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Effects of metronidazole plus amoxicillin as the only therapy on the microbiological and clinical parameters of untreated chronic periodontitis

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Abstract:

Aim: To determine the effect of metronidazole plus amoxicillin (M+A) as the sole therapy, on the subgingival microbiota of chronic periodontitis.

Material and Methods: Twenty-two patients with untreated chronic periodontitis were randomly assigned to a group that received M+A for 7 days, or to a group receiving scaling and root planing (SRP) and two placebos. Clinical measurements including sites with plaque, bleeding on probing (BOP), probing depth (PD) and attachment level (AL) were made at baseline, 3, 6, 9 and 12 months. Subgingival plaque samples were taken from all teeth at baseline 3, 6, 9 and 12 months for the counts of 40 subgingival species using checkerboard DNA–DNA hybridization.

Results: Mean PD was reduced from 2.80 ± 0.45 at baseline to 1.95 ± 0.05 at 12 months ($P < 0.001$) and from 2.39 ± 0.41 to 1.95 ± 0.10 ($P < 0.001$) in the M+A- and SRP-treated patients, respectively. Corresponding values for relative mean AL were 10.07 ± 1.30–9.77 ± 0.34 ($P < 0.001$) and 9.94 ± 0.28–9.77 ± 0.26 ($P < 0.001$). Percentage of sites exhibiting BOP were 40.6 ± 18.3-14.0 ± 1.4 ($P < 0.001$), and 38.5 ± 5.1–19.0 ± 2.8 ($P < 0.001$) in the M+A and SRP groups, respectively. Mean total DNA probe counts and counts of the majority of the 40 test species were significantly reduced over time in both groups, with no significant differences detected at any time point between groups. At 12 months many of the species were still present at significantly lowered levels compared with their baseline counts in both groups.

Conclusions: Changes in clinical and microbiological parameters were similar after receiving systemically administered M+A as the sole therapy or after receiving SRP only.

It is generally accepted that anti-infective therapy is the cornerstone of periodontal treatment (Slots 2002). The goal of periodontal therapy is to preserve the dentition by arresting, slowing down or reversing periodontal destruction and to prevent the recurrence of the disease (Lindhe & Nyman 1987). Periodontal treatment includes surgical and sophisticated regenerative techniques as well as mechanical removal of supra- and subgingival microbial plaques, combined with locally or systemically administered antimicrobial agents in specific groups of patients. A greater proportion of impoverished populations throughout the world are affected by periodontal diseases (World Health Organization, 2006). Most of the patients of undeveloped and developing countries do not receive the benefit from conventional periodontal therapy because financial and human resources are not available to allow access to periodontal care. As there is increasing evidence that periodontal infections negatively affect systemic health (Kuramitsu et al. 2001,

Scannapieco et al. 2001, López et al. 2002), effective control of destructive periodontal disease is essential.

Systemically administered antibiotics used in the treatment of periodontal infections provide periodontal attachment level improvement beyond that observed in subjects receiving mechanical debridement procedures alone. For example, several studies have shown that systemically administered metronidazole employed as an adjunct to scaling and root planing (SRP) in the treatment of periodontal infections offers clinical (Loesche et al. 1984, 1987, 1991, 1992, 1996, Jenkins et al. 1989, Soder et al. 1990, Haffajee et al. 1995) and microbiological benefits over SRP alone (Feres et al. 2001). Metronidazole plus amoxicillin has been used successfully in the treatment of advanced periodontitis, especially with *Actinobacillus actinomycetemcomitans*-associated infections (Van Winkelhoff et al. 1989, Winkel et al. 1998). Further, a 1-week course of systemically administered metronidazole combined with amoxicillin (M+A), as the only therapy, arrested the progression of chronic periodontitis, significantly improved the clinical parameters of the disease and reduced significantly the number of sites with *Porphyromonas gingivalis* and *Prevotella intermedia* compared with the placebo group (López & Gamonal 1998, López et al. 2000). However, no studies have determined the effect of the combination of metronidazole and amoxicillin, as the only periodontal therapy, upon a wide range of subgingival microbial species.

The genesis of this study was due to two factors. First, the recognition that systemically administered antibiotics can have a beneficial effect on clinical parameters of periodontal diseases and may have similar beneficial effects on the subgingival microbiota. Second, there is a need to find cost-effective measures to control periodontal infections in populations where access to periodontal care is limited. Thus, the aim of the present pilot clinical trial was to evaluate the changes in the levels and proportions of 40 bacterial species in the subgingival microbiota of previously untreated chronic periodontitis patients after the administration of metronidazole plus amoxicillin as the only therapy. A group of patients with chronic periodontitis treated with SRP alone were used as control. In addition, the clinical changes brought about by both therapies were evaluated.

Material and Methods

Subject population

Subjects eligible for the study were identified from a population of patients attending a public dental center in Santiago, Chile. Medical and dental histories were obtained and a full-mouth periodontal examination was performed in 32 subjects over 35 years old with chronic periodontitis. Of these, 22 subjects, 15 women and 7 men aged 38–68 years (mean age 46.3 ± 7.8) were selected. Further, these subjects had received no previous periodontal therapy and no personal instruction to prevent periodontal disease.

Inclusion criteria included the following: Subjects were included if they were systemically healthy with at least 18 treatable natural teeth, four of which were molars; at least six pockets ⩾4mm; and concomitant attachment loss ⩾3mm, 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 medication such as antibiotics, steroids, or non-steroidal anti-inflammatory drugs within the previous 6 months; systemic diseases that might affect periodontal disease activity; and allergy to metronidazole or amoxicillin.

All subjects were informed that the aim of the study was to investigate the effect of a systemically administered antibiotic combination to treat periodontal disease. The protocol was approved by the review committee for ethical norms of the Faculty of Dentistry, University of Chile. All subjects signed an informed consent before participating in the study.

Experimental design and treatment

Before the onset of the study, each subject received a supragingival scaling (SGS) to remove gross calculus to allow periodontal probing. Participants were randomly assigned to a test or control group. Randomization was performed using mean pocket depth (PD) to stratify subjects. Subjects were assigned to one of two categories: those with a mean PD <2.5 mm and those with a mean PD⩾ 2.5 mm. Subjects were matched on the basis of the mean PD. Each patient of the matched pair was allocated to the test or to the control group by a coin toss.

Clinical measurements

The following variables were determined at the beginning of the study and every 3 months up to 1 year post-therapy.

Oral hygiene status

The presence of a band of continuous plaque at the cervical portion of the buccal, mesial, lingual and distal surfaces of each tooth was scored by running a probe along the tooth surface. Plaque scores were calculated as the percentage of surfaces examined demonstrating plaque.

Gingival inflammation

Dichotomous measurements of bleeding within 15 s after probing were determined at the same six sites on each tooth at which the probing depth was measured.

Probing depth and relative attachment level measurements

These were taken at six 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 (Florida Probe Corporation, Gainsville, FL, USA). A disk probe was used for relative attachment level recording, and a PD probe was used for probing depth and bleeding on probing recordings. A second set of attachment level and probing depth measurements was taken within 7 days of the first set of measurements. Therefore, a pair of attachment level measurements and probing depths was made every 3 months. The mean of the pair of measurements was used in all the analyses described below. A calibrated examiner monitored the subjects, and all clinical measurements taken were made by the same examiner. As replicate measurements were made at each 3-month evaluation, data were available to determine examiner reproducibility at each subject's evaluation. The examiner performing the measurements did not treat the subjects and he was masked as to which of the groups the patient belonged to.

