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Treatment of periodontitis in smokers with multiple sessions of antimicrobial photodynamic therapy or systemic antibiotics: A randomized clinical trial

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ABSTRACT

Background: The aim of this study was to evaluate the effects of non-surgical periodontal therapies on smokers with chronic periodontitis, involving multiple adjunctive applications of antimicrobial photodynamic therapy (aPDT), and systemic metronidazole (MTZ) with amoxicillin (AMX).
Methods: All participants were treated with scaling and root planing (SRP). Seventeen patients received 400 mg of MTZ and 500 mg of AMX three times per day for 7 days (MTZ + AMX). Additionally, 17 patients received a placebo, and 17 patients were treated with three applications of aPDT (immediately, 48 h and 96 h after SRP). Clinical and microbiological examinations were performed at baseline and at 90 and 180 days post-therapy. Subgingival samples were analyzed using real-time polymerase chain reaction.
Results: After 180 days, the patients in groups MTZ + AMX and aPDT had significantly lower mean probing depths, more clinical attachment level gains and less bleeding on probing. At 180 days, in the moderate pocket there was a reduction in the levels of *Porphyromonas gingivalis* and *Prevotella nigrescens* in the MTZ + AMX group, while group aPDT showed a reduction in *Prevotella nigrescens*. Furthermore, at 180 days, in the deep pocket a reduction in *Porphyromonas gingivalis, Prevotella intermedia* and *Prevotella nigrescens was observed* in group MTZ + AMX, as well as a reduction in the levels of *Prevotella intermedia* and *Prevotella nigrescens* in group aPDT.
Conclusion: In smokers with periodontitis, the MTZ + AMX and aPDT treatments significantly improved the effects of SRP.

1. Introduction

Clinical studies have repeatedly demonstrated that metronidazole (MTZ) plus amoxicillin (AMX) in conventional mechanical therapy for periodontitis is beneficial [1–8]. Despite the proven benefits, this specific indication remains controversial in different clinical situations, especially in relation to other elements of periodontal therapy [9]. Restraint in the use of antibiotics in periodontal therapy has been recommended. Nevertheless, further research is needed to assess the true impact of antibiotics use in periodontal therapy on the development of antibiotics resistance [10].

Antimicrobial photodynamic therapy (aPDT) is characterized by the association of photosensitizing agents with different sources of light, such as lasers or light-emitting diodes (LEDs), with the goal of promoting the generation of reactive oxygen species like free radicals and singlet oxygen, which are cytotoxic to certain bacteria. In the last decade, various studies have evaluated the effects of aPDT as an adjunctive therapy in the treatment of periodontitis [11–17]. At present, however, the literature has been unable to demonstrate the clinical advantages of aPDT [13,16], and several works only showed that aPDT reduced the occurrence of bleeding during the probing of treated sites [11,12,13,15,17].

aPDT could be very useful in patients who present a systemic modifying factor that could alter the periodontal tissue's biological response during tissue repair following conventional periodontal treatment, or modify the progress of periodontal disease [18]. Patients who smoke have an unfavorable response to periodontal treatment, compared to non-smokers [19]. Studies have demonstrated the clinical

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benefits of systemic antibiotics in the treatment of patients who smoke [20–22]. A recent study has revealed that systemic MTZ + AMX treatment yields better outcomes in periodontal therapy than aPDT [23].

Given these facts, the purpose of this study was to examine, clinically and microbiologically, the effects of conventional periodontal therapy in combination with either MTZ + AMX or multiple applications of aPDT in patients who smoke. It was hypothesized that the adjunctive use of multiple aPDT sessions (0, 48 and 96 h), or the systemic use of MTZ + AMX is more beneficial for heavy smokers than conventional periodontal treatment.

2. Material and method

This study consisted of a randomized, prospective, controlled clinical trial with a follow-up period of 180 days. The project received approval from the Human Research Ethics Committee of the Dentistry School of Araçatuba (UNESP) and was registered at the Brazilian Research Platform (CAAE), case number 05,519,412.5.0000.5420/2012. Between May 2013 and February 2015, 51 smoking patients were recruited via the periodontal clinic of the Dentistry School of Araçatuba to participate in the present study.

