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ORIGINAL ARTICLE

Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: microbiological profile

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Abstract The aim of this trial was to investigate changes occurring in the subgingival microbiological composition of subjects with aggressive periodontitis, treated with antimicrobial photodynamic therapy (aPDT), in a single episode, or scaling and root planing (SRP), in a split-mouth design on −7, 0, and +90 days. Ten patients were randomly assigned to either aPDT using a laser source in conjunction with a photosensitizer or SRP with hand instruments. Subgingival plaque samples were collected and the counts of 40 subgingival species were determined using checkerboard DNA-DNA hybridization. The data were analyzed using the method of generalized estimating equations (GEE) to test the associations between treatments, evaluated parameters, and experimental times (α=.05). The results indicated that aPDT and SRP affects different bacterial species, with aPDT being effective in reducing numbers of *A. actinomycetemcomitans* than SRP. On the other hand, SRP was more efficient than aPDT in reducing the presence of periodontal pathogens of the *Red Complex*. Additionally, a recolonization in the sites treated by aPDT was observed, especially for *T. forsythia* and *P. gingivalis.* Under our experimental conditions, this trial demonstrates that aPDT and SRP affected different groups of bacteria, suggesting that their association may be beneficial for the non-surgical treatment of aggressive periodontitis.

Keywords Periodontal diseases/therapy · Photochemotherapy · Photosensitizing agents

A. B. Novaes Jr · H. O. Schwartz-Filho · R. R. de Oliveira
Department of Bucco-Maxillo-Facial Surgery and Traumatology and Periodontology, School of Dentistry of Ribeirão Preto, University of São Paulo,
Ribeirão Preto, SP, Brazil

M. Feres · S. Sato
Department of Periodontology, Dental Research Division, Department of Periodontology, Guarulhos University,
Guarulhos, SP, Brazil

L. C. Figueiredo
Department of Dental Material and Prostheses,
School of Dentistry of Ribeirão Preto, University of São Paulo,
Ribeirão Preto, SP, Brazil

A. B. Novaes Jr (✉)
Faculdade de Odontologia de Ribeirão Preto,
Universidade de São Paulo,
Av. do Café s/n, 14040–904,
Ribeirão Preto, SP, Brazil
e-mail: novaesjr@forp.usp.br

Introduction

Generalized aggressive periodontitis is a rapidly progressing disease that affects otherwise healthy individuals [1, 2] and is characterized by a pronounced episodic and rapid destruction of periodontal tissues, which may result in early tooth loss. Generalized aggressive periodontitis subjects display an inadequate host response to periodontopathogenic bacteria due to an increased expression of a wide variety of immunological and genetic risk factors [3, 4]. The complex interplay between the host risk factors and the periodontal microbiota induces a high susceptibility to periodontal disease.

Besides non-pathogenic species of bacteria and/or extracellular macromolecules, several periodontopathogenic pathogens are associated with periodontal disease, e.g., the aerobic Gram-negative species *Porphyromonas gingivalis*, *Tannerella forsithya, Treponema denticola*, the microaerophilic, Gram-negative *Aggregatibacter actinomycetemcomitans*; and the Gram-negative, facultative aerobic species *Eikenella corrodens* [5–10]. Mechanical removal of the

biofilm and additional use of antibacterial disinfectants or antimicrobial drugs is the conventional approach to treat aggressive periodontitis. More recently, however, there have been reports of bacterial strains becoming resistant due to the frequent use of antimicrobial drugs [11–13]. As a consequence, there is a growing interest in the development of alternative antimicrobial treatment concepts.

Antimicrobial photodynamic therapy (aPDT) could be an alternative to the conventional therapeutic methods. The principle of aPDT is that a photoactivable compound (=the photosensitizer), binds the target cell and becomes activated by light of suitable wavelength. During the activation process, free radicals are formed, which have a toxic effect on the cell. The term photodynamic therapy was established as early as 1900 by Raab [14], who realized that the interaction between acridine, a dye, and visible light in the presence of oxygen killed paramecia.

The bactericidal efficacy of aPDT against periodontal pathogens has been demonstrated in a study using a rat model. The results showed that Toluidine Blue-mediated lethal photosensitization of *P. gingivalis* is possible in vivo, which will result in decreased bone loss [15]. Sigush et al. [16] demonstrated that aPDT using a photosensitizer and a 662-nm laser light source resulted in reduced periodontal signs of redness and reduced bleeding on probing in dogs. The procedure also appeared to significantly suppress *P. gingivalis*.

