Clinical and Microbiologic Effects of Adjunctive Metronidazole Plus Amoxicillin in the Treatment of Generalized Chronic Periodontitis: Smokers Versus Non-Smokers

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Background: The aim of the present study is to evaluate the clinical and microbiologic effects of the adjunctive use of metronidazole (MTZ) and amoxicillin (AMX) in the treatment of smokers and non-smokers with generalized chronic periodontitis (CP).

Methods: Thirty-two smokers and 32 non-smokers were selected and received scaling and root planing (SRP) combined with MTZ (400 mg three times daily) and AMX (500 mg three times daily) for 14 days. Clinical and microbiologic examinations were performed at baseline and 3 months after SRP. Nine subgingival plaque samples per patient were analyzed using checkerboard DNA–DNA hybridization.

Results: Both groups presented a significant improvement in all clinical parameters at 3 months after therapy (P<0.05). Non-smokers showed lower mean number of sites with probing depth (PD) ≥5 mm after therapy. Fewer non-smokers exhibited at least nine of these sites at 3 months after treatment. Non-smokers also presented the greatest reductions in mean PD and gain in clinical attachment between baseline and 3 months after therapy at initially deep (PD ≥7 mm) sites (P<0.01). The most beneficial changes in the microbial profile were observed in the non-smoker group, which showed the lowest proportions of the orange complex at 3 months, as well as a significant increase in the proportions of Actinomyces species after treatment.

Conclusion: Smokers with CP benefit less than non-smokers from treatment by the combination of SRP, MTZ, and AMX. J Periodontol 2014;85:581-591.

KEY WORDS
Amoxicillin; chronic periodontitis; metronidazole; non-surgical periodontal debridement; smoking.

Smoking plays a significant role in the pathogenesis of chronic periodontitis (CP),¹⁻⁴ and it is one of the main and most prevalent risk factors of this infection.³⁻⁵ Positive associations between dose and years of exposure to tobacco products and the severity of periodontal disease have been reported,⁶⁻⁷ with odds ratios varying from 2 to 8, depending on the definition of periodontal disease and smoking history.³⁻⁵,⁸ The negative effects of smoking on bacterial challenge⁹⁻¹³ and the reduced immune-inflammatory response¹⁴⁻¹⁷ have been suggested as possible mechanisms by which smokers are at increased risk of periodontitis.

Several studies have reported greater reduction in probing depth (PD) and gain in clinical attachment level (CAL) in non-smokers compared with smokers after different periodontal therapies, including non-surgical¹⁸⁻²² and surgical approaches.²³,²⁴ Jin et al.²¹ reported a statistically significant greater mean PD reduction in non-smokers (2.4 ± 0.2 mm) compared with smokers (1.1 ± 0.3 mm) at 3 months after scaling and root planing (SRP). In addition, a systematic review evaluating the effect of smoking on non-surgical periodontal therapy has shown that smoking can negatively impact the response to periodontal therapy, with smokers demonstrating less improvement in clinical parameters compared to non-smokers.³⁻⁶,²⁵,²⁶ The mechanisms by which smoking affects periodontal health include the presence of harmful chemicals in smoke, such as nicotine, which can inhibit the immune response and increase bacterial colonization.³⁻⁶,²⁵,²⁶

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therapy demonstrated that the mean difference in PD reduction between smokers and non-smokers in sites with initial PD ≥5 mm were 0.43 mm in favor of non-smokers. Hence, smokers seem to respond less favorably to periodontal therapy, suggesting that these patients might need additional treatments to achieve better and more sustained clinical outcomes.

