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Treatment of experimental periodontal disease by photodynamic therapy in immunosuppressed rats

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Abstract

Background and Objective: The aim of this study was to compare photodynamic therapy (PDT) as an adjunctive treatment of induced periodontitis with scaling and root planing (SRP) in dexamethasone-inhibited rats.

Material and Methods: The animals were divided into two groups: ND ($n = 90$), saline solution treatment; D ($n = 90$), dexamethasone treatment. In the ND and D Groups, periodontal disease was ligature-induced at the first mandibular molar. After 7 days, the ligature was removed and all animals received SRP and were divided according to the following treatments: SRP, saline solution; Toluidine Blue-O (TBO), phenothiazinium dye; and PDT, TBO and laser irradiation. Ten animals in each treatment were killed at 7, 15 and 30 days. The radiographic and histometric values were statistically analysed.

Results: In the ND and D Groups, radiographic analysis showed less bone loss in animals treated by PDT in all the experimental periods than SRP and TBO at 15 days ($p < 0.05$). After a histometric analysis was carried out in the ND and D groups, the animals treated by PDT showed less bone loss in all periods than SRP and TBO after 15 days ($p < 0.05$).

Conclusions: The PDT was an effective adjunctive treatment of induced periodontitis compared with SRP in dexamethasone-inhibited rats.

Key words: animal model; non-surgical periodontal therapy; periodontal disease; periodontal therapy; systemic host effect

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Periodontal disease is the result of the collapse of teeth-supporting structures by the local action of periodontopathogenic microorganisms. These microorganisms release substances that strictly injure periodontal tissues, besides inducing tissue destruction by inflammatory and immunologic responses of the host (Kamma & Slots 2003).

The placement of ligatures around teeth to initiate periodontal tissue loss has been carried out in various animal experimental models. The use of ligature in rats as an experimental model was realized in the present study because many of the same series of events occur in this animal as in the non-human primate (Graves et al. 2008). This experimental model is characterized by accumulation of plaque, flattening and displacement of the gingival crest, increased proliferation of the epithelium into underlying connective tissue and infiltration of mononuclear inflammatory cells. Like human periodontitis, alveolar bone loss in the ligature model is dependent on bacteria, and the destructive phase of ligature-induced experimental periodontitis is associated with a host response. Further, the rat ligature model is sensitive to systemic effects such as drug therapy (Graves et al. 2008).

Systemic factors such as diabetes, tobacco and stress have been found to be associated with severe and/or rapidly

Conflict of interest and source of funding statement

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progressive periodontitis (Breivik et al. 2006). Furthermore, some medications have an impact on the periodontium and its response to bacterial plaque (Seymour 2006).

In the last decades, organ transplant has become an accepted treatment for a range of acquired and congenital disorders. Corticoids are commonly used to treat many different diseases because of their anti-inflammatory effect and immunosuppressant properties. Glucocorticoids link to receptors inside the cell and cause redistribution of the lymphocytes. They also reduce T-cell proliferations, with a decrease in interleukin-2, and also down-regulate interleukin-1 and interleukin-6, thereby curtailing inflammation (Vasanthan & Dallal 2007).

Prolonged therapy with corticoids may favour osteoporosis, which is now regarded as a risk factor for periodontal disease (Seymour 2006). The systemic use of drugs such as non-steroidal anti-inflammatory substances and their possible effects on periodontal disease have been studied (Lipari et al. 1974, Safkan & Knuuttila 1984, Cavagni et al. 2005, Breivik et al. 2006). Experimental studies have demonstrated that the use of corticoid can induce gingival ulceration, upward to downward migration of the epithelium, attachment loss and transeptal fibre disruption (Lipari et al. 1974, Cavagni et al. 2005). In addition, the systemic use of high doses of glucocorticoids leads to fibroblast activity inhibition, collagen and connective tissue loss, with decreased re-epithelization and angiogenesis (Pessoa et al. 2004), a reduction of the number and activity of the osteoblasts and increased osteoclast function (Sattler et al. 2004). However, clinical studies are somewhat equivocal with respect to the effect of systemic glucocorticoids on periodontal tissues (Safkan & Knuuttila 1984, Oettinger-Barak et al. 2007).

The periodontal disease treatment is based on pathogenic microbiota reduction by scaling and root planing (SRP) (Kaldahl et al. 1993). However, the mechanical therapy used may fail to eliminate pathogenic bacteria that are placed into the soft tissue, and also in areas inaccessible to the periodontal instruments, such as furcation area and root depression (Matia et al. 1986, Adriaens et al. 1988).

Systemic disease and adverse drug reactions deal with strategic challenges to the elaboration of a conventional periodontal treatment plan, leading to the use of complementary therapies in order to compensate the intrinsic alterations related to a periodontal repair process. Because of these limitations, adjunctive methods that promote reduction or elimination of periodontal pathogens have attracted the attention of many researchers (Faveri et al. 2006, Derdilopoulou et al. 2007, Kaner et al. 2007, Needleman et al. 2007, Lee et al. 2008). On the other hand, the literature also evidences uncountable researches that demonstrate the selection and resistance of bacteria promoted by the overuse of antimicrobial drugs in the periodontal therapy (VanWinkelhoff et al. 1996).