Treatment procedures

Subjects in both groups received instructions from an experienced perio-

dontist in self-performed oral hygiene measures (IOH): toothbrushing three times a day using the modified Charter's technique with a soft toothbrush and regular toothpaste with fluoride.

SGS and SRP were also administered by the same experienced periodontitst using an ultrasonic scaler (Cavitron SPS, Denstply Detrey, GmbH, Germany) with insert FSI-10. Manual Gracey curettes (Gracey Hu Friedy Instruments, Chicago, IL, USA) were also used in performing root planning, as needed.

The test group received only SGS at all teeth in two sessions, of 45 min each, 3 days apart. Each patient of the test group received 21 tablets of metronidazole 250 mg and 21 tablets of amoxicillin 500 mg, and was asked to take one tablet of each medication every 8 h for 7 days.

The control subjects received SGS and SRP under local anaesthesia. The procedure was scheduled in three or four sessions 3 days apart, according to periodontal disease severity and the number of teeth present. No less than 1 h was assigned to each quadrant. The control subjects received 21 tablets of placebo A and 21 tablets of placebo B, and were asked to take one tablet of each every 8 h for the following week. The antibiotic or placebo therapy was initiated at the first scaling visit.

Subjects in both the test and control groups received a written prescription to request the antibiotics or the placebos from the pharmacy section of the public dental center. Thus, the subject and the examiner who performed the examinations were blinded to the type of medication that the subject had been prescribed. Tablets of placebo A and tablets of placebo B had identical physical characteristics to the tablets of metronidazole and amoxicillin, respectively, and were packed in identical vials.

All subjects were advised not to drink alcoholic beverages during the days of medication. At the beginning of the study and at each monitoring visit, each subject was provided with three toothbrushes and toothpaste.

Post-treatment controls

Subjects were clinically and microbiologically monitored at baseline and at 3-month intervals for a period of 1 year after treatment. At these appointments, the examiner recorded any medical his-tory change, in particular that no antibiotic therapy had been administered, and the clinical periodontal parameters recorded at the baseline visit were repeated. Subgingival plaque samples were taken from the mesial aspect of all teeth, excluding third molars, at these time points. All subjects received full-mouth SGS and a reinforcement of oral hygiene habits after the 3, 6, 9, and 12 months monitoring visits. When performing scaling at these visits, care was taken to limit plaque and calculus removal to the supragingival area only. Residual periodontal pockets were not re-instrumented at the monitoring visits.

Compliance

Subject compliance with the unsupervised usage of the prescribed medication is critical to validate the results of a clinical trial. A decline or disappearance of spirochetes from subgingival plaque has been suggested as a means for measuring patient compliance in taking metronidazole (Loesche et al. 1993). This method was utilized in the present study by examining subgingival plaque samples using a phase contrast microscope. Plaque samples were taken from the distal site of the four most distal molars in each quadrant of each subject in the M+A group, at baseline and at 8 days after initiation of treatment. The sample was suspended in sterile saline with 1% gelatin, and one drop of the dispersed solution was examined with the microscope using phase contrast illumination, at a ×1000 magnification. One to two hundred bacteria were counted from random fields, and the percentage of spirochetes was calculated. An examination to determine the percentage of spirochetes in the subgingival plaque was performed by the periodontologist who administered the treatment (not the clinical examiner). The result of the phase contrast microscopic examination of each patient was kept in a sealed envelope that was opened after the 12-month visit.

Microbiological assessment

Subgingival plaque samples were taken from the mesio-buccal aspect of all teeth, except third molars, in each subject at baseline and at the 3, 6, 9 and 12 months monitoring visits. Counts of 40 subgingival species were determined in each plaque sample using a modifica-tion of the checkerboard DNA–DNA hybridization technique (Haffajee et al. 1997). In brief, after the removal of supragingival plaque, subgingival plaque samples were taken with individual sterile Gracey curettes from the mesial aspect of each tooth. The samples were placed in separate Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and 0.15 ml of 0.5 M NaOH was added. At this point, the samples were stable and were sent to The Forsyth Institute for analysis. The samples were lysed and the DNA was placed in lanes on a nylon membrane using a Minislot device (Immunetics, Cambridge, MA, USA). After fixation of the DNA to the membrane, the membrane was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at $90°$ to the lanes of the device. Digoxigenin-labelled whole genomic DNA probes to 40 subgingival species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes were detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemifluorescence detection. Signals were detected using Atto-Phos substrate (Amersham Life Science, Arlington Heights, IL, USA) and a Storm FluorImager (Molecular Dynamics, Sunnyvale, CA, USA). Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit the detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals were evaluated using the Storm FluorImager and converted to absolute counts by comparison with the standards on the same membrane. Failure to detect a signal was recorded as zero.

Data analysis

Clinical parameters including percentage of sites with plaque, bleeding on probing, mean PD and attachment level were computed for each subject and then averaged across subjects in the two groups at each time point. The significance of differences over time (baseline, 3, 6, 9 and 12 months) was sought using the Friedman test. Differences between groups at each time point were sought using the Mann–Whitney test. Differences from baseline to 3, 6, 9 and 12 months for all clinical para-
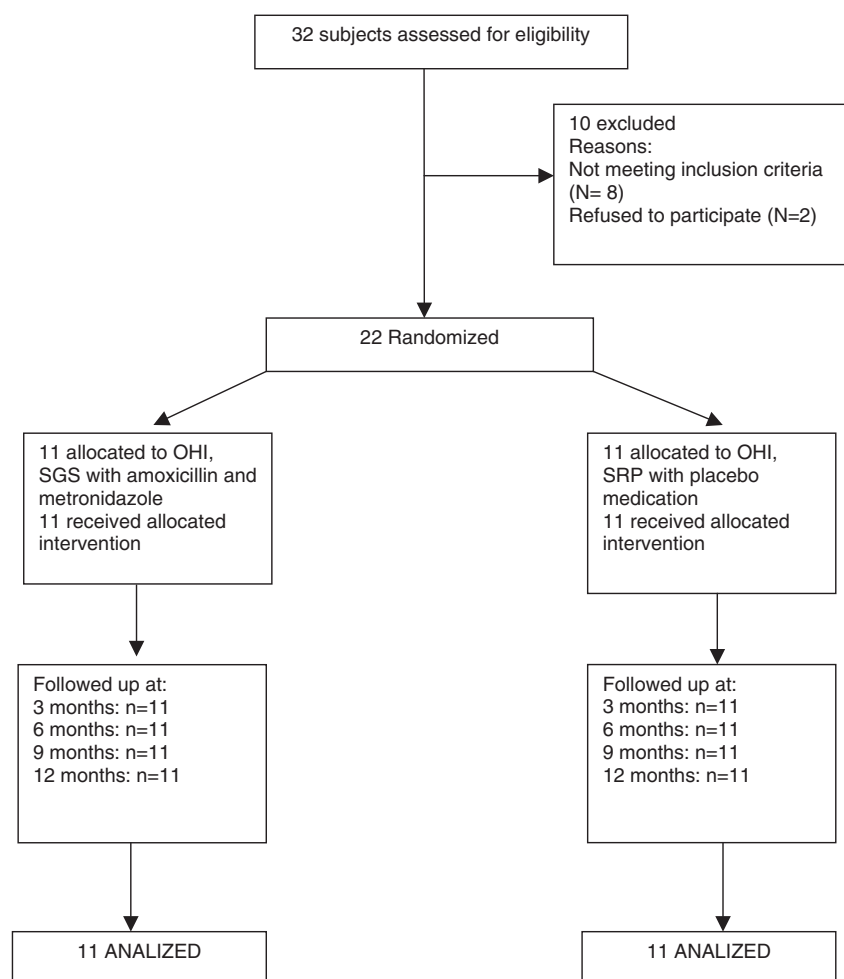
Fig. 1. Flow diagram of the progress through the phases of the study.