Randomized controlled clinical trials have demonstrated that the use of amoxicillin (AMX) and metronidazole (MTZ) as adjuncts to mechanical therapy improves the clinical and microbiologic outcomes of SRP in non-smokers with CP. Certain clinical and microbiologic benefits of SRP + MTZ + AMX in smokers have been described recently by the present authors, and apparently this therapy was less effective in reducing certain putative pathogens from the orange complex compared with a group of non-smokers. However, to date, the effects of this antibiotic protocol have not been directly compared in non-smokers and smokers. Therefore, the aim of this study is to compare the clinical and microbiologic effects of the adjunctive use of MTZ + AMX to SRP in smoker and non-smoker patients with generalized CP. It was hypothesized that non-smokers would reap greater benefit from this combination of therapies than the smokers.

**MATERIALS AND METHODS**

**Sample Size Calculation**

This study is designed to compare the clinical and microbiologic effects of the treatment of smoker and non-smoker patients with SRP + MTZ + AMX (ClinicalTrials.gov identifier NCT01837199). The ideal sample size to ensure adequate power for this clinical trial was calculated considering differences of at least 1 mm between groups for mean CAL gain in initially deep periodontal sites (PD ≥7 mm). It was also determined that the standard deviation of CAL change at deep sites was 1.0 mm based on previous studies of smokers and non-smokers receiving SRP combined with MTZ + AMX. Based on these calculations, it was defined that 26 patients per group would be necessary to provide an 85% power with a α of 0.01 based on a two-sided test. Considering an attrition of ≈20%, it was established that at least 32 patients should be included in each treatment group.

**Patient Population: Inclusion and Exclusion Criteria**

Patient recruitment started in July 2011 and was completed at the end of June 2012. Sixty-four patients (31 males and 33 females, aged 38 to 55 years; mean age: 45.3 years), 32 smokers and 32 non-smokers, with untreated generalized CP were selected from the population referred to the Periodontal Clinic of Guarulhos University (Guarulhos, São Paulo, Brazil). Detailed medical, periodontal, and dental histories were obtained. All eligible patients were informed of the nature, potential risks, and benefits of their participation in the study and signed an informed consent. This study protocol was approved by the Guarulhos University Clinical Research Ethics Committee, Guarulhos, São Paulo, Brazil.

All patients were in good general health and were diagnosed with generalized CP based on the current classification of the American Academy of Periodontology. The inclusion criteria were as follows: 1) ≥35 years of age; 2) presence of at least 15 teeth; and 3) a minimum of six teeth with at least one site each with PD and CAL ≥5 mm, as well as at least 30% of the sites with PD and CAL ≥4 mm and bleeding on probing (BOP). The smokers considered for this investigation had smoked at least 10 cigarettes per day for a minimum of 5 years before the beginning of the study and expressed no interest in quitting smoking. Non-smokers had never smoked. The exclusion criteria were as follows: 1) previous subgingival periodontal therapy; 2) pregnancy; 3) nursing; 4) systemic diseases that could affect the progression of periodontal disease (e.g., diabetes, osteoporosis); 5) long-term administration of anti-inflammatory medications; 6) need for antibiotic premedication for routine dental therapy; 7) continuous use of mouth rinses containing antimicrobials; 8) antibiotic therapy in the previous 6 months; and 9) allergy to MTZ or AMX.

**Experimental Design, Allocation Concealment, and Treatment Protocol**

In this cohort clinical trial, patients were assigned according to their smoking status into smoker and non-smoker groups. All patients received SRP combined with systemic MTZ (400 mg) and AMX (500 mg). Both antibiotics were administered three times per day for 14 days. Before the study began, all patients received full-mouth supragingival scaling and instruction on proper home-care techniques. They were also given the same dentifrice to use during the study period. All patients received full-mouth SRP performed under local anesthesia during four to six appointments lasting ≈1 hour each. Treatment of the entire oral cavity was completed within 10 to 14 days. SRP was performed by one trained periodontist using manual instruments (IB-J). The antibiotic therapies started immediately after the first session of mechanical instrumentation. Guarulhos University Pharmacy prepared the antibiotic pills, sent them to the study

† Colgate Total, Colgate Palmolive, São Bernardo do Campo, Brazil.
coordinator (MFa), who marked the code number of each patient on a set of two packs, and gave them to the examiner (AR). All patients received clinical and microbiologic monitoring at baseline and 3 months after therapy.