2.1. Calculation of the sample size

The sample size was calculated, considering a difference of at least 1 mm in the pocket probing depth (PD) with a standard deviation of 0.9 mm between the groups. It was determined that 13 patients per group would provide 80% power at a 5% significance level [24].

2.2. Patient selection and criteria for inclusion and exclusion

In total, 135 patients from the Periodontal Clinic of the Dentistry School of Araçatuba (UNESP) were screened. Of those, 51 individuals were selected, based on the following criteria: age 30 to 70 years; having smoked more than 10 cigarettes per day for over 5 years; diagnosed with severe generalized chronic periodontitis in at least 6 teeth, including one or several sites with PD ≥ 5 mm; a loss of clinical attachment level (CAL) ≥ 5 mm; a minimum of 30% of the sites with PD and CAL ≥ 4 mm and bleeding on probing (BOP) [24]; the presence of at least one deep periodontal pocket (PD ≥ 7 mm); one moderate pocket (5 ≥ PD < 7 mm) in each quadrant where microbiological samples were acquired; and the presence of at least 15 teeth, not counting third molars [24]. The exclusion criteria were: periapical alterations in the qualified teeth; a medical disorder that required antibiotic prophylaxis or that could influence the treatment response; having received periodontal treatment in the last 6 months; having consumed medicine that affects the periodontal tissue in the last 6 months (antibiotics, anti-inflammatories, anticonvulsants, immunosuppressants or calcium channel blockers); allergy to penicillin or MTZ; pregnancy; ongoing orthodontic treatment; the presence of a metabolic disorder (e.g. diabetes), an immunological disorder or alcoholism; and the use of illicit drugs[24].

2.3. Clinical parameters

A single calibrated examiner (ML) performed all clinical exams and was blinded to the treatments. The calibration procedure was conducted in two patients. In total, 270 sites were analyzed in these two patients (PD) at two different times (with a 7-day interval). The data were submitted to the Kappa concordance test, and the calibration was approved (0.90). Fifteen days before the study began, all patients received instructions on proper techniques for at-home care. Oral hygiene instructions were reinforced at each visit.

Prior to the treatment, as well as 90 and 180 days after it, the examiner recorded the following parameters for six sites on every tooth: the PD, BOP (presence or absence), and the CAL [25]. A periodontal probe with 1-mm marks was used (PCPUNC-15, Hu-Friedy, Chicago, IL, USA). Samples of subgingival biofilm were obtained at baseline, and 90 and 180 days post-treatment from one moderate pocket (5 ≥ PD < 7 mm) and one deep pocket (PD ≥ 7 mm) from each participant. The samples were collected with sterile absorbent paper cones (# 30, Tanari, Manacapuru, AM, Brazil) [26].

2.4. Treatment

The 51 patients were divided into three groups using an online randomizer (www.sealedenvelope.com). Envelopes containing the treatment distribution were prepared by a person independent of the clinical examiner (LHT), and were opened only on the day of treatment after the SRP procedure. Participants were allocated to one of the following groups: **SRP** (N = 17): SRP was carried out with ultrasonic instruments (ProfiNeo, Dabi Atlante, Ribeirão Preto, SP, Brazil) and hand curettes (Gracey curettes, Hu-Friedy, Chicago, IL, USA) for one hour, and 2 placebo pills were administered 3 times per day for 7 days; **MTZ + AMX** (N = 17): SRP was carried out for one hour, followed by the systemic administration of MTZ (400 mg) and AMX (500 mg) 3 times per day for 7 days; and **aPDT (**N = 17): SRP was carried out for one hour, followed by the application of aPDT at two sites (1 moderate pocket and 1 deep pocket) per quadrant immediately, 48 h and 96 h after SRP, followed by the administration of 2 placebo pills 3 times per day for 7 days.