Table 1. Clinical and demographic characteristics at baseline in the two subject groups (mean ± SD)

	SRP only	Amoxicillin + metronidazole
N	11	11
Age	44.55 ± 5.82	48.00 ± 9.78
% Males	37	28
No. of teeth	23.55 ± 1.75	22.18 ± 3.52
% of sites with		
Probing depth 4–6 mm*	6.31 ± 6.17	15.27 ± 9.73
Probing depth >6 mm	0.88 ± 1.80	3.11 ± 5.00
BOP	38.55 ± 18.28	40.55 ± 18.33
Plaque	86.45 ± 13.75	87.18 ± 10.93
% CAL 4–6 mm	38.82 ± 20.70	38.42 ± 16.25
Mean probing depth (mm)*	2.39 ± 0.41	2.80 ± 0.45
Mean CAL (absolute) (mm)	3.73 ± 0.82	3.84 ± 0.56
Mean CAL (relative) (mm)	9.94 ± 0.95	10.07 ± 1.30
% smokers	36	45
N cigarettes/day	3.18 ± 8.95	3.82 ± 5.19

*Significantly different at $p < 0.05$, Mann–Whitney test.
BOP, bleeding on probing; CAL, clinical attachment loss.

meters between the treatment groups were sought using ANCOVA using the baseline values for the subjects as the co-variate.

Counts (levels) of each bacterial species were computed at the sampled teeth for each subject and then averaged across subjects in the two groups separately at each time point. The significance of differences in mean levels of each subgingival species over time was sought using the Friedman test and adjusted for multiple comparisons (Socranksy et al. 1991). For other analyses, the proportions of species within the different microbial complexes described by Socransky et al. (1998) were added up and averaged per subject and averaged across subjects in the two groups separately. Differences between groups at each time point were sought using the Mann–Whitney test. A total of 2522 subgingival plaque samples were analysed, an average of 22.9 per subject per visit.

Results

Figure 1 shows the flow diagram for the different phases of the study. Thirty-two patients were assessed for elegibility. Of these, 10 patients were excluded: six did not meet the inclusion criteria and four refused to participate. Twenty-two patients were randomly allocated to one of the two study groups. All patients received the allocated intervention and no patient was lost to follow-up.

No subject experienced any adverse side effects to the medications employed. No changes in the medical or dental history of patients were detected, and no patient received antibiotics for any other reason during the 1-year monitoring period. The immediate pre-treatment clinical features of all patients are presented in Table 1. The only significant differences in the clinical parameters at baseline between the two groups were the percentage of sites with PD 4–6 mm and the mean PD. Both these parameters were significantly higher in the M+A group.

Compliance

All patients declared they had taken the antibiotics as prescribed. The range of percentage of subgingival spirochetes in patients in the M+A group was between 22% and 39% at baseline. The monitoring of the percentage of subgingival spirochetes indicated that six patients in the M+A group showed a decrease in spirochetes to levels of 2–4% while spirochetes were not detected in subgingival plaque from five patients of the M+A group 8 days after antibiotic administration. The drastic reduction or elimination of spirochetes from subgin-
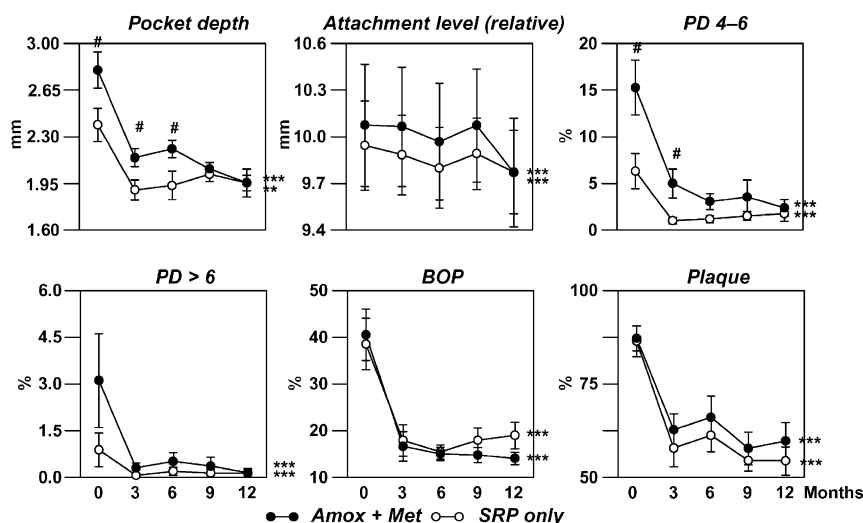
Fig. 2. Plots of the full-mouth mean values (± SEM) for clinical parameters at baseline, 3, 6, 9 and 12 months for subjects treated with scaling and root planing (SRP) only or metronidazole combined with amoxicillin only. The circles represent the mean values and the whiskers represent the standard error of the mean. Values for each parameter were measured at up to 168 sites in each subject, averaged within a subject and then averaged across subjects in each treatment group for each time point. Significance of differences over time was tested using the Friedman test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Significance of differences between groups at each time point was tested using the Mann–Whitney test (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$).

gival plaque found in all the subjects in the M+A group showed that all of them had good compliance with the prescribed medication.

Clinical findings

Figure 2 presents the full-mouth mean values for the clinical parameters at baseline, 3, 6, 9 and 12 months for patients in the M+A group and in the SRP group. There was a statistically significant reduction over time in the mean PD, the relative attachment level, the percentage of sites with bleeding on probing, with PD 4–6 mm and with PD > 6 mm, and the percentage of sites with plaque for both treatment groups. The mean PD values (± SEM) at baseline, 3, 6, 9 and 12 months for the M+A group were 2.80 ± 0.45, 2.14 ± 0.06, 2.20 ± 0.06, 2.06 ± 0.04 and 1.95 ± 0.05 ($p < 0.001$), and for the SRP group were 2.38 ± 0.41, 1.90 ± 0.07, 1.93 ± 0.10, 2.01 ± 0.05 and 1.95 ± 0.10 ($p < 0.001$). The percentage of sites with PD 4–6 mm and with PD > 6 mm were significantly reduced at 3 months after treatment in the SRP and M+A groups ($p < 0.001$) and continued at lowered levels between 3 and 12 months.

Corresponding mean relative attachment level values for the M+A group were 10.07 ± 0.39, 10.06 ± 0.38, 9.96 ± 0.37, 10.7 ± 0.36 and 9.77 ± 0.34

($p < 0.001$), and for the SRP group 9.94 ± 0.28, 9.88 ± 0.25, 9.80 ± 0.25, 9.89 ± 0.22 and 9.77 ± 0.26 ($p < 0.001$). Twelve months after therapy, subjects in the M+A group showed an overall mean gain of attachment of 0.3 mm, and subjects in the SRP group of 0.17 mm. This difference compared with baseline was statistically significant ($p < 0.001$) for both groups.