A controlled clinical trial [17] was designed to compare the effect of aPDT alone and SRP in subjects with aggressive periodontitis. After 3 months of therapy, both treatments yielded comparable outcomes in terms of reducing bleeding on probing, reducing probing pocket depths and gaining clinical attachment levels, suggesting a potential clinical effect of aPDT.

Therefore, the aim of this clinical trial was to investigate the microbiological changes in patients with aggressive periodontitis, after treatment either with antimicrobial photodynamic therapy or scaling and root planning.

Materials and methods

Patient population

The research protocol was reviewed and approved by the institution's Human Research Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo on December 7, 2005 (protocol 05.1.1038.53.9). Ten patients (eight women and two men) aged between 18 and 35 years (mean age 31 years), with clinical diagnosis of generalized aggressive periodontitis were selected. The selected patients had a minimum of 20 teeth (mean of 26 teeth) with at least one tooth in each posterior sextant, and at least one posterior sextant with a minimum of three natural teeth. The subjects also presented with ≥5 mm of attachment loss around at least seven teeth involved, excluding first molars and central incisors [18, 19]. Criteria for exclusion from the study were: (1) periodontal treatment within the last 6 months; (2) systemic diseases that could influence the outcome of therapy; (3) pregnancy; (4) smoking; and (5) ingestion of systemic antibiotics within the last 6 months. All participants signed the informed consent form.

Study design

The study was performed using the split-mouth design. Ten pairs of contra-lateral maxillary single-rooted teeth were included (ten lateral incisors, eight canines, and two premolars). Each tooth of each contra-lateral pair exhibited probing depth of ≥5 mm on at least two sites. In each contra-lateral pair, one tooth was randomly treated, trough a coin to ss, with subgingival scaling and root planing (SRP) using hand instruments whereas the other tooth was treated with aPDT. All patients were treated by the same experienced operator.

Oral hygiene program

Fourteen days prior to treatment, all patients were enrolled in a hygiene program and received oral hygiene instructions, corresponding to their individual need. Supragingival professional tooth cleaning was performed 7 days prior to baseline.

Non-surgical treatments

The mechanical subgingival instrumentation (SRP group) was performed using hand instruments (Gracey curettes, No. 3/4, 5/6, 7/8, 11/12 and 13/14, Hu-Friedy, Chicago, IL, USA). For the aPDT group, a dye/laser system was applied. The system consisted of a hand-held battery-operated diode laser (HELBO® minilaser 2075F dent, HELBO Photodynamic Systems GmbH & Co KG, Grieskirchen, Austria). The laser wavelength was 660 nm with a power of 0.06 W/cm^2 for 10 s and fluency of 212.23 J/cm^2. The dye was a commercial solution based on a phenothiazine chloride (HELBO Blue Photosensitizer®, HELBO Photodynamic Systems). The photosensitizer was applied placing the applicator at the bottom of the periodontal pocket and was continuously deposited in a coronal direction for 1 min followed by copious irrigation with distilled water to remove the excess. Afterwards, the diode laser unit was used with an 8.5-cm-long flexible fiber optic tip curved at an angle of 60° with a spot size 0.06 cm in diameter. The treatment was done in six sites per tooth. The amount of time needed in the SRP group was, on average, 8 min,

while average time spend for the aPDT group was 3 min. All the tooth surfaces were treated either by SRP or aPDT but only the proximal surfaces (mesial and distal) were considered for the microbiologic analysis (total=40 sites).

Collection of plaque samples

Subgingival plaque samples were taken at −7, 0, and at 90 days post-therapy from the proximal pockets (mesial and distal) of the selected teeth. Counts of 40 subgingival species were determined in each plaque sample using the checkerboard DNA-DNA hybridization technique [20, 21]. In brief, after removal of supragingival plaque, subgingival biofilm samples were taken using individual sterile curettes from the proximal surface of each selected tooth and placed into separate microtubes containing 0.15 ml Tris EDTA buffer (10 mm Tris-HCl, 1 mm ethylenediaminetetraacetic acid, pH 7.6). Immediately after, 0.10 ml of 0.5 m NaOH was added to each sample. The samples were boiled for 10 min and neutralized using 0.8 ml of 5 M ammonium acetate. The released DNA was placed into the extended slots of a Minislot 30 apparatus (Immunetics, Cambridge, MA, USA) concentrated onto a 15 × 15-cm positively charged nylon membrane (Boehringer-Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking at 120°C for 20 min. The membrane was placed in a Miniblotter 45 (Immunetics, Cambridge, MA, USA) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 40 subgingival species were hybridized in individual lanes of the Miniblotter. Following hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection and converted to absolute counts by comparison with the regression line determined from data from the standards on the same membrane. Failure to detect a signal was recorded as zero. A total of 40 subgingival samples were evaluated. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Statistical analysis

Statistical analysis was performed using the method of generalized estimating equations (GEE). GEE was employed in place of traditional ANOVA due to the lack of independence among the sites within each patient's mouth [22, 23]. The GEE method is also proper for the analysis of longitudinal data. Through descriptive analysis, mean and standard deviations for each variable depending on factors were presented. This analysis was complemented by graphs containing the confidence intervals for the levels of variation of the factor "time". All analyses were performed at a 0.05 level of significance. A software package was used for all calculations (SPSS version 16.0.0, SPSS, Chicago, IL, USA).