The SRP and aPDT procedures were performed under local anesthesia by one operator (NZA) who was specialized in periodontics and did not know the results of the randomization until SRP completion. The treatment group was concealed from both the therapist and the clinical examiner.

aPDT was performed at two sites: one with 5 ≥ PD < 7 mm (moderate pocket) and another with PD ≥ 7 mm (deep pocket) in each quadrant of each patient. The selected sites were irrigated with 1 ml of methylene blue at 10 mg/ml (Aphoticário Manipulation Pharmacy, Araçatuba, SP, Brazil) using a syringe and a blunt cannula (Becton Dickinson Ind. Ltda., Curitiba, PR, Brazil). After 1 min, the sites were irradiated with a GaAlAs diode laser at 660 nm (Laser Duo, MM Optics, Ltda, São Carlos, SP, Brazil) for 48 s, equaling an energy density of 160 J/cm^2; an energy of 4.8 J, with an output power of 100 mW; and fiber optics of 0.03 cm^2 [24]. The tip of the fiber optic laser was introduced into the area of the periodontal pocket until it reached the bottom of the pocket [24]. The patients and operator used protective glasses during the entire irradiation period.

2.5. Compliance

Patients were called in 8 days after the beginning of the procedure for a clinical evaluation of the treatment. At this time, their medical history, concurrent use of medication and any side effects were noted. Additionally, the patients were asked to bring their medicine bottles with them, to determine total use. They were also asked about side effects of the medication, of which none were reported.

2.6. Microbiological methods

The specimens were stored in 500 µl of phosphate buffered solution, pH 7.0, and frozen at −80 °C [26]. DNA was extracted from the subgingival biofilm samples using the phenol-chloroform protocol and was re-suspended in TE buffer solution (10 mM Tris-HCl; 0.1 mM EDTA, pH 7.5; and 10 µg/ml RNase), as described by Sardi et al. [27]. The DNA was then quantified and adjusted to a concentration of 100 ng/mL, using a spectrophotometer at 260 nm. Next, the samples were analyzed for the detection and quantification of *Porphyromonas gingivalis (P. gingivalis)* [28], *Prevotella nigrescens (P. nigrescens)* [29] and *Prevotella intermedia (P. intermedia)* [28] using the StepOne Real-Time PCR System (ABI, Applied Biosystems, Foster City, CA, USA). The PCR

reaction mixture was composed of 5 µl of SYBR® Green Master Mix (Life Technologies), 0.6 µl of mixed primers (10 µM each), 3.5 µl deionized water and 1 µl of DNA. Standard curves and cycle threshold (CT) values were generated from serial dilutions of DNA from standard ATCC strains. After the final cycle, analysis of the melting temperature (Tm) was carried out for all the amplified samples. The results were normalized against the 16 S rRNA gene, which represented the total bacterial DNA, as a count (ng/mL).

2.7. Statistical analysis

The averages and standard deviations of the PD and CAL were calculated for all sites in the mouth. The categorical BOP data were transformed into percentages, and the averages and standard deviations for the entire mouth were obtained. The reduction in the number of pockets with PD ≥ 4 mm and the percentage of sites with BOP were noted, as well as the reduction in the number of shallow, moderate and deep pockets [24]. The primary outcomes of this study were changes in the mean CAL at 90 and 180 days post-treatment.

Demographic data and clinical parameters were tabulated and submitted to a normality test (Lilliefors Test, $p < 0.05$). Parametric data (PD, CAL and BOP) were submitted to ANOVA ($p < 0.05$), followed by the Tukey test to determine statistically significant relations ($p < 0.05$).Non-parametric data were analyzed via the Kruskal-Wallis test, with associations determined by the Student-Newman-Keuls test ($p < 0.05$). The difference between males and females was evaluated using the chi-square test. Furthermore, the reduction percentages in residual pockets were also analyzed using the Kruskal-Wallis test ($p < 0.05$). The bacterial levels data were submitted to the Mann-Whitney test for comparisons between the groups and the Wilcoxon test for comparisons over time between the different categories of pockets ($p < 0.05$). The numbers of shallow, moderate and deep pockets were subjected to ANOVA and Tukey's range test. All analyses were performed with a significance level of 5% (BioEstat 5.3, Mamirauá Institute, Manaus, AM, Brazil).

