used as an adjuvant to the conventional non-surgical treatment in smoking patients.