Recently, some in vitro (Sarkar & Wilson 1993, Chan Lai 2003, Zanin et al. 2005) and in vivo studies (Kömerik et al. 2003, Sigusch et al. 2005, Almeida et al. 2007, 2008, Andersen et al. 2007, Qin et al. 2007) have showed satisfactory results with the utilization of photodynamic therapy (PDT). This therapy consists of the association of a photosensitizer with an intense light source with the objective to promote cellular death. The photodynamic activity of photosensitizers is based on photooxidative reactions that induce biochemical and morphologic alterations in target cells. When the photosensitizer drug molecule absorbs light from a resonant energy, it is transformed into a single exciting state. Depending on its molecular structure and environment, the molecule may then lose its energy by an electronic or a physical process, thus returning to the ground state, or it may undergo a transition to the triplet exciting state (electron spins unpaired). At this stage, the molecule may once again undergo electronic decay back to the ground state, it may develop a redox reaction with its environment or its excitatory energy may be transferred to molecular oxygen (also a molecular triplet state), leading to the formation of a labile singlet oxygen (type-II reaction). This oxygen reactive species is responsible for irreversible damage on the bacterial cytoplasm membrane, including protein modification, respiratory chain and nucleic acid alterations (Wainwright 1998).

The major advantages of PDT are as follows: it is a specific therapy for target cells, it exerts no collateral effect, initiating its activity only when light exposed, and it supports no resistant bacteria species selection (Maisch 2007), which is quite common with the indiscriminate use of antibiotics (VanWinkelhoff et al. 1996).

The introduction of PDT as an adjunctive periodontal treatment under immunosuppression conditions has not been reported in the literature. Considering that prolonged use of corticoids is associated with a reduction of the number and activity of the osteoblasts (Sattler et al. 2004) and increased osteclastic function (Sattler et al. 2004), the PDT may be an alternative adjunctive method for non-surgical periodontal treatment under immunosuppression conditions.

In this context, the aim of the present study was to evaluate, radiographic, histologically and histometrically, the efficacy of PDT plus conventional mechanical therapy compared with SRP alone of alveolar bone loss of experimental periodontitis induced both in normal and in systemically dexamethasone-inhibited rats.

Materials and Methods

Animals

This study was conducted on 180 adult male Wistar rats (120–140 g). The animals were kept in plastic cages with access to food and water ad libitum. Before 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. 22/06).

Experimental design

Protocol of drug administration

The animals were numbered and divided randomly into two groups of 90 rats each one: the D Group ($n = 90$) received injections of 2 mg/kg (Pessoa et al. 2004) of body weight of dexamethasone (DECADRON® 2 mg, Prodome) (Aché Pharmaceutical Laboratories SA, Campinas, SP, Brazil); the ND Group ($n = 90$), non-dexamethasone – received injections of 2 mg/kg (Pessoa et al. 2004) of body weight of saline solution. The subcutaneous injections were initiated 24 h before the experimental induction of periodontal disease and maintained every 3 days (Cavagni et al. 2005), during all the periods of killing (Fig. 1).

Fig. 1. Experimental design.

The injection was administered on the backs of the animals, next to the cephalic region, and the injections were always been scheduled during the morning period. The animals were weighed weekly with regard to dose maintenance throughout the experimental period.

Protocol of experimental periodontal disease

General anaesthesia was administered by a combination of ketamine (0.4 ml/kg) with xylazine (0.2 ml/kg) via an intra-muscular injection. One mandibular first left molar of each animal in the ND and D Groups was selected to receive the cotton ligature in the submarginal position in order to induce experimental periodontites (Nociti et al. 2000). The contralateral, mandibular first molar in the animals of each group (right side) received neither the ligature nor any treatment. After 7 days of periodontal disease experimental induction, the ligature of the mandibular first left molar was removed in all animals of the ND and D Groups. The left molars were subjected to SRP with manual #13–14 mine five curettes (Hu-Friedy Co. Inc., Chicago, IL, USA) through 10 distal–mesial traction movements in the buccal and lingual aspects. The furcation and interproximal areas were scaled with the same curettes through cervico-occlusal traction movements. SRP was performed by the same experienced operator. The 90 animals of each group (ND and D) were randomly allocated, using a computer-generated table, to the treatments SRP, Toluidine Blue-O (TBO) and PDT. For better standardization, animal 1 was the first choice, followed by 2 and 3, respectively. Thus, the animals of each group (ND and D) were randomly assigned to one of the three treatments (30 animals/treatment): SRP, the mandibular left molars were subjected to SRP and irrigation with 1 ml of saline solution; Phenotiazinium dye (TBO; Sigma Chemical Co., St. Louis, MO, USA), the mandibular left molars were subjected to SRP and irrigation with 1 ml of TBO (100 μg/ml) solution; and PDT, the mandibular left molars were subjected to SRP and irrigation with 1 ml of TBO (100 μg/ml) solution, followed by application of a low-intensity laser (LLLT) after 1 min. (Fig. 1).

PDT treatment

The low-intensity laser used in this study was Gallium–Aluminum–Arsenide (GaAlAs) (GaAlAs; Laser Bio Wave LLLT; Kondortech Equipment, São Carlos, SP, Brazil) with a wavelength of 660 nm and a spot size of 0.07 cm^2. After 1 min. of TBO application, the LLLT was used in three equidistant points at each buccal and lingual aspect of the first mandibular molar in contact with the tissue. The treatment laser was released with a power of 0.03 W at 133 s/point, a power density of 0.428 W/cm^2 and energy of 4 J/point (57.14 J/cm^2/point). The area received a total energy of 24 J. Saline solution and TBO were deposited into the periodontal pocket slowly using a syringe (1 ml) and an insulin needle (13 mm × 0.45 mm) (Becton Dickinson Ind. Ltd., Curitiba, PR, Brazil) without bevel.