Results

The postoperative healing was uneventful in all cases. No complications such as abscesses or infections were observed throughout the study.

All 40 bacterial species evaluated in this trial were detected in different levels before the treatment at day −7. An increase in the mean counts of the majority of the bacterial species in the period between −7 to baseline was observed. As shown in Fig. 1, aPDT reduced the presence of *A. actinomycetemcomitans* significantly more than SRP (p=0.00). At baseline, the mean counts of *A. actinomycetemcomitans* were similar for both groups, 0.33±0.30 for the SRP group and 0.27±0.25 for the aPDT group. After 90 days, a significant reduction in mean counts of *A. actinomycetemcomitans* was observed for the aPDT group (0.02±0.01), while the mean counts for the SRP group remained high (0.26±0.25). On the other hand, SRP was more efficient than aPDT in reducing the presence of periodontal pathogens of the *Red Complex*. These results indicate that aPDT and SRP affect different bacterial species. Additionally, a recolonization in the sites treated by aPDT was observed, especially for *T. forsythia* and *P. gingivalis*. The period from baseline to 90 days was characterized by an increase in mean counts of several bacterial species analyzed, notably, the *Actinomyces* species and the members of the "purple" complex.

Discussion

The present study was designed to investigate microbiological changes in the subgingival microbiota of patients with aggressive periodontitis treated with either aPDT or SRP. It is important to emphasize that this study is the third part of a sequence of studies [17, 24] developed to evaluate the effects of aPDT in the treatment of aggressive periodontitis. Antimicrobial drugs were not used in this trial, so that its adjunctive effect would not interfere with the outcome. Currently, data in the literature suggest that systemically administered antimicrobials can enhance the effects of mechanical therapy in the treatment of aggressive periodontitis as assessed by clinical parameters [25]. However, due to the relative absence of randomized controlled clinical trials including microbiological data, no