3. Results

All 51 patients underwent the proposed treatments. In total, 35 men and 16 women with an average age of 48 years (35–65 years) were treated. From this population, 8 individuals were excluded due to the use of anti-inflammatory medication (3); moving to another city (1); or failing to participate in the third follow-up after 6 months (Fig. 1).

3.1. Clinical findings

Table 1 presents the data of all patients, evaluated at baseline and after 90 and 180 days. In intra-group analysis, significant improvements were observed between baseline and 90 days for the PD parameter in the MTZ + AMX group ($p < 0.05$); and for the PD, CAL and BOP parameters in group aPDT ($p < 0.05$). Between baseline and 180 days, significant improvements were observed in the PD, CAL and BOP parameters in groups MTZ + AMX ($p < 0.05$) and aPDT ($p < 0.05$).

Table 2 shows the PD reduction and CAL gain observed between baseline and 90 days, and between baseline and 180 days. In the analysis of moderate pocket reduction, there was no difference in PD between the groups at any of the evaluated time points ($p = 0.10$). However, there was significant improvement in the CAL in group aPDT compared to group SRP, between baseline and 180 days ($p = 0.01$).

Regarding the presence of residual pockets, there was a decrease with all treatment groups after 90 days (SRP, 45.93 ± 25.22%; MTZ + AMX, 47.85 ± 28.75%; and aPDT, 45.07 ± 22.30%) and 180 days (SRP, 50.63 ± 26.82%; MTZ + AMX, 40.57 ± 24.51%; aPDT, 49.06 ± 24.58%), compared to the corresponding baseline data ($p < 0.01$). Table 3 presents the number of sites with shallow, moderate and deep pockets at baseline, 90 and 180 days into the experiment. Groups MTZ + AMX and aPDT exhibited a significant reduction in the number of moderate and deep pockets during the study.

3.2. Microbiological findings

Comparison of the moderate pockets between time points has revealed a reduction in the levels of *P. gingivalis* and *P. nigrescens* between baseline and 180 days in group MTZ + AMX, while group aPDT had a reduction in *P. nigrescens* at 180 days. With regard to the deep pocket, there was a reduction in *P. gingivalis, P. intermedia* and *P. nigrescens* at 180 days in group MTZ + AMX, and a reduction in the levels of *P. intermedia* and *P. nigrescens* at 180 days and in *P. nigrescens* at 90 days in group aPDT (Table 4).

Quantitative analysis of bacterial presence in the moderate pocket has demonstrated smaller quantities of *P. gingivalis* at 90 ($p = 0.05$) and 180 ($p = 0.008$) days in group MTZ + AMX than in group aPDT, and a smaller quantity of *P. gingivalis* in group MTZ + AMX than in the SRP group at 180 days ($p = 0.03$). In the deep pocket, there were higher levels of *P. gingivalis* in group aPDT than in groups MTZ + AMX ($p = 0.01$) and SRP ($p = 0.03$) at 90 days, and lower levels in the MTZ + AMX group than in groups SRP ($p = 0.002$) and aPDT ($p = 0.003$) at 180 days. The levels of *P. intermedia* were higher in the aPDT group, compared to groups SRP ($p = 0.03$) and MTZ + AMX ($p = 0.002$) at 180 days, while the levels of *P. nigrescens* in the deep pocket were lower in group aPDT than in groups SRP ($p = 0.03$) and MTZ + AMX ($p = 0.005$) at 90 days (Table 4).

4. Discussion

This study evaluated the clinical and microbiological effects of one systemic antibiotic protocol and multiple sessions of aPDT in regular smokers with generalized periodontitis, and compared the outcomes of theses adjunctive therapies with those obtained by conventional SRP. In our analysis we observed that clinically, SRP alone was did not affect PD, BOP or CAL gains after 90 or 180 days. Other clinical studies have demonstrated great pitfalls in the successful periodontal treatment of smokers [30,31]. Contrastingly, the adjunctive treatments evaluated in this study (aPDT and MTZ + AMX) were effective in PD reduction after 90 and 180 days. Moreover, there was an increase of CAL and BOP reduction in group aPDT on days 90 and 180, and in the MTZ + AMX group on day 180. Finally, groups MTZ + AMX and aPDT exhibited a significant reduction in the number of moderate and deep pockets during the study.