Experimental periods

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

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 (6 μm) were obtained in the mesiodistal direction and dyed with haematoxylin and eosin (H&E) or Masson's Trichromic (MT).

Radiographic analysis

Rat left mandibles were removed to determine the degree of bone loss. Standardized radiographs were obtained using digital radiographic images provided by the computerized imaging system Digora (Soredex, Orion Corporation, Helsinki, Finland), which uses a sensor instead of an X-ray film. Electronic sensors were exposed at 70 kV and 8 mA with an exposure time of 0.4 s. The source-to-film distance was 50 cm. The distance between the cementum–enamel junction and the height of alveolar bone was determined for the mesial root surface of the mandibular left first molars (Holzhausen et al. 2002). Bone loss was measured in millimetres for each radiograph in the mask mode three times by the same examiner.

Histological and histometric analysis

Sections dyed by H&E were analysed by light microscopy to establish the bone loss and characteristics of periodontal ligament in the furcation region of first molars. Collagen fibres were analysed in sections dyed by MT.

The area of bone loss in the furcation region was histometrically determined 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 the last sections where the furcation region was evident, five equidistant sections of each specimen block were selected and captured by a digital camera connected to a light microscope. The mean values were averaged and compared statistically. One mask-trained examiner selected the sections for histometric and histological analyses. Another mask-calibrated examiner conducted the histometric analysis. The bone loss at each specimen section was measured three times by the same examiner, on different days, in order to reduce the variation in the data (Almeida et al. 2008).

Intra-examiner reproducibility

Before the radiographic and histometric analyses were performed, the examiner was trained by double measurements of 20 specimens, with a 1-week interval. Paired *t*-test statistics were run and no differences were observed in the mean values for comparison (p-value = 0.51). Additionally,

Pearson's correlation coefficient was obtained between the two measurements and revealed a very high correlation (0.99, $p = 0.000$).

Statistical analysis

The hypothesis that there were no differences in bone loss rate in the furcation region between treatment groups was tested by Bioestat 3.0 software (Bioestat, Windows 1995; Sonopress Brazilian Industry, Manaus, AM, Brazil).

After the normality of radiographic and histometric data was analysed by the Shapiro–Wilk test, intra- and inter-group analyses were carried out using a two-way analysis of variance (ANOVA; $p < 0.05$). When ANOVA detected a statistically significant difference, multiple comparisons were performed using Tukey's test ($p < 0.05$).

Table 1. Mean and standard deviation ($M \pm$ SD) of body weight (g) in each group, treatment and period

Treatments	Periods			
	Initial periods	7 days	15 days	30 days
Non-dexamethasone group (ND)				
SRP	245.85 ± 4.18*	262.28 ± 2.05*,&,†	282.85 ± 1.46*,&,†	306.00 ± 0.81*,&,†
TBO	247.42 ± 5.88*	262 ± 1.41*,&,†	284.28 ± 1.11*,&,†	309.00 ± 1.15*,&,†
PDT	247.28 ± 5.31*	261.42 ± 1.61*,&,†	284.14 ± 2.03*,&,†	307.85 ± 1.95*,&,†
N	90	30	30	30
Dexamethasone (D)				
SRP	246.85 ± 5.6*	218 ± 1.29*,&,†	198.28 ± 1.49*,&,†	177.14 ± 1.34*,&,†
TBO	248.85 ± 6.64*	219.28 ± 1.11*,&,†	199.28 ± 1.11*,&,†	178.28 ± 1.49*,&,†
PDT	246.57 ± 4.92*	219.14 ± 1.21*,&,†	199.14 ± 2.19*,&,†	178.28 ± 1.11*,&,†
N	90	30	30	30

*Significant difference among the experimental periods (initial, 7, 15 and 30 days) in the same group and treatment ($p < 0.05$). ANOVA and Tukey's tests.
&Significant difference between groups in the same treatment and period ($p < 0.05$). ANOVA and Tukey's tests.
†Significant difference between groups and treatments in the same period ($p < 0.05$). ANOVA and Tukey's tests.
SRP, scaling and root planning; PDT, photodynamic therapy; TBO, Toluidine Blue-O.

Results

Clinical analysis

All the ND Group animals, regardless of the treatment, showed no clinical differences in general health, and weight gain within the predicted range for healthy rats (Table 1).

The D Group showed progressive weight loss, at a significant level when compared with the ND animals (Table 1), which show trends of immunossuppression and systemic alterations.

Radiographic analysis

In both groups (ND and D), radiographic examination showed that there was significantly less bone loss in the animals treated by PDT in all experimental periods than SRP and TBO after 15 days (Fig. 2). Inter-group radiographic analysis (ND and D Groups) demonstrated that, in the ND Group, treated with SRP, there was greater bone loss compared with the D Group, treated with PDT at 7 and 30 days (Fig. 2).