**Monitoring of Compliance and Adverse Events**

The patients were asked to bring the packs containing the medication once a week when compliance was checked. The packs contained 21 capsules of each antibiotic, enough for 1 week of medication. During these visits, patients returned the old pack containing the antibiotic and received a new pack of medication. They also answered a questionnaire about any self-perceived side effects of the medication. One study assistant (ROD) conducted this inquiry and were also responsible for calling the patients every 2 days to monitor compliance.

**Clinical Monitoring**

Clinical monitoring was performed by one calibrated examiner (AR) and the treatment was performed by another clinician (IB-J). Thus, the examiner and clinician were masked as to the nature of the treatment groups. Visible plaque (presence or absence), gingival bleeding (presence or absence), BOP (presence or absence), supputation (presence or absence), PD (in millimeters), and CAL (in millimeters) were measured at six sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual) in all teeth, excluding third molars. The same examiner (AR) participated in a calibration exercise that was performed in 10 non-study patients every 2 days to monitor compliance. The calibration exercise was previously described by Mestnik et al.³⁴ The standard error of measurement was calculated, and the intraexaminer variability was 0.17 mm for PD and 0.20 mm for CAL.

**Investigator Calibration**

The same examiner (AR) participated in a calibration exercise that was performed in 10 non-study patients with CP. The calibration exercise was previously described by Mestnik et al.³⁴ The standard error of measurement was calculated, and the intraexaminer variability was 0.17 mm for PD and 0.20 mm for CAL.

**Microbiologic Monitoring**

**Sample collection.** Subgingival plaque samples were collected at baseline and 3 months after SRP from nine non-contiguous interproximal sites per patient. The selected sites were randomized in different quadrants and subsets according to baseline PD, three samples in each of the following categories: 1) shallow (PD ≤3 mm); 2) intermediate (PD = 4 to 6 mm); and 3) deep (PD ≥7 mm). After the clinical parameters had been recorded, the supragingival plaque was removed, and the subgingival samples were taken with individual sterile curets§ and immediately placed in separate centrifuge tubes containing 0.15 mL TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 7.6]). One hundred microliters of 0.5 M NaOH were added to each tube, and the samples were dispersed using a vortex mixer.

**Checkerboard DNA–DNA hybridization.** Counts of 40 bacterial species were determined in each sample, using the checkerboard DNA–DNA hybridization technique.³⁴,³⁵ The microbiologic analysis was performed entirely at the Laboratory of Microbiology of Guarulhos University. The samples were boiled for 10 minutes and neutralized using 0.8 mL 5 M ammonium acetate. The released DNA was then placed into the extended slots of a minislot apparatus,¶ concentrated on a 15 × 15-cm positively charged nylon membrane,¶ and fixed to the membrane by baking it at 120°C for 20 minutes. The membrane was placed in a miniblotter§ with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes for 40 bacterial species were hybridized in individual lanes of the miniblotters.** After hybridization, the membranes were washed at high stringency, and the DNA probes were detected using the antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. The last two lanes in each run contained standards at concentrations of 10³ and 10⁶ cells of each species. Signals were converted to absolute counts by comparison with the standard lanes on the membrane. The sensitivity of the assay was adjusted to permit the detection of 10⁴ cells of a given species by adjusting the concentration of each DNA probe.

**Primary and Secondary Outcome Variables**

The primary outcome variable of this study was the mean CAL change at 3 months after SRP in sites with initial PD ≥7 mm. Secondary outcome variables were differences between groups for the following parameters: 1) number and percentage of patients with low, moderate, and high risk for disease progression; 2) mean CAL and PD changes in the full-mouth as well as in sites with initial PD between 4 to 6 mm; 3) mean number and percentage of sites/patients with PD ≥5 and ≥6 mm; 4) percentage of sites with BOP, plaque accumulation, gingival bleeding, and supputation; and 5) differences in the occurrence of adverse events and differences between therapies for the mean changes in levels of the 40 bacterial species analyzed and changes in the mean proportion of the microbial complex.

