Repeated Metronidazole and Amoxicillin Treatment of Periodontitis. A Follow-Up Study

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Background: The prevailing concept is that little or no clear benefit is derived from antibiotic therapy in chronic periodontitis. Studies to determine the effect of metronidazole plus amoxicillin (M+A) on adult periodontitis are questionable because standard design for clinical trials was usually not used. In addition, there is no information about the effect of M+A as the sole therapy for periodontitis.

Methods: A randomized, triple-blind, controlled clinical trial was used to determine the effect of systemic administration of M+A, as the sole therapy, in progressive adult periodontitis. Forty-six subjects with moderate to advanced adult periodontitis who showed ≥2 mm attachment loss in at least 2 sites in the previous 2 months were entered in the study. Subjects were randomly distributed to a group who received 21 tablets of metronidazole 250 mg plus amoxicillin 500 mg, or to a group receiving a placebo (1 tablet every 8 hours for 1 week). Patients were examined every 2 months for 12 months. The M+A or placebo regimen was repeated at 4 and 8 months. No effort was made to change the oral habits of patients and they received no additional therapy. Differences between groups were assessed using the Mann-Whitney U test. The differences at every 2-month interval within each group were assessed using the ANOVA test.

Results: Seven subjects abandoned the study; at 12 months the M+A group had 20 subjects and the placebo group 19. There were no significant differences in the clinical parameters at baseline between the 2 groups. After 2 months and thereafter, the M+A group showed significant clinical improvement while the placebo group showed a progressive deterioration of periodontal status. At 12 months compared to baseline, subjects of the M+A group showed: 1) a significant overall mean attachment gain of 0.43 mm ($P = 0.005$); 2) a significant decrease of active sites ($P \leq 0.03$); 3) a significant increase of sites gaining attachment level ($P \leq 0.01$); 4) a significant reduction of pocket depth ($P \leq 0.00006$); and 5) a significant decrease in percentage of bleeding on probing sites (BOP) ($P \leq 0.0005$). Significant differences between both groups at all 2-month evaluations were found in overall mean attachment level ($P \leq 0.000004$), in percent of active sites ($P \leq 0.03$), and in percent of BOP sites ($P \leq 0.02$). Sites exhibiting ≥2 mm of attachment loss in 2 successive or alternate evaluations, and periodontal abscess were noticed only in the placebo group.

Conclusions: A 1-week course of systemic M+A every 4 months, as the only therapy, arrests the progression of adult periodontitis and significantly improves the clinical parameters of the disease. *J Periodontol 2000;71:79-89.*

KEY WORDS

Triple-blind studies; clinical trials, controlled; metronidazole/therapeutic use; amoxicillin/therapeutic use; periodontitis/drug therapy.

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Periodontal diseases are caused by subgingival infection and microbiological studies have suggested that these diseases are associated with specific pathogens.[1] Treatment of periodontal diseases has been based largely on mechanical root debridment to eliminate subgingival plaque. This therapy has been found to be effective for the majority of patients. In the last 20 years mechanical therapy has been frequently used with antimicrobial agents systemically or locally delivered to increase the success of periodontal therapy. The use of antibiotics to control periodontal infection has been based on the experimental evidence that suggests that these diseases are caused by specific pathogens, mainly anaerobic and Gram-negative microorganisms.[2]

Clinical studies support the use of systemically administered antibiotics in the treatment of localized juvenile periodontitis[3-7] and refractory periodontitis.[8-13] The prevailing concept is that little or no clear additional benefit is derived from antibiotic therapy in chronic adult periodontitis,[14-17] even though there are several other studies[18-24] that have shown favorable results of adjunctive antibiotic therapy on adult periodontitis. Metronidazole has been extensively studied as an antimicrobial for treatment of periodontitis, and its ability to improve periodontal status has been evaluated in several clinical trials.[21-32] However, the use of metronidazole as an adjunctive therapy in adult periodontitis is still controversial. A review on the effects of metronidazole in the treatment of periodontal diseases concluded that "its routine use in conjunction with root planing is unnecessary, whereas drug administration helped arrest disease progression in patients refractory to conventional therapy."[33] Metronidazole used as a monotherapy is not supported by some clinical trials,[28,32] but a recent meta-analysis study[34] of the effect of systemic metronidazole as an adjunct to scaling and root planing concluded that its use may offer a benefit in the treatment of adult periodontitis. Results of several studies concerning the beneficial effect of metronidazole are questionable because well-defined evaluations were absent. A double-blind format, which is the standard design for clinical trials, was not used, and disease activity was not documented before the antibiotic was administered. Metronidazole plus amoxicillin (M+A) has been used successfully in the treatment of advanced periodontitis, especially with *A. actinomycetemcomitans*-associated infections.[35-38] In these studies, M+A has demonstrated a positive effect on periodontitis in presence of good oral hygiene and subgingival debridement. At present, insufficient studies have been done using M+A to determine the success of this therapy, and there is no information about its effect on adult periodontitis in the absence of any other therapy.