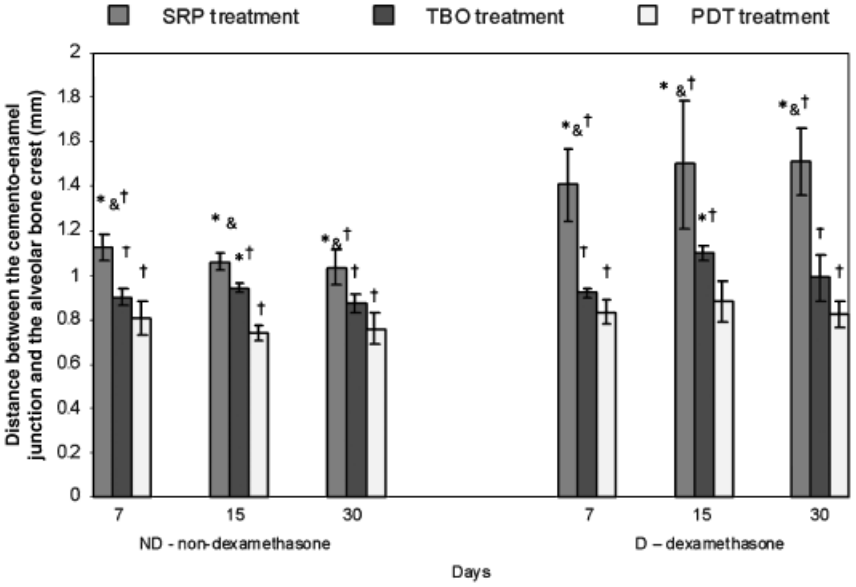

Fig. 2. Mean and standard deviation ($M \pm$ SD) of the radiographic data of the distance between the cemento-enamel junction and the alveolar bone crest (mm) on the mesial surface of the mandibular first molars in each group, treatment and period.

Histological analysis

SRP treatment

At 7, 15 and 30 days, most specimens in the ND Group that received the SRP treatment showed connective tissue with a high number of neutrophils in degeneration, bone tissue with thin bone trabeculae and resorption areas. At 7, 15 and 30 days, most specimens in the D Group that received SRP treatment showed disorganized connective tissue with a small number of fibroblasts. There were bone resorption areas with thin bone trabeculae and an intense inflammatory infiltrate (Fig. 3). The cementum surface in most specimens showed resorption areas.

TBO treatment

At 7, 15 and 30 days, most specimens in the ND and D (Fig. 4) Groups, which received the TBO treatment, showed organized bone and connective tissues, with a moderate number of fibroblasts. The periodontal ligament and cementum areas showed normal characteristics.

PDT treatment

At 7, 15 and 30 days, in most specimens in the ND and D (Fig. 5) Groups that received the PDT treatment, the periodontal ligament was found to be intact, organized with parallel collagen fibres and lack of an inflammatory infiltrate. The bone tissue showed organization with thick bone trabeculae and no signs of resorption. The cementum surface did not show resorption areas.

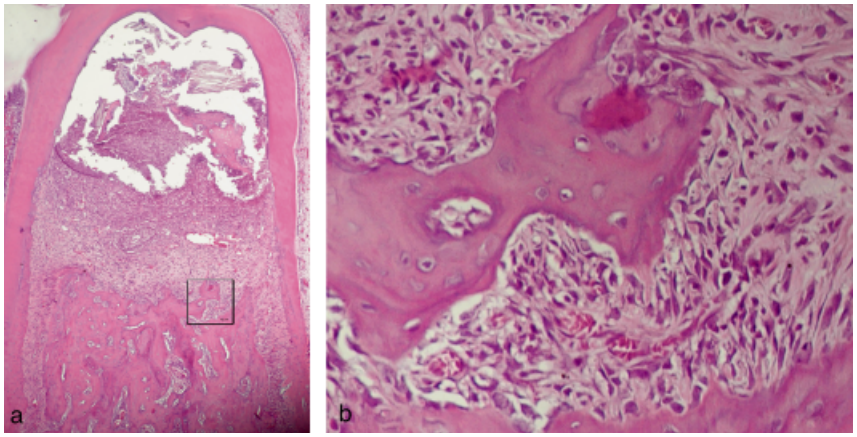

Fig. 3. Photomicrograph illustrating the periodontal ligament area and bone loss in the furcation region of the mandibular first molar in the D Group, treatment SRP 15 days – apical third into the furcation region – Areas of bone resorption with thin bone trabeculae (H&E; original magnification: a, ×12.5; b, ×40). H&E, hematoxylin and eosin SRP, scaling and root planing.

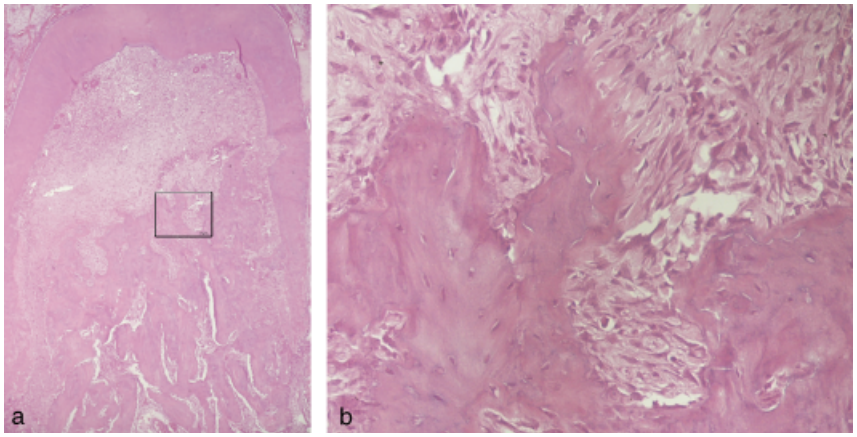

Fig. 4. (a) Photomicrograph illustrating the periodontal ligament area and bone loss in the furcation region of the mandibular first molar in the D Group, treatment TBO 15 days – apical third into the furcation region – Areas of bone resorption with thin bone trabeculae and disorganized connective tissue (H&E; original magnification: a, ×12.5; b, ×40). H&E, hematoxylin and eosin; TBO, Toluidine Blue-O.