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³⁴ UNC periodontal probe, Hu-Friedy, Chicago, IL.
§ Mini Gracey curets (11/12), Hu-Friedy.
¶ Minislot 30 apparatus, Immunetics, Cambridge, MA.
† Boehringer Mannheim, Indianapolis, IN.
# Miniblotter 45, Immunetics.
** Miniblotter 45, Immunetics.
**Statistical Analyses**

Each individual clinical parameter was computed per patient and then across patients in both groups. Changes in PD and CAL in sites with initial PD = 4 to 6 mm and ≥7 mm or the mean number/percentage of sites with PD ≥5, or ≥6 mm were averaged separately within the PD categories per patient and then across patients in each group. The significance of differences within each group (during the course of the study) was sought using paired Student t test and between groups (at each time point) using the Student t test. Adjustments for multiple comparisons were performed using Bonferroni correction for changes from baseline to 3 months for mean CAL gain and PD reduction in initially deep sites (PD ≥7 mm). The X² test was used to compare differences in frequency of sex, patients exhibiting different categories of residual sites at 3 months after therapy, and self-perceived adverse effects. Mean counts (×10⁵) of individual bacterial species were averaged within each patient and then across patients in both groups. The percentage of the total DNA probe counts was determined initially in each site and then per patient and averaged across patients in the two groups. The significance of differences between groups for the microbiologic parameters was sought using the Mann-Whitney U test. The Wilcoxon signed-rank test was used to detect statistically significant differences within each group between the time points. Adjustments for multiple comparisons were performed when the 40 bacterial species were evaluated simultaneously. The level of significance was set at 5%.

**RESULTS**

**Individual Retention, Compliance, and Adverse Effects**

There were no dropouts during the course of the study period. All patients returned for the 3-month follow-up visit. Thus, a total of 64 patients completed the study, 32 in each group (smokers and non-smokers). Figure 1 presents the flowchart of the study design. All patients reported that they completed the course of the antibiotics, and this information was confirmed by pill counts. Five patients...
from both groups reported adverse events (diarrhea and vomiting) during the study. No significant differences were observed between groups for the number of patients reporting adverse events ($P > 0.05$). All patients reported that they would start the treatment again if necessary.

**Clinical Findings**

Table 1 presents the demographic characteristics and the full-mouth mean clinical data for the clinical parameters evaluated at baseline and at 3 months after therapy. There were no significant differences between groups for any parameter at baseline ($P > 0.05$), except the mean number of cigarettes per day ($P < 0.05$). All therapies led to a significant decrease in mean PD, CAL, and the percentage of sites with visible plaque, gingival bleeding, BOP, and suppuration. At 3 months, the full-mouth mean PD and mean percentage of sites with BOP was statistically significantly lower in the non-smoker group compared with the smoker group.

The mean PD reduction and CAL gain between baseline and 3 months after therapy are presented in Table 2. Non-smokers taking MTZ + AMX exhibited a greater reduction in PD and gain in CAL (primary outcome variable) in initially deep sites (PD ≥7 mm; $P < 0.01$) compared with smokers. They also showed the greatest reduction in the mean PD of initially intermediate sites (PD = 4 to 6 mm).