A controlled, randomized clinical trial was designed to determine whether systemic M+A administered to subjects with adult progressive periodontitis has the ability to arrest the progression of the disease. The study was used to establish whether a 1-week-course of M+A as the only treatment, given 3 times every 4 months, would have any effect on arresting the progression of moderate to advanced adult periodontitis. The null hypothesis tested was that clinical parameters in patients with adult progressive periodontitis treated with M+A do not show statistical differences from patients treated with a placebo.

Earlier, we reported the clinical and microbiological results after 2 and 4 months of a 1-week course of M+A as the only therapy in progressive adult periodontitis.[39] That report included the results found on a test group of 23 subjects and a placebo group of 21 subjects. The present report describes the clinical results of the 12-month study of 20 subjects in the M+A group and 19 subjects of the placebo group.

MATERIALS AND METHODS

Sixty patients, 18 men and 42 women (36 to 68 years old; mean age 43.6 ± 8 years), were selected from the pool of patients attending a Public Dental Service and the Periodontics Clinic at the Dental School of the University of Chile in Santiago. All patients had at least 14 treatable natural teeth, 4 of which were molars; at least 4 pockets ≥4 mm; and at least 6 sites with attachment loss >3 mm, as well as radiographic evidence of moderate to advanced destructive periodontal disease.

Exclusion criteria included previous periodontal treatment; medical conditions requiring premedication with antibiotics for periodontal probing; administration of medications such as antibiotics, steroids, or nonsteroidal anti-inflammatory drugs within the previous 6 months; and systemic diseases that might affect periodontal disease activity. Prior to the start of the study each subject received a supragingival scaling to remove gross calculus thus allowing access for probing. Patients were then enrolled in a disease-monitoring phase designed to detect periodontal activity by measuring attachment loss. The criteria of active destruction used for this study was defined as a 2 mm or greater loss of attachment in at least 2 sites, or the occurrence of a periodontal abscess. Patients were monitored every 2 months until periodontal activity was detected.

All patients were told that the aim of the study was to investigate the effect of a new antibiotic combination to treat periodontal disease. All patients signed an informed consent before participating in the study, and the protocol was approved by the review committee for ethical norms of the Faculty of Dentistry, University of Chile. The protocol research stated that if a patient showed one or more teeth exhibiting attachment loss in 2 successive or alternate evaluations or a periodontal abscess during the study period, the tooth

or teeth would then be treated with root planing and excluded from the study.

Clinical Measurements

The following variables were determined at the beginning of the study and each 2 months after:

Oral hygiene status. The presence of continuous plaque at the cervical portion of the buccal, mesial, lingual, and distal surfaces of each tooth was recorded. Plaque scores were calculated as the percentage of surfaces examined demonstrating plaque.

Gingival inflammation. Dichotomous measures of bleeding within 30 seconds after probing was determined at the same 6 sites on each tooth on which the probing depth was measured.

Probing depth and relative attachment level measurements. These were made at 6 sites on each tooth at the mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual positions of every tooth present with the exception of third molars. Two models of an automated probe were used.‡ A disk probe was used for relative attachment level recording, and a pocket depth probe was used for probing depth and bleeding on probing recordings. The probe had a titanium tip 0.4 mm in diameter; the measurement interval was 0.2 mm; and the applied load was 20 g. Probing depth and attachment level measures were made in molars with furcation involvement only in the vertical dimension. A second attachment level measurement and probing depth were taken within 7 days of the first measurement. Therefore, a pair of attachment level measurements and probing depths were made every 2 months.

The mean of the pair of measurements was used in all the analyses described below. Two calibrated examiners monitored the subjects, but all clinical measurements taken on each individual were made by the same examiner at each time period. Since replicate measurements were made at each 2-month evaluation, each examiner was provided with data to determine his reproducibility at each subject's evaluation.