There was also a significant decrease in the percentage of sites that bled on probing in both treatment groups. The mean values at time points from baseline to 12 months were 40.54 ± 5.52, 16.60 ± 3.15, 15.00 ± 1.44, 14.75 ± 1.62 and 14.0 ± 1.35 ($p < 0.001$) for M+A-treated subjects, and were 38.54 ± 5.1, 17.90 ± 3.41, 15.41 ± 1.51, 17.96 ± 2.57 and 18.99 ± 2.84 ($p < 0.001$) for SRP-treated subjects. There were no significant differences between the two treatment groups at the different time points in any of the clinical parameters, except for mean PD at baseline, 3 and 6 months and percentage of sites with PD 4–6 mm at baseline and 3 months.

Figure 3 presents the mean baseline and 12 months values for PD and relative attachment level for each subject in each treatment group. All subjects receiving M+A exhibited mean PD reduction while 10 of 11 subjects receiving SRP only showed mean PD reduc-

tions at 12 months. The PD reduction appeared to be greater in the M+A group. One subject in the M+A group and four subjects receiving SRP only showed mean attachment level loss at 12 months. The greatest attachment level gain appeared to occur for the subjects in the M+A group, particularly in those subjects with the greatest mean baseline attachment loss.

The mean change from baseline to each time point for each clinical parameter for both groups is presented in Fig. 4. After adjusting for baseline values of the clinical parameter, the changes in all the clinical parameters were very similar in both treatment groups.

The mean percentage of sites with attachment level gain > 2 mm at 12 months was higher in the M+A group (3.79 ± 1.33) than in the SRP group (1.14 ± 0.65), but the difference was not statistically significant (Fig. 5). No site with attachment loss greater than 2 mm was detected in any subject in either group from baseline to 12 months.

Microbiological findings

Figure 6 presents the mean total DNA probe count at baseline and at the post-therapy time points for subjects in the two treatment groups. Mean counts were significantly reduced over time in both groups. However, there were no significant differences at any time point between groups.

Figures 7 and 8 present the mean counts ($\times 10^5 \pm$ SEM) of the 40 subgingival species at baseline, 3, 6, 9 and 12 months for the SRP- and M+A-treated subjects, respectively. The majority of the test species were significantly reduced over time in both treatment groups, with the major reductions occurring between baseline and 3 months. At 12 months many of the species were still present in significantly lowered levels compared with their baseline counts in both groups. In the SRP group, only mean counts of *P. intermedia*, *Actinomyces naeslundii* genospecies 2, *Leptotrichia buccalis* and *Neisseria mucosa* did not show a statistically significant reduction after treatment. In the M+A group mean counts of *Actinomyces gerencseriae*, *Actinomyces israelii*, *A. naeslundii* genospecies 2, *Actinomyces odontolyticus*, *Eikenella corrodens*, *Campylobacter gracilis*, *Fusobacterium nucleatum*
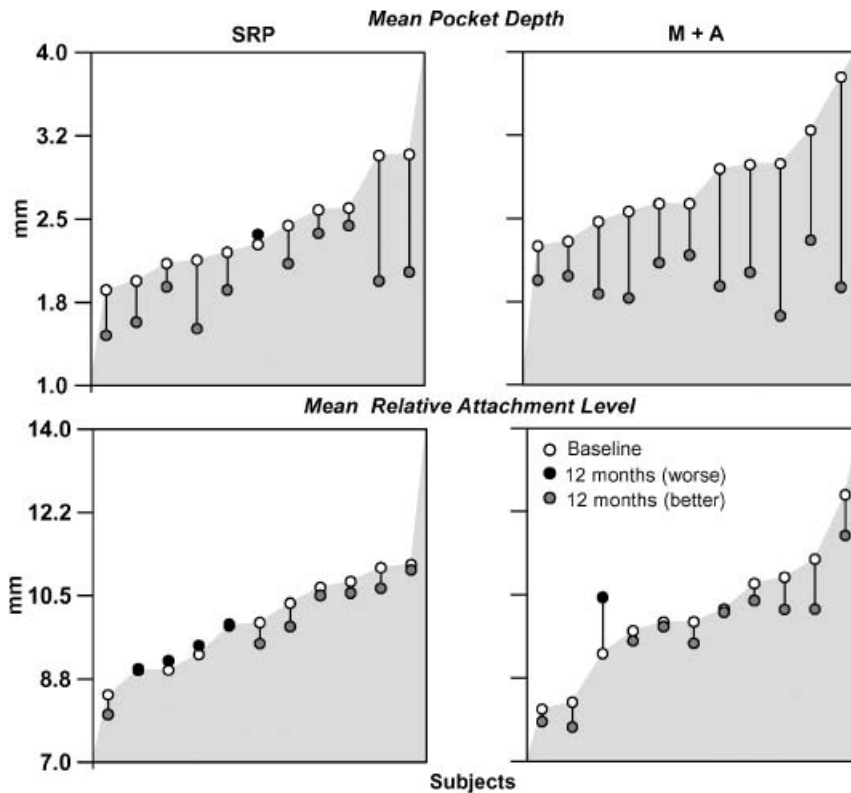
Fig. 3. Plots of the mean values for PD and relative attachment level at baseline and 12 months in each subject in both treatment groups. The two left panels provide data for subjects in the scaling and root planing (SRP) only group, while the right panels present data for the metronidazole combined with amoxicillin (M+A) group. The upper panels represent mean PD values and the lower panels represent mean relative attachment level. Each circle represents the mean value for an individual subject. The open circles represent the mean values at baseline for each subject, and the black and grey circles represent the mean values for the same subject at 12 months. The grey circles in the shaded area represent a decrease from baseline values, while the black circles in the unshaded area represent an increase from baseline values.
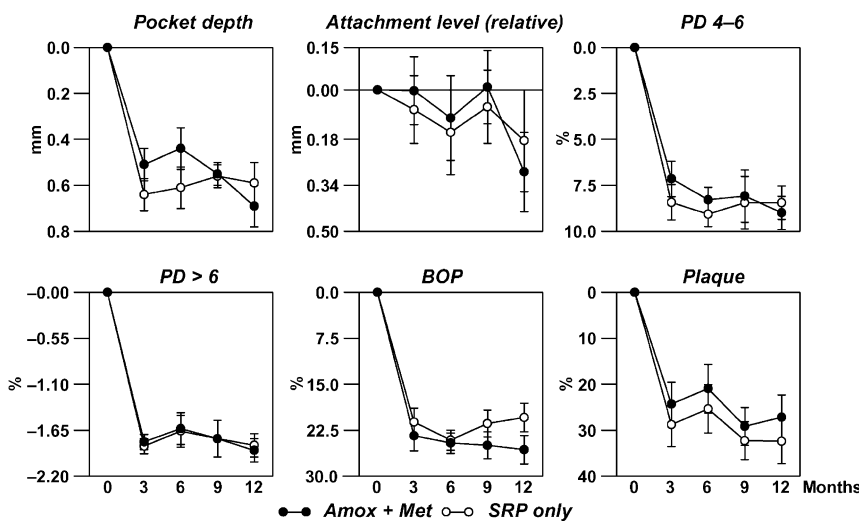