Table 3 presents the mean number and mean percentage of sites with PD ≥5 mm and PD ≥6 mm at 3 months after treatment. Both groups exhibited

### Table 1.

**Demographic Characteristics and Mean ± SD Full-Mouth Clinical Parameters at Baseline and 3 Months After Therapy**

<table>
<thead>
<tr>
<th>Variables</th>
<th>SRP + MTZ + AMX Non-Smokers (n = 32)</th>
<th>SRP + MTZ + AMX Smokers (n = 32)</th>
<th>$t$ Test ($P$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)*</td>
<td>15/17</td>
<td>16/16</td>
<td>0.8025</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.59 ± 8.52</td>
<td>42.86 ± 7.16</td>
<td>0.0609</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>0.0 ± 0.0</td>
<td>14.41 ± 4.46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>3.97 ± 0.58$^a$</td>
<td>4.17 ± 0.58$^a$</td>
<td>0.1225</td>
</tr>
<tr>
<td></td>
<td>2.70 ± 0.32$^b$</td>
<td>3.12 ± 0.41$^b$</td>
<td><strong>0.0000</strong></td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>4.75 ± 0.80$^a$</td>
<td>4.84 ± 0.80$^a$</td>
<td>0.5456</td>
</tr>
<tr>
<td></td>
<td>3.93 ± 0.90$^b$</td>
<td>4.07 ± 0.79$^b$</td>
<td>0.3024</td>
</tr>
<tr>
<td>% sites with plaque accumulation</td>
<td>73.5 ± 17.8$^a$</td>
<td>74.6 ± 18.2$^a$</td>
<td>0.8090</td>
</tr>
<tr>
<td></td>
<td>34.8 ± 22.5$^b$</td>
<td>44.0 ± 21.6$^b$</td>
<td>0.0807</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% sites with gingival bleeding</td>
<td>45.8 ± 27.3$^a$</td>
<td>42.2 ± 34.2$^a$</td>
<td>0.6576</td>
</tr>
<tr>
<td></td>
<td>19.8 ± 16.9$^b$</td>
<td>22.4 ± 21.9$^b$</td>
<td>0.8097</td>
</tr>
<tr>
<td>% sites with BOP</td>
<td>73.0 ± 18.8$^a$</td>
<td>78.9 ± 23.6$^a$</td>
<td>0.0522</td>
</tr>
<tr>
<td></td>
<td>31.2 ± 23.7$^b$</td>
<td>55.4 ± 25.3$^b$</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>% sites with suppuration</td>
<td>0.91 ± 1.16$^a$</td>
<td>1.5 ± 2.6$^a$</td>
<td>0.0997</td>
</tr>
<tr>
<td></td>
<td>0.03 ± 0.09$^b$</td>
<td>0.03 ± 0.12$^b$</td>
<td>0.1832</td>
</tr>
</tbody>
</table>

The significance of differences between baseline and 3 months after therapy was assessed using paired Student $t$ test (different letters indicate significant differences between time points). The significance of differences between groups at each time point was assessed using the Student $t$ test and $\chi^2$ test (*). $P$ values with a statistically significant difference are bold.
a significant reduction in the number/percentage of these sites during the course of the study ($P<0.05$). The non-smoker group had statistically significantly fewer sites with PD $\geq 5$ mm compared with the smoker group at 3 months after therapy. Although not statistically significant, the non-smoker group showed fewer sites with PD $\geq 5$ mm with BOP ($n=5.15$) compared with the smoker group ($n=8.0$) (data not shown).

Data for residual sites at patient level are presented in supplementary Table S1 in the online Journal of Periodontology. The top of supplementary Table S1 was organized according to the individual risk profile for periodontal disease progression proposed by Lang and Tonetti, as follows: 1) low risk, no more than four sites with PD $\geq 5$ mm; 2) moderate risk, five to eight sites with PD $\geq 5$ mm; and 3) high risk, at least nine sites with PD $\geq 5$ mm. Fewer patients in the non-smoker group ($n=9, 28.2\%$) still had high risk for disease progression at 3 months after therapy (at least nine sites with PD $\geq 5$ mm) compared with the smoker group ($n=18, 56.3\%$). Conversely, 16 patients in the non-smoker group and nine in the smoker group showed low risk for disease progression (no more than four sites with PD $\geq 5$ mm) at the end of the study period. Although not statistically significant, this same trend was observed for different thresholds (none, one to two, or $\geq 3$) of residual sites with PD $\geq 6$ mm.