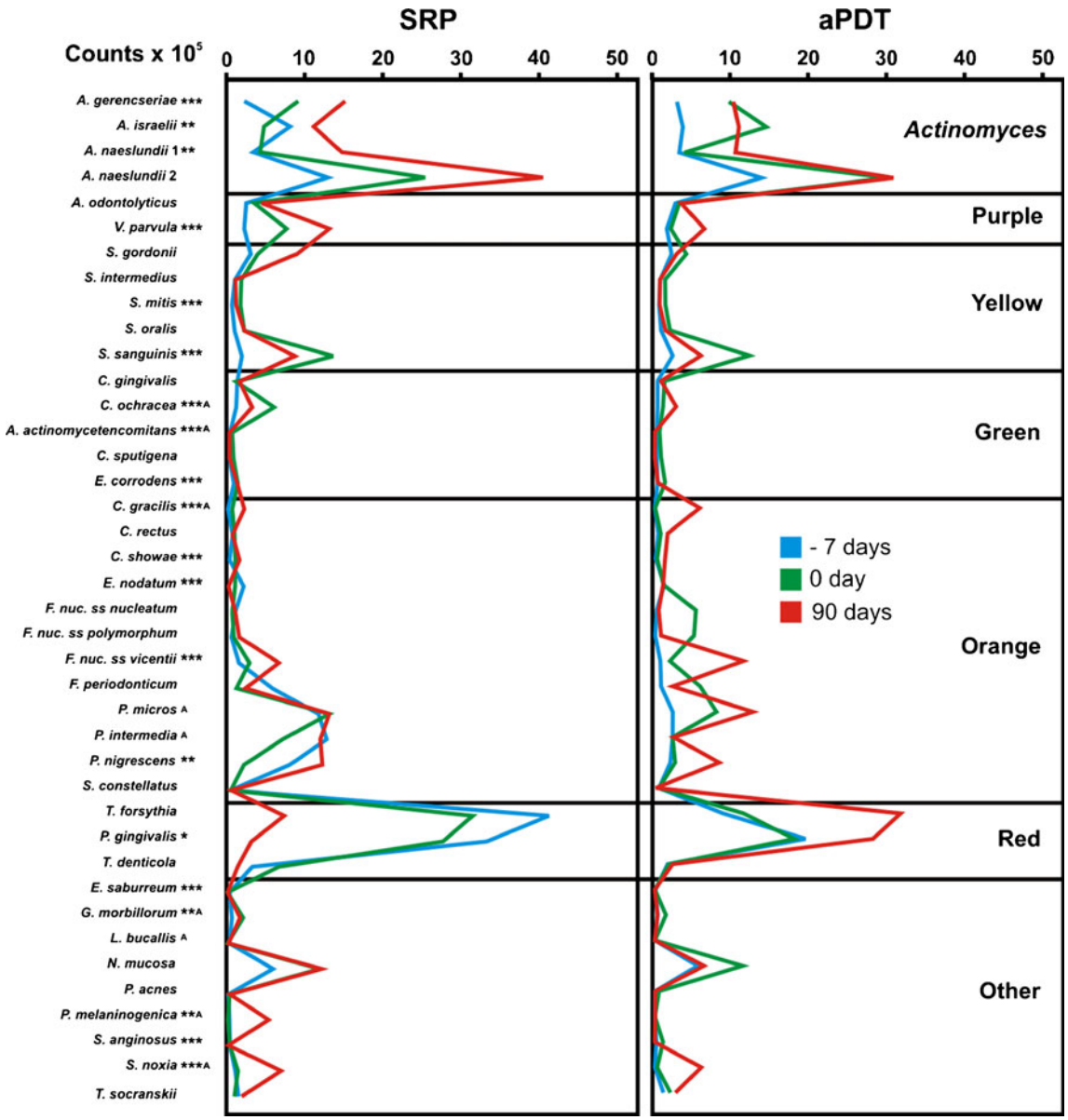

Fig. 1 Mean counts ($\times 10^5$) of 40 bacterial species at −7 days, 0, and 90 days in subjects in each of the two treatment groups. Significance of differences between the treatments is marked with the letter "A" ($p<0.05$) and differences over time were marked as $*p<0.05$, $**p<0.01$ and $***p<0.001$

guidelines exist regarding the appropriate antimicrobial regime and time of administration for this particular group of patients. Possibly, one of the reasons for the low number of studies is the low prevalence of aggressive periodontitis [26] and the consequent difficulty in selecting participants for studies. Furthermore, aggressive periodontitis is often diagnosed at an advanced stage of the disease, when the prognosis for non-surgical treatment may be unfavorable.

The results of this study showed that both treatment modalities may lead to statistically significant improvements in bacterial counts when comparing both treatments and experimental times. Additionally, the observation that the postoperative healing was uneventful in all cases throughout the study period indicates that non-surgical periodontal treatment using aPDT is well tolerated by the patients.

It has been clearly demonstrated that periodontitis is an infectious disease [27, 28] and the current concept for treatment is based on eliminating the infection. Flemmig et al. [29] demonstrated that SRP alone with systemically administered antimicrobial drugs did not result in satisfactory clinical and microbiological outcomes. Sigush et al. [30] showed that in generalized aggressive periodontitis, systemically administered antimicrobial drugs, even if

preceded by the complete removal of supra and subgingival contaminants including root planning, do not lead to good long-term results regarding probing depth reduction clinical attachment gain or bacterial eradication, even unless reinstrumentation of the affected roots is performed in an additional step.

In addition, manual scaling and root planing can often be difficult and time-consuming due to the complex and adverse root morphology when working blindly at deep pocket sites [31]. Since periodontal debridement requires a certain level of skill, time, and endurance, it seems appropriate to develop an easy-handling technique that allows one to achieve a highly efficient and time-saving removal of contaminants, with less effort on behalf of the clinician.

The effect of SRP on the subgingival microflora has been investigated in several studies as previously described [32, 33]. There is general agreement that this procedure, in addition to improving clinical parameters, reduces the microbial load and results in a shift towards a more health-compatible microflora [34–36]. Darby et al. [36] also showed that SRP was effective in reducing the presence of periodontal pathogens in generalized aggressive periodontitis patients. However, there are conflicting reports on the ability of SRP to completely eradicate or suppress important periodontal pathogens like *A. actinomycetemcomitans*, which have been shown to remain in periodontal pockets after non-surgical therapy [37, 38]. Bacterial recolonization or regrowth in the subgingival environment is anticipated after SRP, even shortly after treatment, and it is suggested that, in order to prevent a return to pretreatment levels of pathogens, regular supportive periodontal therapy is essential [32]. It is important to emphasize that the remaining teeth did not receive subgingival mechanical instrumentation during the course of the study, which may have contributed to the recolonization of the treated teeth after the 3-month follow-up.