Histometric analysis

The histometric data are shown in Table 2. In the ND Group, statistical analysis revealed greater bone loss in the SRP treatment (1.12 ± 0.13, 0.90 ± 0.27, $1.00 \pm 0.16\,mm^2$) when compared with the TBO treatment at 7 ($0.67 \pm 0.14\,mm^2$) days. In comparison with the PDT treatment (0.54 ± 0.06, 0.56 ± 0.13, $0.53 \pm 0.05\,mm^2$), there was greater bone loss in the SRP ($p < 0.05$) treatment in all experimental periods (Fig. 6a). The furcation areas treated with PDT showed a significant reduction of bone loss ($p < 0.05$), when compared with the TBO treatment ($0.95 \pm 0.21\,mm^2$) at 15 days (Fig. 6b and c).

In the D Group, statistical analysis of histometric data showed greater bone loss in the SRP treatment at 7, 15 and 30 days (Fig. 6d) (1.65 ± 0.15, 1.71 ± 0.11, $1.5 \pm 0.25\,mm^2$) when compared with the TBO treatment (0.74 ± 0.12, 1.06 ± 0.10, $0.75 \pm 0.31\,mm^2$) (Fig. 6e) and the PDT treatment (0.60 ± 0.10, 0.59 ± 0.13, $0.57 \pm 0.10\,mm^2$) (Fig. 6f). Histometric analysis demonstrated more significant bone loss in the TBO treatment compared with the PDT treatment at 15 days ($p < 0.05$).

Histometrically, inter-group analysis (ND and D Groups) in the ND Group, treated with SRP (1.12 ± 0.13, $1.00 \pm 0.16\,mm^2$), showed greater bone loss compared with the D Group, treated with PDT, at 7 ($0.60 \pm 0.10\,mm^2$) and 30 days ($0.57 \pm 0.10\,mm^2$).

Discussion

The aim of this study was to compare the influence of PDT as an adjunctive treatment on induced periodontitis in rats inhibited with dexamethasone. To investigate the in vivo effect of PDT on periodontal disease, we established the periodontal disease model in rats caused by natural infection, simulating clinical situation conditions as closely as possible. In the present study, the induced periodontal disease was characterized by clinical signs of gingival inflammation, as oedema, redness and attachment loss of tooth gingival tissue. In dexamethasone-inhibited animals (D), the clinical signs of gingival inflammation were more exacerbated, characterized as: greater bone loss in the furcation region, connective tissue disorganization, discrete fibroblasts and an intense inflammatory infiltrate in all experimental periods, when compared with non-inhibited rats (ND).

The animals treated with this drug showed lethargy, haematoma and alopecia at the time they were killed. Furthermore, there was a significant weight reduction in the present study; this probably occurred because the drug decreased the gastrointestinal nutrient absorption (Metzger et al. 2002). These alterations were already shown by other authors (Labelle & Schaffer 1966, Lipari et al. 1974), indicating a trend towards immunossuppression and systemic alterations.

The results of the present study have also demonstrated that group D animals showed greater bone loss in the furcation area, as well as more disorganized connective tissue when compared with group ND animals. These alterations were described in other studies that have also evaluated the effects of corticoid on periodontal tissues (Lipari et al. 1974, Cavagni et al. 2005).

On the other hand, a clinical study has not demonstrated the influence of corticosteroid therapy on the clinical parameters of periodontal disease in patients suffering from neurological diseases (Safkan & Knuuttila 1984). The use of high doses of corticoid leads to a reduction in the number and activity of the osteoblasts and an increase in osteoclast functions (Sattler et al. 2004). It also reduces gastrointestinal calcium

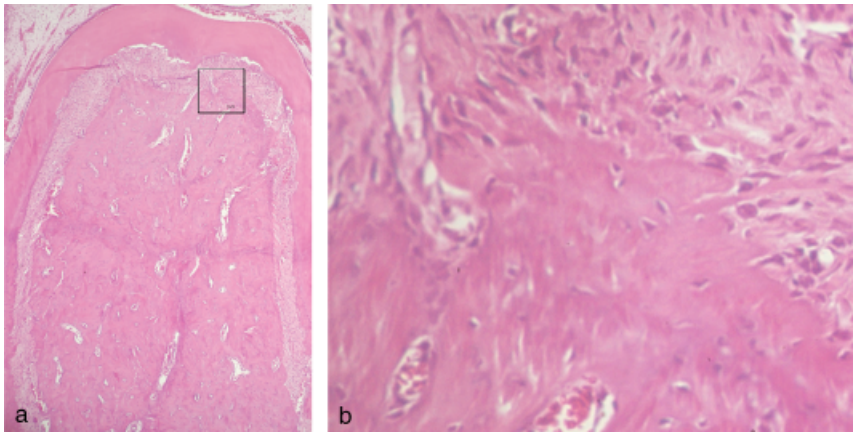

Fig. 5. (a) Photomicrograph illustrating the periodontal ligament area and bone loss in the furcation region of the mandibular first molar in the D Group, treatment PDT 15 days – coronary third into the furcation region – thick bone trabeculae without signs of resorption (H&E; original magnification: a, ×12.5; b, ×40). PDT, photodynamic therapy; H&E, hematoxylin and eosin.