### Table 2.

**Mean ± SD PD Reduction and CAL Gain From Baseline to 3 Months After Therapy**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>t Test ( (P) value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SRP + MTZ + AMX</td>
<td>SRP + MTZ + AMX</td>
</tr>
<tr>
<td></td>
<td>Non-Smokers (n = 32)</td>
<td>Smokers (n = 32)</td>
</tr>
<tr>
<td>Full-mouth</td>
<td>SRP (mm), 0 to 3 months</td>
<td>1.27 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>CAL (mm), 0 to 3 months</td>
<td>0.80 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>bPD 4 to 6 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD (mm), 0 to 3 months</td>
<td>1.76 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>CAL (mm), 0 to 3 months</td>
<td>1.12 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>bPD $\geq 7$ mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD (mm), 0 to 3 months</td>
<td>3.44 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>CAL (mm), 0 to 3 months</td>
<td>2.64 ± 0.85</td>
</tr>
</tbody>
</table>

The significance of differences between groups at each time point was assessed using the Student $t$ test. Adjustments for multiple comparisons were performed using Bonferroni correction for changes from baseline to 3 months for mean CAL gain and PD reduction in initially deep sites (PD $\geq 7$ mm). $P$ values with a statistically significant difference are bold ($P \leq 0.025$ was considered statistically significant). bPD = baseline PD.

### Table 3.

**Mean ± SD Number (mean ± SD percentage) of Sites With PD $\geq 5$ mm and PD $\geq 6$ mm at Baseline and 3 Months After Therapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>t Test ( (P) value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SRP+MTZ+AMX</td>
<td>SRP+MTZ+AMX</td>
</tr>
<tr>
<td></td>
<td>Non-smokers (n = 32)</td>
<td>Smokers (n = 32)</td>
</tr>
<tr>
<td>PD $\geq 5$ mm</td>
<td>Baseline</td>
<td>42.87 ± 17.35a (33.9 ± 14.4)</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>7.78 ± 7.58b (6.3 ± 6.1)</td>
</tr>
<tr>
<td></td>
<td>$\Delta 0$ to 3 months</td>
<td>35.09 ± 15.19 (27.7 ± 12.9)</td>
</tr>
<tr>
<td>PD $\geq 6$ mm</td>
<td>Baseline</td>
<td>22.87 ± 13.18a (19.0 ± 11.5)</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>3.06 ± 3.58b (2.5 ± 2.9)</td>
</tr>
<tr>
<td></td>
<td>$\Delta 0$ to 3 months</td>
<td>19.80 ± 12.06 (16.6 ± 10.6)</td>
</tr>
</tbody>
</table>

The significance of differences between baseline and the follow-up visits was assessed using paired Student $t$ test (different letters indicate significant differences between time points). The significance of differences between groups at each time point was assessed using the Student $t$ test. $P$ values with a statistically significant difference are bold.
**Microbiologic Findings**

Four species (Veillonella parvulla, Capnocytophaga ochracea, Campylobacter gracilis, and Selenomonas noxia) at baseline presented significantly higher levels in smokers compared with non-smokers (data not shown; \( P < 0.001 \)). Figure 2 presents the mean counts \( (\times 10^5) \) as well as the mean changes of the 40 species evaluated during the course of the study. The species were grouped according to the microbial complexes described by Socransky et al.\(^{38}\) In general, counts of most of the host-compatible species did not change significantly from baseline to 3 months after therapy for both groups (Actinomyces species, purple, yellow, and green complexes). *Actinomyces oris* was the only species that presented a statistically significant increase in levels in the non-smoker group. A reduction in mean counts of several periodontal pathogens from the red and orange complexes was observed, especially in the non-smoker group \( (P < 0.05) \). *Eubacterium nodatum* was the only species from the orange complex that was reduced in the smoker group, whereas six species (C. gracilis, E. nodatum, Fusobacterium nucleatum ssp. nucleatum, F. nucleatum ssp. vincentii, Prevotella intermedia, and Prevotella nigrescens) were reduced in the non-smoker group. The counts of the three pathogens from the red complex, *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola*, were significantly reduced in both groups \( (P < 0.05) \). Overall, the mean changes at 3 months after therapy on the levels of the 40 bacterial species evaluated were quite similar between the two groups, except several orange complex species, which were less affected by therapy in smokers. *F. nucleatum ssp. nucleatum*, *Fusobacterium periodonticum*, and *Porvimonas micra* presented a statistically significantly lower mean reduction in smokers.