Subjects were monitored every 2 months until periodontal activity was detected. The mean time of disease-monitoring phase was 4 months with a range of 2 to 8 months. Upon the detection of disease activity, subjects were entered into the treatment phase.

Forty-four of the 60 subjects exhibited at least 2 sites with ≥2 mm attachment loss, and 2 subjects showed periodontal abscess during one of the 2-month periods. Participants were randomly assigned to a group receiving 21 tablets of metronidazole 250 mg plus amoxicillin 500 mg, or 21 tablets of a placebo, and asked to take 1 tablet every 8 hours for the following week. M+A tablets and placebo tablets had the same appearance and were packed in identical containers labeled Tab A and Tab B. Neither examiners nor patients were aware of the identity of the tablets. Patients were warned verbally not to consume alcoholic beverages until one day after completing the medication. Smokers were randomly assigned to both groups. Every 2 months, clinical examinations were again recorded until completion of the 12-month monitoring period. The drug or placebo therapy was repeated 2 additional times after 4-month intervals; that is at 4 and 8 months after baseline (Table 1).

Table 1.

Experimental Schedule

	Months						
	0	2	4	6	8	10	12
Examination	●	●	●	●	●	●	●
M+A or placebo	●		●		●		

After the 2-, 6-, and 10-month visits, patients were asked to return the pill containers. Compliance with the medication was checked by interviews and counting the remaining tablets. Throughout the study, no effort was made to change the oral hygiene habits of patients, and they received no additional periodontal therapy during the 12-month period.

Statistical Analyses

Differences of clinical measurements between experimental and control groups at baseline were assessed using the *t* test. The variables evaluated in the present study were changes in mean attachment level and the percentage of sites that gained or lost ≥2 mm attachment in each subject. Change in attachment level was computed for each site, averaged within a subject, and then averaged across individuals in experimental and control groups. Change in probing depth from pre- and post-therapy, percentage of sites bleeding on probing, and percentage of surfaces with plaque accumulation were tested in the same way. The significance of differences between the 2 groups was assessed using the Mann-Whitney U test. The test of comparison of proportions from independent samples[40] was used to determine significant differences in proportions of subjects of the M+A group and subjects of the placebo group that showed an overall gain or loss in clinical attachment. The significance of differences in attachment level, probing depth, bleeding on probing, and plaque accumulation every 2 months post-therapy within each group was assessed using the ANOVA test for univariate repeated measures. The significance level was set at $P<0.05$. The statistician who performed the statistical analysis was masked about the identity of the

‡ Florida Probe Corporation, Gainesville, FL.

experimental group. A statistical package§ was used in all data analyses.

RESULTS

No patient experienced any adverse side effects to medication. Two patients of the placebo group were dropped from the study at 2 months because they had taken an antibiotic for medical reasons. Two patients of the placebo group, and 3 patients of the M+A group, decided to abandon the study after 6 and 8 months, respectively, because they did not have time for the control examinations. All these patients were excluded from data analysis. Thus, the M+A group consisted of 20 subjects and the placebo group of 19 subjects who attended every 2-month control during the 12-month period.

The immediate pretreatment clinical features of all subjects are presented in Table 2. No age or gender differences existed between the 2 groups. There were no significant differences in the clinical parameters at baseline between the 2 groups. Table 3 presents the changes in the mean of clinical parameters in the M+A group, and Table 4 in the placebo group from baseline until 12 months.

Table 2.

Clinical Characteristics (mean ± SD) of M+A and Placebo Patients Prior to Treatment

Characteristic	M+A (n = 20)	Placebo (n = 19)
Age (years)	46.7 ± 6.8	44.1 ± 8.8
N missing teeth	5.62	4.01
% males	15	15.78
Mean probing depth (mm)	2.93 ± 0.92	2.66 ± 0.66
Mean attachment level (mm)	3.4 ± 1.63	3.2 ± 1.2
% active sites	3.3 ± 1.63	3.14 ± 1.45
% sites with		
Plaque	62.2 ± 18.9	57.7 ± 17.8
Bleeding on probing	33.2 ± 14.6	29.1 ± 14.2
Probing depth <4 mm	84.32 ± 9.8	82.87 ± 7.1
Probing depth 4-6 mm	12.2 ± 8.9	14.2 ± 8.8
Probing depth >6 mm	3.48 ± 2.6	2.88 ± 1.56
% smoking subjects		
5-10 cigarettes/day	25	31.57
>10 cigarettes/day	15	10.5