Fig. 4. Adjusted mean values (± SEM) for the change in the six clinical parameters from baseline to 3, 6, 9 and 12 months. There were no significant differences between groups at any time point after adjusting for the baseline value of each clinical parameter. The circles represent the mean and the whiskers represent the SEM after adjusting for baseline values using ANCOVA.
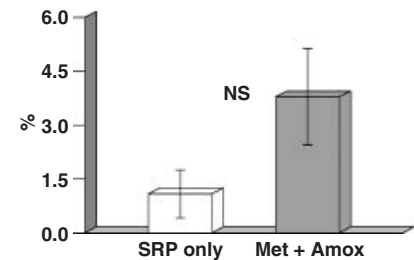
Fig. 5. Mean percentage of sites gaining > 2 mm from baseline to 12 months in the two treatment groups. The bars represent the mean and the whiskers represent the SEM. The difference between groups was not significant using the Mann–Whitney test.

ss nucleatum, *P. intermedia*, *Prevotella nigrescens*, *Gemella morbillorum*, *L. buccalis* and *N. mucosa* were not significantly reduced over time. In general, the overall pattern of reduction was similar in both treatment groups. There were no significant differences in mean counts for any of the test species between the treatment groups at baseline or at any of the time points. The microbial profiles of the 40 test species at each time point in each treatment group are superimposed in Fig. 9. The profiles demonstrate the clear reductions in subgingival counts in both treatment groups over time, particularly from baseline to 3 months.

Remarkably reduced mean counts of pathogens of the ''red complex'', *Tannerella forsythensis*, *P. gingivalis* and *Treponema denticola*, were detected up to 12 months post-therapy in the M+A and SRP groups (Fig. 10). The reduction of mean counts of the red complex species, at the different time points, was very similar in both groups. Figure 11 shows the proportions of the microbial complexes over time in the two treatment groups. The pies have been sized relative to the baseline total counts of the SRP group. For the SRP group, the *Actinomyces* increased significantly, the green and orange complexes decreased significantly and the red complex changed significantly decreasing from baseline to 6 months and then increasing somewhat to 12 months. For the M+A group, the *Actinomyces* increased significantly, the yellow and green complexes decreased significantly.

Discussion

The aims of the current study were (1) to evaluate the changes in the subgingival

microbiota of previously untreated chronic periodontitis patients after the administration of M+A, as the only therapy, and (2) to determine if systemic antibiotics as the only treatment would provide a clinical benefit for patients in undeveloped countries as this form of treatment would be cheaper and easier to administer than the mechanical thera-pies performed in the dental office. Although patients treated with SRP served as controls, the present study cannot be considered as a study to test equivalence between treatments because of the low number of subjects in each group. In addition, the maintenance treatment given at 3-month intervals to patients in both groups did not include subgingival debridement, which is not comparable with the standard mainte-nance care for periodontitis. Subgingi-val debridement as part of the main-tenance treatment was not performed in the current study so as not to mechani-cally disturb the subgingival microflora, as performed in previous studies testing the effect of different treatments (Car-valho et al. 2004, 2005).

The subjects of the current study were representative of the population of patients with chronic periodontitis who requested treatment in public health centers in Santiago. They were subjects who had never received periodontal therapy or personal instruction to pre-vent periodontal disease. These subjects exhibited a mean clinical attachment loss (CAL) >3.70 mm and more than 30% of sites with CAL $\geqslant 5$ mm, indicat-ing that they had moderate to severe periodontitis (Flemming 1999). The subjects of the present study also had higher levels of the red complex species than similar patients of the United States and Brazil (López et al. 2004).

Current periodontal therapy strongly emphasizes the reduction of specific periodontal pathogens (Slots & Rams 1996, Socransky & Haffajee 2002).
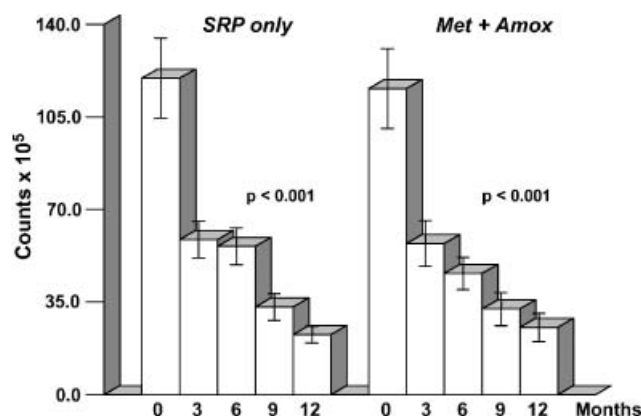

Fig. 6. Mean total DNA probe counts ($\times 10^5$, $\pm$ SEM) in subgingival plaque samples taken at baseline, 3, 6, 9 and 12 months from subjects in the two treatment groups. Mean counts were computed for a subject for each visit and then values were averaged across the subjects at each time point in the two treatment groups separately. The whiskers indicate the SEM. Significance of differences over time was sought using the Friedman test. There were no significant differences between groups at any time point.
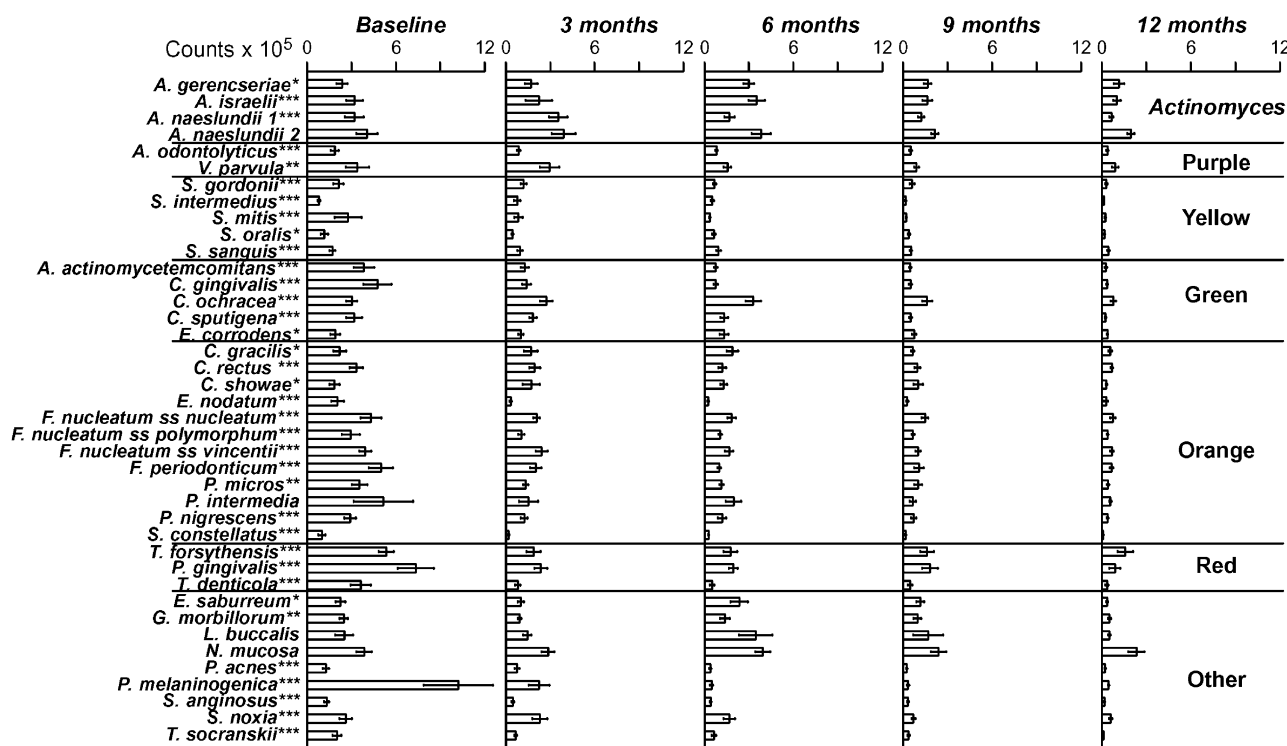