Based on this study, it can be concluded that the MTZ + AMX combination employed for 7 days may be clinically beneficial. In line with the findings of the present work, other studies have shown that the use of an adjunctive therapy with MTZ or MTZ + AMX has clinical and microbiological treatment benefits in non-smoking patients with chronic periodontitis [2,32–34] as well as in patients who smoke [22,30]. There is still no consensus on antibiotic protocol in smokers [35], although certain studies have used the combination of MTZ + AMX for a period of 7 [30] or 14 [22,25] days. Another study suggested that smokers may specifically benefit from being treated with a combination of MTZ + AMX in the non-surgical phase, and that this reduces the need for surgery [8].

In the present study, group MTZ + AMX showed a significant decrease in BOP at 180 days, and at 90 and 180 days in group aPDT. However, these results may be controversial because the vasoconstrictive action of nicotine causes periodontal disease to be hidden due to suppression of the classic signals of gingival inflammation, although bleeding on probing may occur [36]. Because BOP occurred less patients who smoked than in non-smokers, we believe that, regardless of therapy, this clinical parameter is altered by cigarette consumption. In this study, except for repeated oral hygiene instructions, no additional therapy was allowed in any of the patients prior to the SRP treatment [37,38]. Nevertheless, there is an association between smoking and

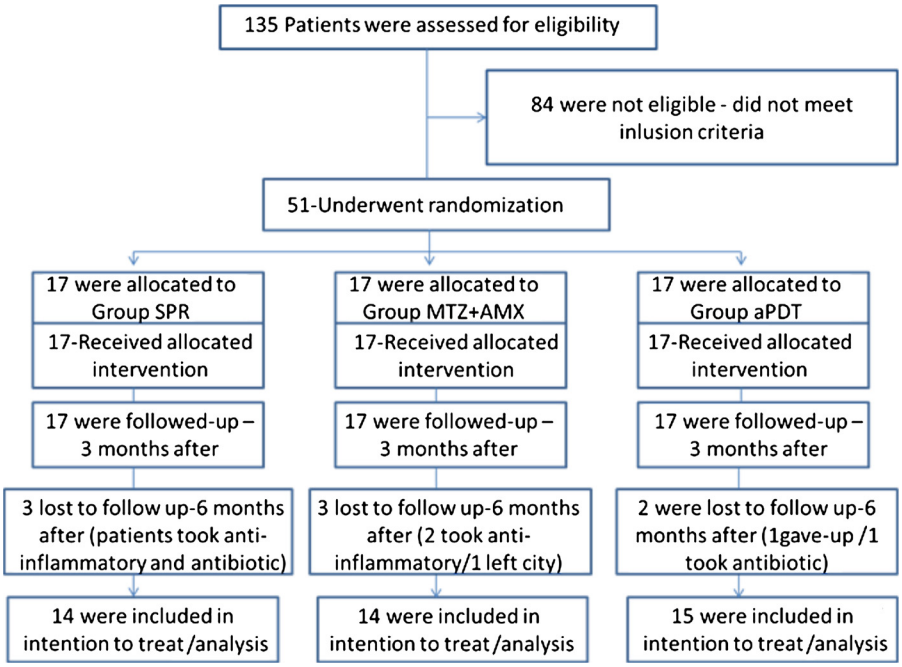

Fig. 1. Flow chart of the study design.

gingival bleeding, and between the number of years smoked and gingival bleeding, controlling for age [39]. For the purpose of this study, patients with a daily consumption of 10 cigarettes or more over a period of at least 5 years prior to the research, were selected. The treated patients had a mean age of 45 years and had smoked for more than 15 years.