Compliance

Assessment of tablet ingestion compliance was determined by counting the remaining pills in the returned containers at 2-, 6-, and 10-month visits. All patients returned the containers empty and reported they had taken the medication as indicated. But, after finishing the study period, the results showed that 3 subjects of the M+A group had a low response to therapy in contrast with all the other subjects in that group. It was decided to re-examine these 3 subjects to determine if they had complied with the instructions. The 3 subjects were separately re-interviewed and it was explained that results of their treatment were different from other subjects, and that this suggested non-compliance with instructions. All 3 acknowledged that they had taken all the tablets, but that on some days, they had forgotten to take the drug according to instructions. Lack of compliance was thus verified in the 3 subjects who showed a lower response to treatment.

When the values of the clinical parameters at every 2-month control were compared to the baseline values within each group, there were more significant changes within the M+A group than within the placebo group.

Clinical Attachment Level

While subjects in the M+A group showed a greater improvement in mean attachment level after beginning therapy, there were subjects in each group showing mean loss of attachment. Two months after beginning the therapy, 19 subjects in the M+A group (95%) and 4 subjects in the placebo group (21%) showed an overall mean gain of attachment; this difference was statistically significant ($P = 0.001$). At 4 months, 18 subjects (90%) in the M+A group and 3 subjects (16%) in the placebo group exhibited an average gain in attachment; this difference was also significant ($P = 0.001$). One subject of the placebo group showed no change in attachment level at 2 months, and 2 subjects of the same group exhibited no change at 4 months. At 12 months, only 1 subject of the M+A group (5%) exhibited an overall mean loss of attachment (0.16 mm), while in the placebo group 16 subjects (84%) exhibited an overall mean loss of attachment, and this difference was statistically significant ($P = 0.001$). The overall mean attachment gain for subjects treated with M+A was 0.26 mm at 2 months, increasing to 0.46 mm at 12 months, and this difference was significant ($P = 0.009$). In contrast, the placebo group showed a significant increase of the mean attachment loss from 0.13 mm at 2 months to 0.43 mm at 12 months ($P = 0.005$). The overall mean attachment level values for subjects of the M+A group improved significantly compared to those in the placebo group, and

§ Systat 7.0, SPSS, Chicago, IL.

Table 3.

Changes in Clinical Parameters in the M+A Group (n = 20) Every 2 Months Until 12 Months Post-Therapy

Parameter (% of sites)	Baseline	2 Months	4 Months	6 Months	8 Months	10 Months	12 Months
With plaque	62.2 ± 18.9	61 ± 15.5	62.6 ± 16.2	62.7 ± 18.2	59.1 ± 15.2	57.6 ± 15.2	55.7 ± 12.9
Bleeding on probing	33.2 ± 14.6*	20.3 ± 12.3*	20.3 ± 8.7*	18.7 ± 8.5*	17.6 ± 8.3*	16.5 ± 6.9*	16.1 ± 7*
Active sites	3.3 ± 1.63†	1.17 ± 1.24†	0.95 ± 0.87†	1.01 ± 0.93†	1.25 ± 1.03†	0.66 ± 0.74†	0.95 ± 0.88†
Gaining attachment level	0.57 ± 1.03‡	1.48 ± 1.8	2.32 ± 1.93‡	1.25 ± 1.79	1.63 ± 1.58‡	2.05 ± 2.04‡	2.01 ± 1.48‡
Mean attachment gain (mm)	0	0.26 ± 0.27§	0.25 ± 0.22	0.35 ± 0.29	0.39 ± 0.31§	0.45 ± 0.35§	0.46 ± 0.38§
Mean probing depth (mm)	2.93 ± 0.92‖	2.64 ± 0.81‖	2.57 ± 0.71‖	2.42 ± 0.65‖	2.42 ± 0.58‖	2.38 ± 0.61‖	2.35 ± 0.60‖

* Baseline versus: 2 months ($P = 0.0001$), 4 months ($P = 0.0005$), 6 months ($P = 0.0002$), 8 months ($P = 0.0001$), 10 months ($P = 0.0002$), and 12 months ($P = 0.0001$).
† Baseline versus: 2 months ($P = 0.001$), 4 months ($P = 0.0002$), 6 months ($P = 0.0004$), 8 months ($P = 0.0005$), 10 months ($P = 0.0001$), and 12 months ($P = 0.0003$).
‡ Baseline versus 4 months ($P = 0.0001$), 8 months ($P = 0.03$), 10 months ($P = 0.0004$), and 12 months ($P = 0.004$).
§ 2 months versus 8 months ($P = 0.05$), 10 months ($P = 0.01$), and 12 months ($P = 0.009$).
‖ Baseline versus 2 months ($P = 0.0004$), 4 and 8 months ($P = 0.0006$), 6 months ($P = 0.0003$), 10 and 12 months ($P = 0.0002$).