In the present study, the mean microbial counts decreased significantly in both groups, however, it seems that aPDT and SRP affected different groups of bacteria. The aPDT was more effective in reducing counts of *A. actinomycetemcomitans*, probably because the photosensitizer is able to penetrate through the epithelium and connective tissue [15], which also *A. actinomycetemcomitans* can infiltrate through. On the other hand, SRP was able to reduce the pathogens of the "red complex" such as *T. forsythia*, *P. gingivalis*, and *T. denticola*, which is in accordance with other studies that used a similar approach [39, 40]. Also, both therapies led to an increase in counts of certain putative beneficial species such the *Actinomyces* and pathogens of the "purple complex".

Another aspect that should be kept in mind is that the aPDT sites did not receive subgingival debridement, leaving the biofilm undisturbed. This may have hampered the penetration of the photosensitizer, thereby reducing its effect and leading to the increase in counts of the "red complex" after 3 months. According to this statement, combining therapies may be indicated since the treatments tested present distinct mechanisms of action on the microbiota and thus might have synergistic or even additive effects. SRP would physically lower the biomass of bacteria on the tooth surface and in the periodontal pocket, while aPDT may present a different spectrum of activity due its non-invasive nature and ability to eliminate microorganisms causing alteration in membranes and/or plasma membrane proteins and DNA damage.

These results were very difficult to compare since there are no similar clinical studies dealing with aggressive periodontitis. Conversely, an in vitro study [41] evaluating the use of aPDT on oral bacteria showed that the combination of a photosensitizer with low-power laser irradiation was effective in killing *A. actinomycetemcomitans, P. gingivalis*, and *F. nucleatum*. In a similar in vitro study [42], complete elimination of *A. actinomycetemcomitans, P. gingivalis*, and *F. nucleatum* was also possible if aPDT was used against bacteria organized in biofilms.

However, a direct comparison of the mentioned microbiologic findings to those from the present study is difficult. It is well known that the results of in vitro studies cannot always be extrapolated to the human situation; therefore, they need to be interpreted with caution [43]. Furthermore, different types of photosensitizers, laser beam devices, and wavelengths were used in the aforementioned studies, which makes direct comparison very difficult [44].

The standard treatment for aggressive periodontitis remains highly unspecific, depending mostly on the mechanical debridement of the affected root surfaces in conjunction with antimicrobial drugs. However, a small, although relevant proportion of sites and patients do not respond adequately to this therapy [45]. Antimicrobial drugs may further suppress the periodontal pathogens and increase the benefits obtained by conventional mechanical treatment. Numerous systemic and local antimicrobial agents have been evaluated for the treatment of periodontitis with various degrees of success [46–49]. A lack of effectiveness of some of the antibiotics used may be due to the development of drug-resistant strains [50, 51]. On the other hand, due to its localized and noninvasive nature, the side-effects associated with many antimicrobial drugs (e.g., gastrointestinal disturbance) are unlikely to occur with aPDT. Furthermore, development of resistance to aPDT would appear to be unlikely since its bactericidal activity is due to singlet oxygen and other reactive radicals such as hydroxyl, which affect a range of cellular targets [52–54].

When interpreting the microbiologic effects obtained with aPDT, the possible effects due to the application of the

photosensitizer itself should be considered. Moreover, it should be emphasized that there are very limited data from controlled clinical studies comparing aPDT in conjunction with non-surgical periodontal therapy to aPDT alone, SRP alone, or photosensitizer alone (i.e., used without light activation). Additionally, further studies with a large sample size are necessary before any definitive conclusions can be drawn about the possible clinical and microbiological benefits of aPDT used in conjunction with non-surgical therapy. The frequency of the aPDT application is another possible explanation for the results obtained in this trial. The manufacturer suggests that aPDT treatment should be performed repeatedly during the first weeks of healing to enhance the antimicrobial effect. However, in this study, a single episode of aPDT was performed to avoid an additional confounding factor (i.e., frequency of applied treatment), which could influence the results obtained. Future studies are needed to clarify if and to what extent multiple applications of aPDT might enhance the outcome of therapy.

Conclusions

Under our experimental conditions, this clinical trial demonstrates that a single episode of aPDT and SRP affected different groups of bacteria. aPDT was more effective in reducing the counts of *A. actinomycetemcomitans*. These results suggest that a combination of both treatment methods would be indicated for the non-surgical treatment of aggressive periodontitis.

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