Regarding aPDT, some studies in humans have not shown clinical advantages in PD reduction after a single session of chronic periodontitis treatment [13,16]. However, other studies have demonstrated that this therapy was capable of reducing BOP at the treated sites [11,12,15]. Moreover, studies that evaluated the effect of multiple aPTD applications in the periodontal treatment of residual pockets also observed clinical improvements in PD reduction and reduced bleeding [40,41]. In contrast, research that performed three applications of aPDT in residual pockets on the first, third and seventh day after SRP did not demonstrate clinical improvements in relation to the PD and CAL parameters, but did demonstrate a more substantial reduction in BOP at 3 and 12 months [17]. These differences in results could be attributed to differences in the employed methods and analyses, such as the pre-irradiation time; the assessed parameters; and the mode, number and frequency of laser irradiations. In this study, three aPDT applications with intervals of 48 h in the moderate and deep pockets were performed, based on the results of another study of the same group [24]. The pre-irradiation time was 1 min, and irradiation was performed during 48 s in each site with a diode laser (660 nm; energy density 160 J/cm^2).

Regarding the use of aPDT in the periodontal treatment of smokers, a clinical study evaluated the effect of one aPDT session for periodontitis treatment; significant reductions in IL-1β levels one week after the treatment and in MMP-8 levels after twelve weeks were observed,

Table 1
Demographic characteristics and the mean and standard deviation (M ± SD) of the clinical parameters (PD, CAL and BOP) of the full mouth at baseline and 90 and 180 days after treatment.

Variable	SRP group[a] N = 14	MTZ + AMX group[a] N = 14	aPDT group[a] N = 15	p-value[b]
Age (years)	46.2 ± 6.7	48.9 ± 5.1	48.8 ± 3.9	0.80
Women, n(%)	4 (28.6%)	5 (35.7%)	5 (33.3%)	0.91
Cigarettes per day	14.93 ± 3.52	18.93 ± 3.50	15.00 ± 3.27	0.01; 0.93; 0.01
PD (mm)				
Baseline	4.19 ± 0.85	4.04 ± 0.38	4.02 ± 0.42	0.83; 0.84; 0.99
90 days	4.84 ± 0.62	3.59 ± 0.59[c]	3.58 ± 0.36[c]	0.51; 0.29; 0.70
180 days	3.85 ± 0.73	3.48 ± 0.35[c]	3.54 ± 0.33[c]	0.10; 0.23; 0.64
CAL (mm)				
Baseline	4.68 ± 0.94	4.64 ± 0.37	4.55 ± 0.39	0.54; 0.92; 0.60
90 days	4.51 ± 0.80	4.21 ± 0.58	4.12 ± 0.36[c]	0.53; 0.12; 0.35
180 days	4.47 ± 0.80	4.12 ± 0.38[c]	4.11 ± 0.34[c]	0.17; 0.13; 0.92
BOP %				
Baseline	76.48 ± 22.68	87.57 ± 9.33	87.88 ± 9.61	0.14; 0.13; 0.97
90 days	69.12 ± 19.44	79.45 ± 12.60	69.40 ± 13.08[c]	0.23; 0.71; 0.11
180 days	68.89 ± 22.02	70.66 ± 18.89[c]	59.69 ± 17.97[c]	0.94; 0.11; 0.09

[a] Mean ± standard deviation or number of participants (percentage).
[b] p-value for SRP vs MTZ + AMX, SRP vs aPDT, or MTZ + AMX vs aPDT.
[c] Statistically significant difference compared to baseline in the same group.

Table 2
The mean and standard deviation (M ± SD) of the PD reduction and CAL gain in the moderate and deep pockets between baseline and 90 days and between baseline and 180 days.