Table 4.

Changes in Clinical Parameters in the Placebo Group (n = 20) Every 2 Months Until 12 Months Post-Therapy

Parameter (% of sites)	Baseline	2 Months	4 Months	6 Months	8 Months	10 Months	12 Months
With plaque	57.7 ± 17.8*	57.3 ± 17.3	64.4 ± 19.1	63.4 ± 16.4	64.4 ± 14.8*	61.3 ± 11.5	65 ± 13.7
Bleeding on probing	29.1 ± 14.2†	31.4 ± 15.8	33.3 ± 17.6	34 ± 17.4†	35.7 ± 17.7	36.4 ± 16.5†	38.2 ± 16.4†
Active sites	3.14 ± 1.45	3.06 ± 2.09	2.8 ± 1.72	4.13 ± 3.05	2.98 ± 3.09	2.83 ± 1.74	3.36 ± 1.75
Gaining attachment level	0.53 ± 0.78	0.60 ± 1.35	0.88 ± 1.17	0.50 ± 0.82	0.59 ± 0.74	0.38 ± 0.52	0.13 ± 0.34
Mean attachment loss (mm)	0	0.13 ± 0.18‡	0.23 ± 0.32	0.32 ± 0.34‡	0.32 ± 0.38‡	0.38 ± 0.39‡	0.43 ± 0.43‡
Mean probing depth (mm)	2.66 ± 0.66§	2.71 ± 0.64	2.72 ± 0.69	2.74 ± 0.72	2.84 ± 0.72§	2.83 ± 0.77§	2.95 ± 0.82§

* Baseline versus 8 months ($P = 0.02$).
† Baseline versus: 6, 10, and 12 months ($P \leq 0.03$).
‡ 2 months versus: 6 months ($P = 0.005$), 8 months ($P = 0.02$), 10 months ($P = 0.007$), and 12 months ($P = 0.005$).
§ Baseline versus 8 months: ($P = 0.01$), 10 months ($P = 0.04$), and 12 months ($P = 0.007$).

the differences between both groups were highly significant ($P \leq 0.000004$) at all the 2 month-evaluations (Tables 3 and 4).

Active Sites

The proportion of sites losing ≥2 mm attachment level, or "active" sites, in the M+A group decreased significantly from 3.30% at baseline to 1.17% ($P = 0.0014$) at 2 months after the administration of the first course of M+A. After 2 months there was a sustained decrease in the proportion of active sites in the M+A group until the end of the study (Table 3). The differences between the proportion of active sites in the M+A group in all the 2-month controls compared to baseline value were statistically significant ($P \leq 0.0005$). The proportion of active sites in the placebo group showed small and non-statistically significant changes from day 0 to 12 months. The difference of the proportion of active sites between the M+A group and the placebo group was highly significant at 2 months ($P = 0.002$), at 4 months ($P = 0.0003$), at 6 months ($P = 0.0003$), at 8 months ($P = 0.030$), at 10 and at 12 months ($P = 0.0001$) (Tables 3 and 4).

Sites Showing Gain in Attachment Level

Subjects receiving M+A showed a greater percentage of sites exhibiting ≥2 mm of attachment gain at 2 months after beginning the therapy and thereafter, compared to the placebo group (Table 3). The proportion of sites gaining attachment level in the placebo

group did not show significant changes from 2 to 12 months (Table 4). The percentage of sites gaining attachment level was significantly higher in the M+A group compared to the placebo group, and the difference between both groups was significant at 4 months ($P = 0.008$), at 8 months ($P = 0.01$), at 10 ($P = 0.001$), and at 12 months ($P = 0.0001$).