Table 2. Mean and standard deviation ($M \pm SD$) of histometric data of bone loss area (mm^2) in the furcation region of the mandibular first molars in each group, treatment and period

Treatments	Periods		
	7 days	15 days	30 days
Non-dexamethasone group (ND)			
SRP	$1.12 \pm 0.13^{*,\&,\dagger}$	$0.90 \pm 0.27^{*,\&}$	$1.00 \pm 0.16^{*,\&,\dagger}$
TBO	$0.67 \pm 0.14^{\dagger}$	$0.95 \pm 0.21^{*,\dagger}$	$0.74 \pm 0.26^{\dagger}$
PDT	$0.54 \pm 0.06^{\dagger}$	$0.56 \pm 0.13^{\dagger}$	$0.53 \pm 0.05^{\dagger}$
N	30	30	30
Dexamethasone (D)			
SRP	$1.65 \pm 0.15^{*,\&,\dagger}$	$1.71 \pm 0.11^{*,\&,\dagger}$	$1.50 \pm 0.25^{*,\&,\dagger}$
TBO	$0.74 \pm 0.12^{\dagger}$	$1.06 \pm 0.10^{*,\dagger}$	0.75 ± 0.31
PDT	$0.60 \pm 0.10^{\dagger}$	0.59 ± 0.13	$0.57 \pm 0.10^{\dagger}$
N	30	30	30

*Significant difference with PDT treatment in the same period and group ($p<0.05$). ANOVA and Tukey's tests.
&Significant difference between groups in the same treatment and period ($p<0.05$). ANOVA and Tukey's tests.
†Significant difference between groups and treatments in the same period ($p<0.05$). ANOVA and Tukey's tests.
SRP, scaling and root planning; PDT, photodynamic therapy; TBO, Toluidine Blue-O.

absorption, which, in turn, results in lower calcium blood levels, and triggers PTH secretion that leads to systemic bone resorption (Suzuki et al. 1983).

However, another clinical study on liver transplantation patients has demonstrated that the doses of glucocorticoids had no effect on alveolar bone loss, although there was an inverse relationship with the duration of treatment (Oettinger-Barak et al. 2007).

Corticoids can lead to a delay in the healing process (Pessoa et al. 2004, Tenius et al. 2007) by decreased angiogenesis and capillary proliferation, which reduces blood flow (Leibovich & Ross 1975, Pierce & Lindskog 1989, Fässler et al. 1996, Durmus et al. 2003). They also interfere in phagocytosis and antigen digestion, inhibiting macrophage migrations and stabilizing lysosome, preventing the release of proteolytic enzymes. In addition, they modify fibroblast functions, delaying their migration, damaging type-I and type-II pro-collagen synthesis by modifying mRNA and mitotic activity (Salmela 1981, Autio et al. 1994).

The number of researches related to the PDT antimicrobial effects has increased. This therapy consists of an association of a photosensitizing agent with a light source, being initially used for oncology treatment (Tomaselli et al. 2001). Studies have shown favourable results using PDT principles against microorganisms involved in periodontitis (Yilmaz et al. 2002, Kömerik et al. 2003, Sigusch et al. 2005, Qin et al. 2007) and periimplantitis (Shibli et al. 2003).

In the analysis of the histometric evaluation results, the ND and D groups, which received PDT treatment, showed less significant bone loss when compared with the animals treated only with SRP in all the experimental periods. The PDT treatment has also shown effectiveness in bone loss reduction in both animal groups when compared with TBO-treated animals, in a 15-day period.

In both groups (ND and D), radiographic examination showed that there was significantly less bone in the animals treated by PDT in all experimental periods than SRP and TBO after 15 days, confirming the histometric results.

The results obtained in the present study are in accordance with the literature studies that showed PDT effectiveness in periodontal treatment both in animals (Kömerik et al. 2003, Sigusch et al. 2005, Almeida et al. 2007, Qin et al. 2007) and in humans (Andersen et al. 2007, Braun et al. 2008). Results of recent studies in humans are controversial with respect to the beneficial effects of PDT as an adjunctive therapy to non-surgery periodontal treatment (Braun et al. 2008, Christodoulides et al. 2008). Braun et al. (2008) showed that, in patients with chronic periodontitis, the clinical outcomes of conventional subgingival debridement can be improved by adjunctive PDT treatment. Another human study (Christodoulides et al. 2008) showed that the PDT failed to result in an additional improvement in probing depth, clinical attachment level and microbiologic changes, but it resulted in a significantly higher reduction in bleeding scores. This discrepancy in the results may be explained by the different methodologies used in the studies such as: drug concentrate ion, period of maintenance of the drug within the tissue, time for biological response, pH of the environment (tissue/tooth interface), presence of exudate, gingival fluid, mode and frequency of drug application (irrigation, slow-release gel) (Wilson 2004).