Figure 3 shows changes in the proportions of the microbial complexes in the two groups at baseline and 3 months after treatment. The microbial profiles were profoundly affected by treatments, and the most beneficial changes were observed in the non-smoker group. These patients showed a significant reduction in the proportions of red and orange complexes from baseline to 3 months after therapy, as well as an increase in the proportion of beneficial *Actinomyces* species, purple, green and yellow complexes. In the smoker group, the treatment led to a statistically significant reduction of the mean proportions of the red complex and an increase in the proportions of purple and green complexes. At 3 months after treatment, there was a significantly lower proportion of the orange complex species and a higher proportion of the *Actinomyces* species in the non-smoker group compared with the smoker group \( (P < 0.05) \). In addition, between baseline and 3 months after therapy, non-smokers presented a greater change in the proportion of orange complex (reduction of 11.9 percentage points) compared with the smoker group (increase of 0.1 percentage points). Conversely, non-smokers showed a deeper increase in the proportions of the host-compatible *Actinomyces* species (16.9 percentage points) than the smokers (4.2 percentage points) \( (P < 0.05) \).

**DISCUSSION**

To the best of the authors' knowledge, this is the first clinical trial especially designed to directly compare the adjunctive effects of MTZ +
AMX in the treatment of smokers and non-smokers with generalized CP. These short-term results indicated that the clinical and microbiologic benefits observed with the use of this therapeutic protocol were more profound in non-smokers than in smokers.

At 3 months after therapy, non-smokers who submitted to SRP plus MTZ + AMX exhibited statistically significantly greater reduction in mean PD and gain in mean CAL in initially deep sites, as well as lower mean number of sites with PD ≥5 mm (Tables 2 and 3) compared with the smokers who submitted to the same therapy. In addition, data for the remaining deep sites with PD ≥5 mm at the individual level were also most informative (see supplementary Table S1 in the online Journal of Periodontology). Sixteen (50%) non-smokers and only nine (28.2%) smokers achieved a low risk for future disease recurrence according to Lang and Tonetti37 at 3 months, i.e., presented at most four sites with PD ≥5 mm. It should be emphasized that the absence of sites with PD ≥5 mm after treatment is an important clinical endpoint of therapy.30,39

Therefore, the lower effect of the periodontal therapy tested in the present study in reducing these residual sites in smokers should be taken into consideration.

The fact that smokers respond less favorably to mechanical non-surgical periodontal therapy than non-smokers has been suggested previously.18-22 However, information in the literature is very scarce in terms of the effects of the adjunctive use of MTZ + AMX to SRP in the treatment of smokers. Available data come from clinical trials that have included some smokers in the control and/or test groups16,26,40 and from one study that evaluated only smokers.31 Together, the results of these studies as well those from the present investigation demonstrate that smokers normally exhibit, on average, from 50% to 75% of the clinical improvements achieved by the non-smokers,3 even when MTZ and AMX were used, as observed in the present investigation.