Probing Depth

Treatment with M+A resulted in an overall mean reduction in probing depth of 0.29 mm at 2 months and 0.58 mm at 12 months. The difference between the baseline probing depth and the probing depth assessed in all the following 2-month evaluations in the M+A group was statistically significant ($P \leq 0.0006$) (Table 3). The mean PD in the placebo group showed a steady increase from 2.66 mm at baseline to 2.95 mm at 12 months (difference + 0.29) (Table 4). The difference in the increased mean PD in the placebo group was significant at 8 months ($P = 0.010$), at 10 months ($P = 0.044$), and at 12 months ($P = 0.0074$) when compared to baseline value. The difference in the mean probing depth between the 2 groups was significant only at 12 months ($P = 0.015$).

Percentage of Bleeding on Probing Sites

The percentage of BOP sites in the M+A group decreased significantly at 2 months compared to baseline ($P = 0.0001$), and from there showed a sustained decreasing trend up to the 12-month control. The differences of the percentage of BOP between day 0 and all the following evaluations were significant ($P \leq 0.0005$) in the M+A group. In contrast, the percentage of bleeding sites in the placebo group increased from 2 months to 12 months, and the differences from baseline were significant at 6 months ($P = 0.038$), at 10 months ($P = 0.031$), and at 12 months ($P = 0.0079$). The differences of the proportion of BOP sites between M+A group were significant at all the 2-month evaluations ($P \leq 0.02$).

Proportion of Surfaces With Plaque

At baseline and at the various 2-month examinations the frequency of tooth surfaces with plaque was high in both groups (Tables 3 and 4). There was no significant increase in the percentage of surfaces with plaque in the placebo group at 2, 4, 6, 10, and 12 months. Only at 8-month control was the difference of the surfaces with plaque significantly higher compared to day 0 ($P = 0.021$) in the placebo group. In the M+A group, the surfaces with plaque remained almost unchanged at 2, 4, and 6 months. At 8, 10, and 12 months there was a slight and non-statistically significant decrease in surfaces with plaque compared to day 0. The differences between patients treated with M+A and patients treated with placebo as regards the proportion of surfaces with plaque were significant only at 12-month examination ($P = 0.037$) (Tables 2 and 3).

Teeth Showing Repeated Attachment Loss

A total of 24 sites in 4 patients of the placebo group exhibited ≥2 mm of attachment loss in 2 successive or alternate evaluations, and 6 teeth showed periodontal abscess in 4 patients in the placebo group. Teeth with 1 or more sites showing attachment loss in 2 successive or alternate evaluations, or teeth affected by abscess were treated with root planing and eliminated from the data used to compile results in the successive patient evaluations. In the M+A group no patient was found with sites exhibiting ≥2 mm of attachment loss in 2 successive or alternate evaluations, nor was any tooth affected by abscess noticed during the study period.

DISCUSSION

The present research intended to fulfill all the requirements of a standard design for clinical trials;[41-45] i.e., 1) a control group equivalent to the test group in all the significant variables associated with periodontal disease was employed; 2) subjects were randomly distributed to treatment groups; 3) a triple-blind format was used, since the statistician who performed the statistical analysis, as well as the examiners and the patients, were masked as to the identity of the experimental group; and 4) the patient was adopted as the unit of study.[46] All the subjects included in the present study were entered in the therapy phase only after they had shown previous disease progression assessed by the detection of at least 2 sites losing ≥2 mm attachment level or the occurrence of a periodontal abscess. This criterion has been employed in a number of other clinical studies.[11,13,47-49] In addition, every possible precaution was taken to minimize changes due to experimental error. Thus, an automated force-controlled probe and calibrated examiners were used. The same operator made the examinations on each individual at each time period. Replicate measurements at each patient evaluation and longitudinal measurements were taken.

The data of the present investigation indicated that a 1-week-course of systemic M+A, as the only treatment administered every 4 months, in subjects with moderate to advanced progressive adult periodontitis not only arrested the progression of periodontitis, but was also effective in obtaining a significant overall mean attachment gain of 0.46 mm and significant overall mean probing depth reduction of 0.58 mm. In addition, M+A significantly reduced the percentage of bleeding sites, the proportion of active sites, and increased the proportion of sites gaining attachment level. Four months after beginning the M+A therapy there was an additional slight and non-significant