The beneficial effect of PDT adjunctive to conventional mechanical treatment of periodontal disease, both in dexamethasone-inhibited and in non-inhibited rats, was probably caused by the photo-destructive effects on the different periodontal pathogenic species,

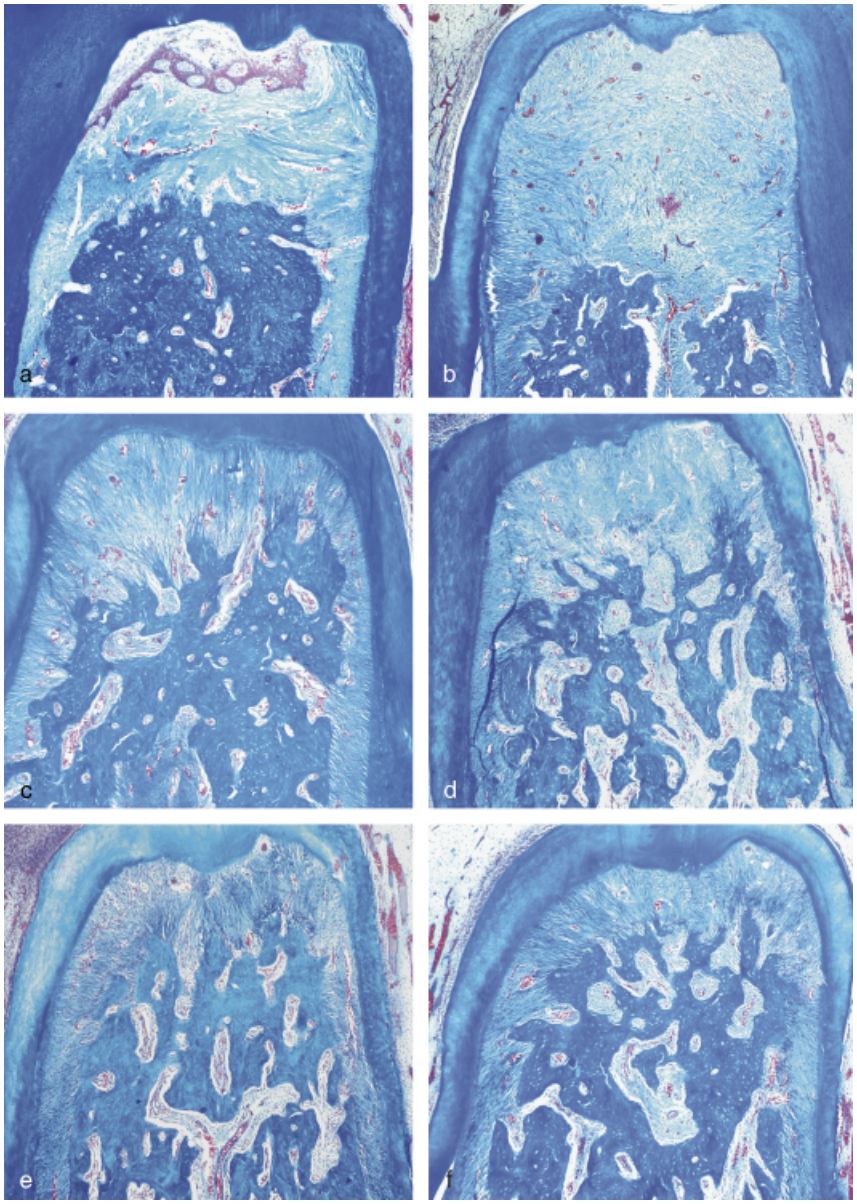

Fig. 6. Photomicrograph illustrating bone tissue in the furcation region of the mandibular first molar in the different Groups (ND and D) and treatments: (a) ND Group, treatment SRP 15 days; (b) D Group, treatment SRP 15 days; (c) ND Group, treatment TBO 15 days; (d) D Group, treatment TBO 15 days; (e) ND Group, treatment PDT 15 days; (f) D Group, treatment PDT 15 days (original magnification ×12.5; Masson's Trichromic). SRP, scaling and root planing; PDT, photodynamic therapy; TBO, Toluidine Blue-O.

mediated by a type-I reaction (initiated by superoxide, anionic hydroxyl or free radicals) or by a type-II reaction (initiated by a singlet oxygen) (Ochsner 1997, Wainwright 1998). These oxygen-reactive species are responsible for irreversible damage on the bacterial cytoplasmatic membrane, including protein modification, respiratory chain and nucleic acid alterations (Wainwright 1998).

The isolated use of TBO (100 μg) in group ND rats also promoted less bone loss in the furcation area when compared with SRP treatment, at 7 days, and in group D, in all experimental periods, different from the results found by Kömerik et al. (2003), when using TBO isolated with 0.01, 0.1 and 1 mg/ml (10, 100 and 1000 μg/ml), where the morphometric analysis showed no significant difference in bone loss level. However, in the microbiologic analysis, a reduction of *Porphyromonas gingivalis* was observed at a concentration of 1 mg/ml (1000 μg/ml) after 4 and 8 min. of photosensitizing drug use. In the present study, the TBO treatment was carried out after the conventional mechanical therapy, which was not done in that study (Kömerik et al. 2003).