Fig. 7. Bar charts of the mean counts ($\times 10^5$, $\pm$ SEM) of the 40 test species in subgingival plaque samples taken at baseline, 3, 6, 9 and 12 months in the SRP only group. Mean counts for each species were computed for a subject for each visit and then values were averaged across the 11 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Friedman test. $*p<0.05$, $**p<0.01$, $***p<0.001$ after adjusting for multiple comparisons.
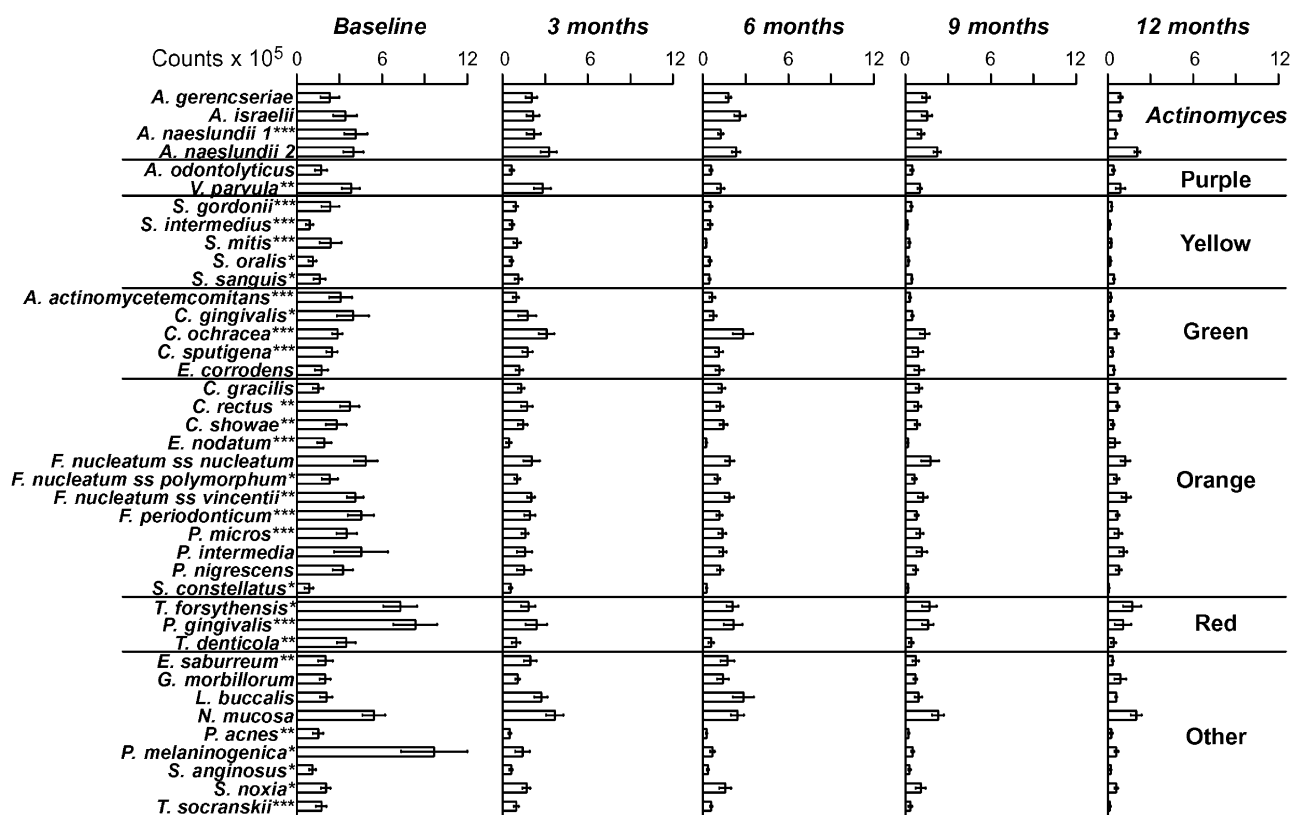
Fig. 8. Bar charts of the mean counts ($\times 10^5$, $\pm$ SEM) of the 40 test species in subgingival plaque samples taken at baseline, 3, 6, 9 and 12 months in the M+A only group. Mean counts for each species were computed for a subject for each visit and then values were averaged across the 11 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Friedman test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ after adjusting for multiple comparisons.

However, current periodontal therapies differ in their ability to suppress periodontal pathogens. A study evaluating the effect of SRP on the clinical and microbiological parameters of periodontal diseases (Haffajee et al. 1997) found that SRP appeared to have a modest effect on the composition of the subgingival microbiota. Only the red complex species, *P. gingivalis*, *T. forsythensis* and *T. denticola* were significantly decreased in the cited study, and none of them or any other species was undetectable post-therapy when SRP was used as the only treatment. Even though SRP had a moderate effect on the composition of the subgingival microbiota, a significantly post-SRP clinical improvement was observed (Haffajee et al. 1997). The results of the present investigation showed that, on average, periodontal therapy consisting of plaque control and SRP was effective in achieving an overall significant gain in clinical attachment level of 0.17 mm, a significant reduction in the mean PD, percentage of sites with bleeding on probing (BOP) and percentage of sites with plaque. Additionally, SRP therapy produced a significant reduction of most of the periodontal pathogens, including the red complex species. All the changes in the clinical and microbiological parameters were maintained up to 12 months after therapy.