Variable	SRP group[a] N = 14	MTZ + AMX group[a] N = 14	aPDT group[a] N = 15	p-value[b]
Moderate pocket				
PD (mm)				
0–90 days	0.66 ± 0.46	0.89 ± 0.68	0.92 ± 0.66	0.10
0–180 days	0.68 ± 0.49	0.86 ± 0.55	1.15 ± 0.51	0.10
CAL (mm)				
0–90 days	0.52 ± 0.70	1.03 ± 0.55	0.88 ± 0.67	0.06; 0.06; 0.99
0–180 days	0.68 ± 0.61	0.76 ± 0.67	1.20 ± 0.64	0.58; 0.01; 0.06
Deep pocket				
PD (mm)				
0–90 days	2.04 ± 1.12	1.88 ± 1.76	2.50 ± 1.76	0.31
0-180 days	2.40 ± 1.14	2.40 ± 1.55	3.54 ± 2.43	0.31
CAL (mm)				
0–90 days	2.19 ± 1.95	1.79 ± 1.83	3.04 ± 2.50	0.33
0–180 days	2.38 ± 1.91	2.43 ± 1.60	3.49 ± 2.61	0.33

[a] Mean ± standard deviation.
[b] p-value for SRP vs MTZ + AMX, SRP vs aPDT, or MTZ + AMX vs aPDT.

Table 3
Mean ± SD of number of sites with shallow (PD > 3 and < 5 mm), moderate (PD ≥ 5 and < 7 m m) and deep pockets (PD ≥ 7 mm) at baseline, 90 and 180 days after treatment.

Variable	Groups SRP N = 14	MTZ + AMX n = 14	aPDT n = 15
PD > 3 and < 5 mm			
Baseline	31.21 ± 12.02[a]	45 ± 15.69	49.13 ± 17.22[b]
90 days	41.21 ± 16.94	35.23 ± 12.17	42.06 ± 19.42
180 days	38.36 ± 14.95	39.61 ± 12.41	44 ± 18.85
PD ≥ 5 and < 7 mm			
Baseline	38.86 ± 19.59	30 ± 14.88[A]	26.6 ± 14.14[A]
90 days	24.36 ± 13.45	19.61 ± 14.88	16.87 ± 9.83
180 days	23.36 ± 14.29	15.77 ± 9.87[B]	15.13 ± 8.47[B]
PD ≥ 7 mm			
Baseline	9 ± 9.66	7.92 ± 5.48[A]	6.33 ± 5.07[A]
90 days	5.93 ± 7.18	4.31 ± 3.57	2.47 ± 2.64[B]
180 days	6.28 ± 8.19	2.69 ± 3.06[B]	2.6 ± 3.48[B]

[A,B]Different capital letters indicate significant differences between time points.
[a,b],Different small letters indicate significant differences between groups.

Table 4
The mean (median) and standard error of the levels (ng/mL) of periodontal pathogens evaluated in the total DNA (16 S) at baseline and 90 and 180 days.

Variable	SRP group[a]	MTZ + AMX group[a]	aPDT group[a]	p-value[b]
Moderate pocket				
P. gingivalis				
Baseline	1.21 (0.39)0.57	5.2 (0.02) 4.15	1.13(0.04)0.62	0.734;0.621;0.949
90 days	4.97 (0) 4.79	0.3(0)0.21	0.97(0.16)0.45	0.390;0.496;0.05
180 days	0.45(0.15) 0.22	0.37 (0)0.32[d]	1.20(0.26)0.55	0.03;0.488;0.008
P. intermedia				
Baseline	0.31(0)0.3	0.01(0)0.01	0.17(0.01)0.1	0.511;0.186;0.063
90 days	0	0.04(0)0.02	0.02(0)0.01	0.311;0.555;0.650
180 days	0	0	1.58(0)1.49	1;0.175;0.175
P. nigrescens				
Baseline	0.33(0) 0.23	0.09(0)0.03	1.79(0)1.78	0.511;0.780;0.270
90 days	0.12(0)0.1	0.1(0)0.06[e]	0.01(0)0	0.579;0.964;0.496
180 days	0.02(0) 0.01	0[d]	0 [d]	0.511;0.440;1
Deep pocket				
P. gingivalis				
Baseline	0.88(0.36) 0.36	1.44 (0.04) 0.63	1.33(0.51)0.49	0.650;0.618;0.683
90 days	0.45 (0) 0.21	0.15(0)0.11	2.51(0.49)1.51	0.550;0.03;0.01
180 days	1.08 (0.16) 0.63	0.1(0)0.05[d]	2.79(0.54)1.60	0.002;0.836;0.003
P. intermedia				
Baseline	0	0.17(0)0.02	0	0.259;0.751;0.158
90 days	0.02(0) 0.02	0.42(0)0.39[e]	0.01(0)0.01	0.202;0.342;0.339
180 days	0	0 [d]	0.07(0)0.03 [d]	1.0;0.003;0.002
P. nigrescens				
Baseline	0.03(0) 0.02	0.1(0)0.06	0.05(0)0.03	0.259;0.525;0.533
90 days	0.07(0) 0.07	0.01(0.01) 0.07[e]	0[c]	0.094;0.03;0.005
180 days	0	0 [d]	0[d]	0.756;0.446;0.722