The TBO used as a photosensitizing drug in PDT has rarely been evaluated in vivo in periodontitis treatment (Kömerik et al. 2003, Qin et al. 2007). Several studies have demonstrated that gram-positive bacteria are susceptible to photodynamic inactivation, but gram-negative bacteria (Malik et al. 1990, Usacheva et al. 2001) are significantly resistant to many photosensitizers used in PDT. In the present study, TBO was used as a photosensitizer because it interacts with LPS, present in the cell membrane of gram-negative bacteria, more significantly than methylene blue, although the absorption band of the methylene blue is more resonant with the emitted radiation of the laser used in the present study (660 nm) (Usacheva et al. 2003, Wilson 2004).

There are reports in the literature on the bactericide activity of TBO in light absence (Usacheva et al. 2001). The results of this study demonstrated that the furcation treated with PDT at 7 and 30 days showed less bone loss than the TBO treatment in both groups (ND and D), but no statistically significant differences were found. This can be explained by an increased penetration of the drug into periodontal tissues, through the epithelium and connective tissue, after a predicted removal of the sulcular epithelium following SRP procedures. These results could have occurred due to its interaction with LPS present in the cell membrane of gram-negative bacteria (Usacheva et al. 2003), along with biofilm disorganization caused by SRP.

On the other hand, the TBO at 15 days showed a relative increase in bone loss, but not a statistically significant difference between 7 and 30 days. This result is probably because the TBO concentration was not so efficient on biofilm reduction disorganized by SRP at 15 days, besides the inflammatory response and bone resorption may be more at 15 days. The bacterial endotoxins, cytotoxins and other pathogenic substances are released from the biofilm and diffused into the adjacent soft tissues, where they elicit an inflammatory response, resulting in tissue disruption and degradation to periodontal tissue (Kornman et al. 1997, Page et al. 1997). It was also evident in the present study that group D animals, which

received PDT treatment, showed less bone loss compared with group ND animals, which received only SRP treatment, at 7- and 30-day periods. The beneficial effects of PDT in the periodontal disease could be explained not only by the local anti-microbial activity, described previously, but also by the increased angiogenesis that supplies more oxygenation to the area (Benstead & Moore 1989).

Another possible explanation for the results could be the biomodulation action of the low-intensity laser isolated. Studies have reported that the use of this source accelerates bone repair, exerts an anti-inflammatory effect, favours the cellular chemotaxis (Houreld & Abrahamse 2007) and promotes local vasodilatation and angiogenesis (Pessoa et al. 2004). Thus, it could provide increased oxygen diffusion through the tissue (Surinchak et al 1983, Al-Watban & Zhang 1997), which favours the repair process because the collagen secretion by fibroblasts in the extracellular space occurs only in the presence of high rates of oxygen pressure (Reenstra et al. 2001).

The systemic corticoid use has been indicated in low and high doses for many treatments such as mucocutaneous and respiratory disease, tendinitis, bursitis, arthritis and cysts in general (American Academic of Periodontology 2003); it is also used in all levels of immunotherapy, based on the need and regimen prescribed by the individual practitioner (Vasanthan & Dallal 2007). One of the side effects of this drug is the increased infection risk because of the inhibition effects of cellular immunity, which could cause more severe periodontal damages (Lipari et al. 1974, Cavagni et al. 2005), as demonstrated in this study.

Considering these facts, the application of alternative or adjunctive periodontal therapeutics to SRP conventional treatment, such as the use of systemic antibiotics, has been indicated, in spite of the disadvantage of the development of bacterial drug resistance (VanWinkelhoff et al. 1996). In this context, the use of local bactericide agents would be an alternative adjunctive technique for periodontitis treatment. The concept of PDT is plausible and could bring forth new therapy concepts for periodontal disease, principally in immunosuppressed patients, who present challenges for treatment strategies (Meisel & Kocher 2005).

The periodontal treatment has a local limitation, such as effectiveness of mechanical instrumentation in areas that are difficult to access, e.g., the furcation region. This limitation does not apply to the PDT as it is based on a photosensitizer associated with light emission, such as laser irradiation. Another advantage of the PDT is that it has no side effects, initiating its activity only when exposed to a light source and preventing resistant bacteria species selection (Maisch 2007).

Within the limits of this study, it can be concluded that PDT was effective as an SRP adjunctive treatment for bone loss reduction in induced experimental periodontitis when compared with non-surgical conventional treatment, both in normal rats and in systemic dexamethasone-inhibited animals. The TBO use isolated is also effective as an adjunctive periodontal treatment for bone loss reduction in both normal rats and in dexamethasone-inhibited rats. These encouraging results suggest that further experimental and clinical studies must be carried out to determine effective parameters of irradiation and drug concentration for clinical applicability in the periodontal treatment of immunosuppressed patients.

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Clinical Relevance

Scientific rationale for the study: Prolonged therapy with corticoids may be a risk factor for periodontal disease. In such cases, only the SRP can fail in periodontal treatment. The PDT has showed satisfactory results with an adjunctive periodontal treatment, but its application in immunosuppressed patients has not been reported in the literature.
Principal findings: The PDT was effective as an SRP adjunctive treatment for bone loss reduction in induced experimental periodontitis both in normal rats and in systemic dexamethasone-inhibited animals.
Practical implications: The PDT might provide further treatment possibilities to non-surgical conventional treatment in immunosuppressed patients.