In agreement with the clinical results, non-smokers presented the most favorable changes in the subgingival microbial profile after treatment. Both groups showed a striking reduction in the mean levels of the three red complex pathogens (T. forsythia, P. gingivalis, and T. denticola), as well as in the proportions of this complex (Figs. 2 and 3). These results are in agreement with studies that have also demonstrated the adjunctive effects of these two antibiotics in reducing red complex species in populations mainly comprising smokers16,26,31 or non-smokers with CP.26,29,41

When the putative pathogens from the orange complex were evaluated, it was observed that although the therapy used was effective in reducing this complex in non-smokers, this effect was not observed in the smoker group (Fig. 3). Indeed, the only species from this complex that was shown to be reduced in smokers with statistical significance was E. nodatum (Fig. 2), and interestingly, the Fusobacterium species were only reduced in non-smokers (Fig. 2). In fact, the proportion of the orange complex did not change between the two time points (31.0% and 31.1%, respectively) in smokers, whereas in the non-smoker group, this complex presented a significant reduction from baseline to 3 months (30.8% and 18.9%, respectively) (Fig. 3). The lack of effect of the
mechanical therapy, either combined with this antibiotic regimen or not, in suppressing individual levels of orange complex species or the mean proportion of this complex in smokers has been suggested previously. \cite{29,31} The present authors reported previously that species from the orange complex might persist after periodontal therapy in smokers, even when MTZ + AMX were used as adjuncts to mechanical treatment. At 3 months after therapy, smokers who received SRP combined with MTZ + AMX showed increased mean proportions of this complex, which went from 30.9% to 32.2% between baseline and 3 months. Those who received SRP only showed an even higher increase in these pathogens, from 29.1% to 36.7%. \cite{31} Because the suppression of this complex is one of the important endpoints of the periodontal therapy, \cite{31,43} it may be speculated that the poorer clinical effects observed in smokers in the present study are associated with the lack of efficacy of treatment in reducing the orange complex, more specifically \textit{Fusobacterium} species.

There was a trend toward an increase in some host-compatible species, particularly those from the purple and green complexes in both groups. These results are in agreement with previous reports. \cite{29,31} However, it is interesting to note that the proportions of the \textit{Actinomyces} species, highly associated with periodontal health, showed a statistically significant increase after therapy only in the non-smoker group.

Several biologic pathways, such as the alteration of the neutrophil function, antibody production, local effects of nicotine, differences in the subgingival microbiota, or in the production of inflammatory mediators, have been suggested as possible causes for the poorer response to therapy observed in smokers when compared with non-smokers with CP. \cite{18,22,31} and therefore could have influenced the clinical and the microbiologic response for the smoker group in the present study. Another possible explanation, particularly associated with the treatment regimen used in the present study, is that smoking might interfere with the bioavailability of MTZ in plasma. \cite{49} Montalli et al. \cite{49} detected a statistically significant reduction in plasmatic MTZ concentration in smokers compared with non-smokers. Because there is a positive correlation between the bioavailability of MTZ in plasma and GCF, \cite{50} the antibiotic concentration in the subgingival environment may be impaired by smoking, leading to the reduced clinical and microbiologic effects of the therapy observed in the present study. However, this hypothesis regarding antibiotic bioavailability in GCF of smokers should be further explored.

Overall, the results of this study confirm that smokers respond less favorably to periodontal therapy than non-smokers. Interestingly, even the very potent therapy used in this study—which has shown excellent results for the treatment of non-smokers—is not as effective for the treatment of smokers. These results suggest that other adjunctive therapies, such as lasers, alternative antibiotic protocols, or even host modulators focusing on the enhancement of the healing process, could be further explored for improving the clinical and microbiologic outcomes of this group of patients.

**CONCLUSIONS**

Smokers with CP treated with SRP + AMX + MTZ show less favorable clinical and microbiologic outcomes than non-smokers. Other treatment modalities should be studied for the treatment of these patients.

**ACKNOWLEDGMENT**

The authors report no conflicts of interest related to this study.

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Submitted April 24, 2013; accepted for publication June 5, 2013.