The data in the present investigation support the extensively reported beneficial effect of SRP alone on clinical parameters (Badersten et al. 1981, 1984, Kaldahl et al. 1996) and on the subgingival microbiota (Haffajee et al. 1997) in chronic periodontitis. The effects of SRP on the subgingival microbiota in the present study confirm the decrease in levels of *P. gingivalis* and *T. denticola* after SRP reported in other studies (Haffajee et al. 1992, Simonson et al. 1992, Shiloa & Patters 1994). The mean total DNA probe count and the total mean counts of 36 out of the 40 subgingival species studied were significantly reduced 3 months after SRP alone, and at 12 months after SRP many of these species were still present in significantly lowered levels compared with their baseline counts, including the red complex species and *A. actinomycetemcomitans*. Our results do not agree with other studies that have shown that the prevalence and levels of *A. actinomycetemcomitans* are minimally affected by SRP alone (Gunsolley et al. 1994, Mombelli et al. 1994, Haffajee et al. 1997). In the present study, mean counts of subgingival *A. actinomycetemcomitans* were significantly reduced in the SRP group at 3 months and continued at significantly lowered levels compared with their baseline values, and were maintained up to 12 months. Thus, SRP as the sole therapy in patients of the current study appeared to be more effective in reducing periodontal pathogens and other species in the subgingival microbiota than the results of previous studies (Ali et al. 1992, Gunsolley et al. 1994, Mombelli et al. 1994, Haffajee et al. 1997). The major reason for the difference between the results of the current study and previous investigations may be that the subjects in this study had never received periodontal therapy. Thus, the levels of subgingival species, both pathogenic and non-patho-
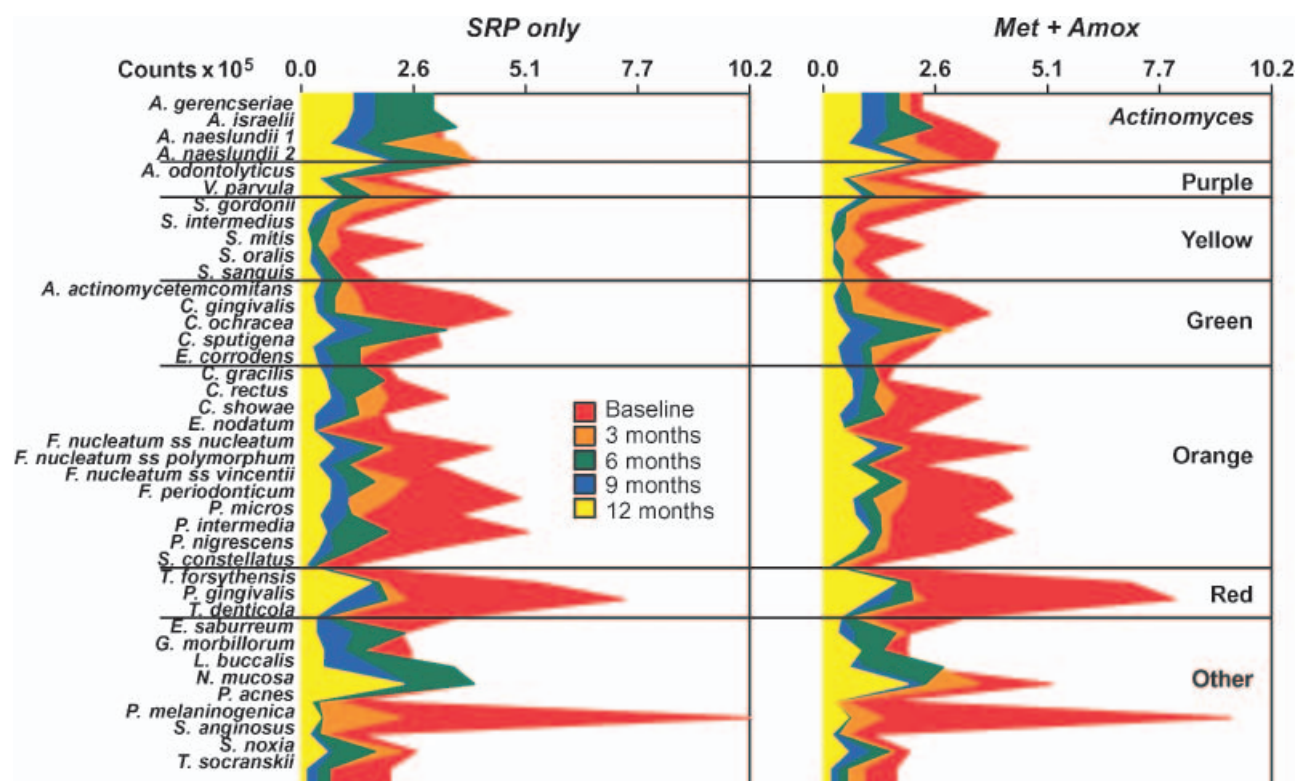
Fig. 9. Mean subgingival microbial profiles at baseline, 3, 6, 9 and 12 months for samples from subjects in the two treatment groups. Mean counts of each species were averaged within a subject at each time point and then averaged across subjects in the two treatment groups separately for each time point. The mean profiles are superimposed to provide a clearer depiction of the reductions that took place in both groups after therapy.
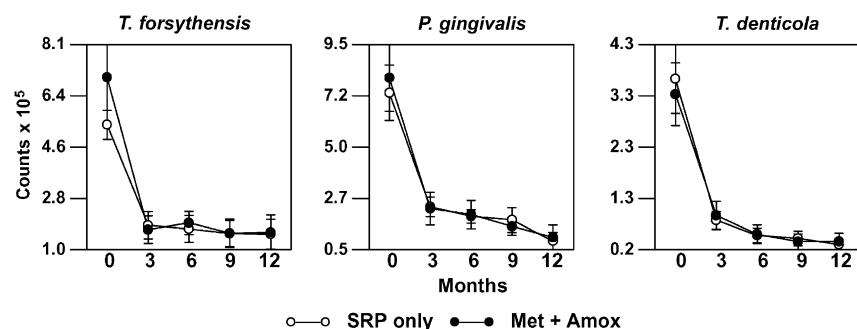

Fig. 10. Mean counts ($\times 10^5$, $\pm$ SEM) of the red complex species, *Tannerella forsythensis*, *Porphyromonas gingivalis* and *Treponema denticola*, at baseline, 3, 6, 9 and 12 months for the two treatment groups. Counts of each species were averaged across sites in each subject and then averaged across subjects at each time point for the two groups separately. The circles represent the mean values and the whiskers represent the SEM. Significance of differences over time was sought using the Friedman test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ after adjusting for multiple comparisons. There were no significant differences between treatment groups at any time point.

genic, were higher than in subjects who had received prior periodontal therapy. It is easier to reduce high levels of organisms to lower levels than to reduce lowered levels to even lower or undetectable levels. In the present investigation, it may be surmised that SRP decreased the subgingival microbial burden as a result of tissue inflamma-tion. This in turn would have diminished the gingival crevicular fluid flow that is needed for the growth of subgingival microbiota. In addition, home care procedures and supragingival plaque and calculus removal at 3-month intervals may have helped to sustain these changes.

Systemic M+A, as the sole therapy, also significantly improved clinical parameters in subjects in the present study. The M+A therapy appeared to arrest the progression of periodontitis, as only one subject in the test group showed mean attachment loss after 12 months. Further, this therapy was effective in obtaining a significant over-all mean attachment gain of 0.30 mm and a significant overall mean probing depth reduction of 0.85 mm. In addition, M+A significantly reduced the percentage of bleeding on probing sites and the proportion of sites gaining attachment level was higher than in subjects receiving SRP alone, even though the difference between groups was not statistically significant. The greater PD reduction and greater attachment level gain observed in the M+A group than the SRP group (Fig. 2) may be explained by the higher percentage of sites with PD 4–6 mm and higher mean PD at baseline in subjects in the M+A group. It has been shown that systemic metro-nidazole as an adjunct to SRP for chronic periodontitis is more effective in reducing PD and clinical attachment level at sites with an initial PD of 4–6 mm (Elter et al. 1997). In addition, SRP appears to cause a slight loss of
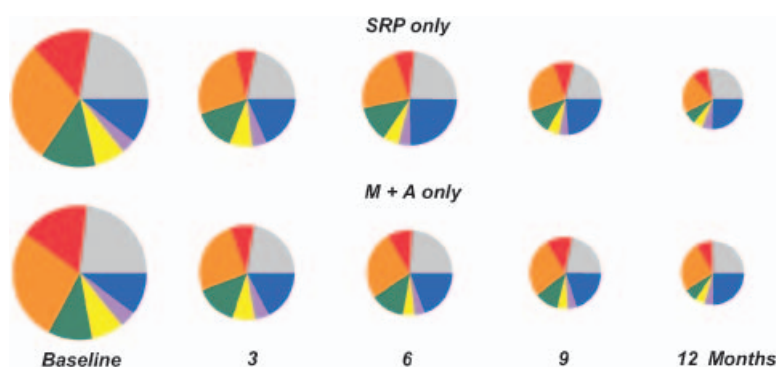
Fig. 11. Proportions of microbial complexes over time in the two treatment groups. The pies have been sized relative to the baseline total counts of the scaling and root planing groups.

attachment level at the shallow pocket (Hung & Douglass 2002).