[a] Mean (median) standard error.
[b] p-value for SRP vs MTZ + AMX, SRP vs aPDT, or MTZ + AMX vs aPDT according to the Mann-Whitney test ($p < 0.05$).
[c] Statistically significant difference between baseline and 90 days in the same group according to the Wilcoxon test ($p < 0.05$).
[d] Statistically significant difference between baseline and 180 days in the same group according to the Wilcoxon test ($p < 0.05$).
[e] Statistically significant difference between 90 and 180 days in the same group according to the Wilcoxon test ($p < 0.05$).

which indicates a potentially positive effect on periodontal cicatrization. Nonetheless, aPDT did not display any clinical advantages in terms of reducing the PD and increasing the CAL [42]. These findings may explain why there was a significant CAL gain in the moderate pockets in group aPDT in the present study.

Moreover, a higher number of compromised sites, in combination with a significant reductions in bone height has been observed in heavy smokers [39]. This fact could also be explained by the fact that in heavy smokers, human gingival fibroblasts and cell proliferation decrease, whereas cytotoxicity increases with higher concentrations of cigarette smoke condensate [43].

Our microbiological analysis has revealed that antibiotic therapy was effective in reducing the levels of all of the evaluated microorganisms. Furthermore, aPDT was effective in reducing the levels of *P. intermedia* and *P. nigrescens.* In the moderate pockets in particular, at 180 days, antibiotic therapy reduced the levels of *P. gingivalis* and *P. nigrescens*, while aPDT reduced *P. nigrescens*. In the deep pocket, there was a reduction in the levels of *P. gingivalis, P. intermedia* and *P. nigrescens* at 180 days in the MTZ + AMX group, compared to baseline, while group aPDT showed a reduction in the levels of *P. intermedia* and *P. nigrescens* at 180 days and in *P. nigrescens* at 90 days. In contrast, SRP treatment did not cause a significant reduction in the levels of microorganisms, compared to baseline.

With regard to clinical studies that have evaluated the effect of aPDT on pathogenic microorganisms, the literature reports varying results where some studies demonstrate a reduction in these microorganisms [16,17], others do not [13,41]. Relative to this study, other studies of antibiotic therapy in smokers have demonstrated that the MTZ + AMX combination causes a proportional reduction in *Tannerella forsythia, P. gingivalis* and *Treponema denticola* [22,25], and that MTZ alone provokes a reduction in *Aggregatibacter actinomycetemcomitans, P. gingivalis* and *P. intermedia* [44].

Based on the comparative evaluation we carried out, it can be said that the conventional mechanical SRP treatment should be complemented by an adjunctive treatment in smokers. Additionally, the two evaluated therapies proved effective and promoted additional benefits in smokers, with the advantage of aPDT being a local therapy that does

not promote bacterial resistance or side effects.

Thus, based on the methodology used, it can be concluded that the adjunctive use of MTZ + AMX, or three sessions of aPDT constitutes an effective therapy for the treatment of periodontitis in smokers. In addition, the use of antibiotic therapy by itself was an effective treatment for reducing the levels of pathogenic periodontal microorganisms. Nonetheless, more randomized controlled clinical trials need to be performed to evaluate the effects of antibiotic therapy and other aPDT protocols in patients who smoke.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

All authors declare that they have no conflict of interest.

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