improvement in the clinical parameters compared to the 2-month control (Table 3), but in each following 2-month evaluation, after repeating the administration of M+A at 4 and 8 months, a steady and sustained improvement in all the clinical parameters was noticed. In contrast, the placebo group showed a progressive deterioration of periodontal status (Table 4). The decision to administer M+A every 4 months was made based on the results of studies[50-54] that showed that recolonization of gingival sulcus by periodontopathogens begins around 2 months after mechanical or antibiotic therapy has finished. Based on these studies, we speculated whether M+A could eliminate or reduce the main pathogens of the periodontal infection, in which case the effect would last at least 2 months. Since microbial recolonization usually begins 2 months after therapy, the microorganisms would take at least 2 additional months to reach the level of colonization necessary to produce detectable clinical changes in the periodontal tissues. In addition, Lindhe et al.[21] reported that repeated dosages of metronidazole achieved clinical and microbiological results equivalent to root planing. Therefore, the regimen to administer M+A was chosen to imitate the initial phase of conventional therapy and the supportive therapy at 4-month intervals.

Since patient compliance with the unsupervised usage of prescribed medication is critical to validate the results of a clinical trial, the present study tried to enhance patients' compliance by maintaining a good relationship with study subjects. The method to assess patient compliance with the medication by interviews and tablet count, as used in the present study, has been found not reliable.[55] Loesche et al.[56] found 44% of patients non-compliant in a double-blind clinical trial involving the unsupervised usage of metronidazole. Compliance may be assured by having the patients take the medication under supervision, but this is impractical with ambulatory patients. Monitoring declination or disappearance of spirochetes from subgingival plaque has been suggested as a means of measuring patient compliance in taking metronidazole.[56] This method was not applied in the present study because it would have been necessary to use another operator, other than the 2 examiners, in order to maintain the blindness of the trial.

Successful periodontal treatment is dependent on a substantial reduction of the commensal flora, the complete eradication of the exogenous periodontopathogens in the subgingival flora, or a decrease in the proportion of pathogens to a level manageable by the host.[57] The significant improvement in clinical parameters observed with the use of M+A in patients who did not receive any other form of therapy is related to suppression or elimination of the pathogens associated with their disease. A microbiological study done in the same patient population in the present study has been published.[39] According to that study, the improvement of the clinical parameters 2 months after M+A therapy began was associated with a significant decrease in percentages of sites harboring high levels of *P. gingivalis,* and with the elimination of sites with high and moderate levels of *P. intermedia*.

Systemic periodontal antimicrobial therapy is based on the premise that specific microorganisms cause destructive periodontal disease and that the antimicrobial agent in the periodontal pocket must reach the concentrations necessary to eliminate the pathogens.[58] It has also been suggested that in the treatment and control of periodontal disease, the selective elimination of pathogenic microorganisms, rather than the subgingival microbiota *in toto,* may be preferred.[59] Metronidazole has been shown to have a pronounced effect on the subgingival microbiota of periodontal lesions in humans,[23,24,31,33,38] to affect plaque development and gingivitis in dogs[60,61] and prevent development of periodontitis in monkeys.[62] It has been found that after multiple 250 mg doses, metronidazole can reach a concentration of 26.7 ug/mL in the gingival crevicular fluid,[32,63] and a single oral dose of 750 mg of metronidazole give range concentrations of 8.7 to 13.8 ug/mL in gingival crevicular fluid,[64] that exceed the minimal inhibitory concentration for most anaerobic oral microorganisms.[65,66] Otherwise, amoxicillin appears to be very effective against most periodontal pathogens[66,67] and exhibits high antimicrobial activity at levels that occur in gingival crevicular fluid.[63] The combination of M+A has been found to be an effective adjunctive therapy for the elimination of *A. actinomycetemcomitans,*[35-38] and it would be expected to target a broad spectrum of periodontal organisms with metronidazole inhibiting the anaerobes, and amoxicillin inhibiting the facultative and aerobic bacteria.[68] In several clinical studies, systemic metronidazole has been shown to augment the clinical effect of mechanical periodontal treatment in adult periodontitis patients[21,23,24,34] and the administration of metronidazole, when poor hygiene was practiced, did not enhance the effects of scaling.[15,69] Metronidazole therapy without concomitant scaling and root debridement provides very short-lived clinical and microbial benefits.[28,32] The significant improvement of clinical parameters in patients treated with M+A as the only treatment may be ascribed to the synergistic effect between amoxicillin and metronidazole against periodontal pathogens.[70] The combined effect of M+A may overcome the low efficacy of metronidazole when used in the absence of a predominant anaerobic infection, or as the only therapy.

There are very few published studies on the use of the combination of M+A on adult periodontitis. Thus, it is difficult to compare the results from this and

other studies, since the subject population and the methods used were different. In addition, these other studies[35-38] did not employ control groups, nor use a double-blind format which is the standard design for clinical trials. They also lacked documentation of disease activity before the antibiotic therapy was administered.[38]

The main intention of the present investigation was to study the effects of an antimicrobial combination in the treatment of moderate to advanced adult periodontitis, and not to replace the mechanical therapy by antibiotic therapy. However, according to the results of the present study, M+A could be applied to prolong or amplify the effect of the mechanical treatment in adult periodontitis patients, and in some cases could be used to enhance or replace the mechanical treatment. The serial antibiotic regimen used in the current study could provide a useful protocol for treatment of problematic periodontal patients with advanced disease who do not respond to oral hygiene instructions. Nevertheless, more long-term clinical and microbiological studies are necessary to determine if the combination of M+A as antimicrobial periodontitis therapy may replace traditional mechanical treatment in some patients, extend the interval of time between visits, or shorten the time that the conventional therapy takes. Many studies have shown that patients who are not properly maintained following active therapy frequently develop recurrent periodontitis including additional attachment loss.[71,72] Since periodontitis has a propensity for recurrence, there is need for regimens which can produce a more sustained clinical stability without the requirement of frequent retreatment. If an antimicrobial drug can effectively reduce the dependency on long-term supportive therapy to maintain periodontal health, a larger segment of the population would benefit from periodontal therapy.

The significant improvement in patients treated with M+A is surprising, considering that there was no additional periodontal treatment during the 12 months, and no efforts were made to instruct the patients in supragingival plaque control. The average of the percentage of surfaces with dental plaque, which was 57.8% at baseline in the placebo group and 62% in the M+A group (Tables 2 and 3), indicates that all the subjects of the present study had poor oral hygiene. The percentage of surfaces with plaque increased slightly during the 12-month period in the placebo group, and decreased (also slightly) in the M+A group. The difference was significant between both groups only at 12 months. This significant difference at 12 months suggests that M+A administered every 4 months may reduce supragingival plaque. Supragingival plaque control has been shown to be a major factor in determining success after conventional therapy[73-76] and it strongly influences both clinical and microbiological outcomes when antibiotics are used.[77] All these studies have suggested that failing to obtain an appropriate level of plaque control after therapy may reduce the success of therapy. Otherwise, there is a significant discrepancy in the literature regarding the effect of dental plaque removal and of supragingival plaque control on the subgingival microflora.[78] Since no therapy other than systemically administered M+A was used in the present study, the arrested progression of periodontitis and the improvement of clinical parameters in the subjects can only be ascribed to the antimicrobial effects of M+A. The results of the present study suggest that not removing subgingival and supragingival plaque may have no influence on the outcome of the therapy if the antimicrobial therapy eliminates or reduces the pathogenic microorganisms of the subgingival microbiota. The possible changes in subgingival microflora ascribed to the effect of M+A are apparently independent of the presence of supragingival plaque. However, the possibility that after the repeated administration of antimicrobials, the antibiotic levels in saliva may have had some effect on the microbial composition of supragingival plaque, cannot be excluded. Metronidazole has been found in saliva at levels which are much higher than that required for inhibition of *Treponema, Bacteroides,* and other anaerobic organisms.[64]

It has been suggested that the effect of antibiotics on subgingival microflora is transient due to a major effect of the antimicrobial on bacteria that are in suspension or not adhering to the biofilm subgingival dental plaque.[79] Otherwise, an in vitro study[80] indicated that biofilm-associated *P. gingivalis* may be resistant to metronidazole in concentrations which are usually reached by systemic administration. However, in the present study, the additional courses of M+A appeared to have overcome the transient antimicrobial effect of the first course of M+A on the subgingival bacteria which existed within the subgingival biofilm, as evidenced by the significant improvement in clinical parameters of periodontitis. However, it is necessary to do additional microbiological studies to determine, in the long term, the changes of the subgingival microbiota which may occur after giving the treatment described in the current study. In addition, it is necessary to determine whether the therapeutic modality used in the present study can produce bacterial resistance.

CONCLUSIONS

A 1-week-course of systemic administration of metronidazole plus amoxicillin, every 4 months, as the only therapy, in patients with moderate to advanced progressive adult periodontitis, is effective in arresting the progression of disease. In addition, M+A therapy sig-

nificantly improves clinical attachment level, reduces probing depth, and bleeding on probing sites.

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