The current study was designed to determine the effect of M+A as the only therapy at different time points up to 12 months. The review committee for ethical norms of the Faculty of Dentistry at the University of Chile stated that patients treated with M+A as the only therapy should receive SRP after 12 months of follow-up irrespective of the effects of the antibiotic treatment. Thus, for ethical reasons, it was not possible to follow up the patients of the M+A group after 12 months to determine whether the no-removal of subgingival calculus would increase the risk of recurrence of periodontitis.

The success of periodontal treatment depends on a substantial reduction of the colonizing microbiota, particularly a decrease in the proportion of pathogens to a level manageable by the host (Bollen & Quiriynen 1996). Treatment with M+A alone significantly reduced the mean total DNA probe count and the overall mean count of 28 of the 40 subgingival species studied, including the red complex species, up to 12 months post-therapy. Mean counts of the 40 species were significantly reduced over time in both treatment groups and no significant differences at any time point between groups were detected. Therefore, the significant improvement in clinical parameters observed in subjects treated with M+A alone and in patients treated with SRP alone in the present study was probably due to the marked reduction of the pathogens associated with their infection.

Several studies have evaluated the effect of metronidazole alone (Loesche et al. 1984, 1991, 1992, Jenkins et al. 1989, Feres et al. 2001) or combined with amoxicillin (Van Winkelhoff et al.

1989, Winkel et al. 1998) as an adjunct of SRP for the treatment of periodontal infections, but only a few studies have evaluated the effect of metronidazole alone (Lindhe et al. 1983) or of the combination of M+A as the only therapies for periodontal infections (López & Gamonal 1998, López et al. 2000). The results of the present investigation agree with those of previous studies (López & Gamonal 1998, López et al. 2000) that showed that M+A as the sole therapy in progressive untreated adult periodontitis reduced the proportion of *P. intermedia* and *P. gingivalis* and allowed a significant improvement in clinical conditions.

The effect of the M+A treatment on the subgingival microbiota appears to be more effective and more lasting in reducing periodontal pathogens than either metronidazole or amoxicillin alone used as an adjunct of SRP. A study aiming to determine the changes in subgingival microbial profiles in chronic periodontitis patients receiving either systemically administered amoxicillin or metronidazole, as an adjunct of SRP (Feres et al. 2001), found that both antibiotics were useful in rapidly lowering counts of periodontal pathogens. However, there was a tendency for counts of *T. forsythensis* and *T. denticola* to increase in some amoxicillin-treated subjects. After withdrawing the amoxicillin, "red complex" species started to rebound. However, in the metronidazole-treated group, the "red complex" species remained at low levels after cessation of therapy and at 90–360 days these species were still in much lower proportions compared with baseline. Feres et al. (2001) also found that "orange complex" species were markedly decreased during metronidazole or amoxicillin administration, but

tended to increase once the antibiotics had been withdrawn. At 90 days the proportions of the "orange complex" species were comparable with baseline values in both antibiotic treatment groups. In the present study, subjects in the M+A group as well as those in the SRP group showed a significant reduction of the mean total DNA probe counts of subgingival species at 3, 6, 9 and 12 months post-therapy compared with baseline. Systemic antibiotic therapy in the treatment of periodontal infections is based on the premise that specific microorganisms cause destructive periodontal disease (Van Winkelhoff et al. 1996), and it may be better that the antimicrobial agent selectively eliminates the pathogenic microorganisms than the subgingival microbiota in toto. However, at present no periodontal treatment has been able to selectively eliminate only the periodontal pathogens without changing the proportions of "host compatible species". The results of the current study agree with most studies that have evaluated the effect of SRP on periodontal infections and have shown that there is a positive response to this treatment in most chronic periodontitis patients. Metronidazole has also been shown to have a pronounced effect on the subgingival microbiota (Loesche et al. 1981, Feres et al. 2001) and penetrates the gingival crevice fluid and saliva (Pähkla et al. 2005). After multiple 250 mg doses, metronidazole can reach a concentration of 26.7 μg/ml in the gingival crevicular fluid (Van Oosten et al. 1986), and a single oral dose of 750 mg of metronidazole gave concentrations of 8.7–13.8 μg/ml in gingival crevicular fluid (Walker et al. 1985) that exceed the minimal inhibitory concentration for most anaerobic oral microorganisms. Amoxicillin appears to be also very effective against most periodontal pathogens (Sutter et al. 1983, Goodson 1994, Feres et al. 2001), and exhibits high antimicrobial activity at the levels achieved in gingival crevicular fluid (Walker et al. 1983). The combination of M+A has been found to be an effective adjunctive therapy for the elimination of *A. actinomycetemcomitans* (Van Winkelhoff et al. 1989, Flemming et al. 1998, Winkel et al. 1998) and reduced the number of sites with *P. gingivalis* and *P. intermedia* (López & Gamonal 1998).

Patients of the current study were randomly assigned to the test or to the

control group. Although proper random assignment prevents selection bias, it does not always guarantee that the groups are totally equivalent at baseline, as it occurred in the present study. Any differences in some baseline characteristics, as occurred in the present study, may be the result of chance rather than bias (Altman & Dore 1990). The only significant differences in the clinical parameters in patients at baseline were the percentage of sites with probing depth 4–6 mm and the mean probing depths that were higher in the M+A group. However, probing depth measurements are considered a secondary outcome in clinical trials and are less valid than clinical attachment levels measurements (Carlos et al. 1987). Changes in clinical attachment level are recognized as the gold standard in determining success in the treatment of periodontitis (Gutman & Tylenda 1997).

Patient compliance with the usage of prescribed medication is critical to validate the results of a clinical trial. The present study tried to enhance compliance by maintaining a good relationship with the study subjects. Methods to assess patient compliance with medication intake commonly used in clinical studies, such as interviews and tablet count, have been found to be unreliable (Greenber 1984). Compliance may be assured by having the patients take the medication under supervision, but this is impractical with ambulatory patients. Monitoring reduction or disappearance of spirochetes from subgingival plaque, as used in the current study, has been shown to be a reliable method of measuring patient compliance in taking metronidazole (Loesche et al. 1993). The drastic reduction or disappearance of spirochetes in patients of the test group after 8 days of taking M+A suggested good compliance with taking the prescribed medication. There may be some concern that supragingival scaling rather than the M+A may have reduced the population of spirochetes. This contention is supported by the in vitro bactericidal effects of dental ultrasonic cleaning on spirochetes (Thilo & Baehni 1987). However, the results of studies on the bactericidal effects of ultrasonic scalers upon the subgingival microflora are conflicting (O'Leary et al. 1997). All the studies aiming to determine the effect of ultrasonic instrumentation on spirochetes have used in vitro models simulating the subgingival environment of the periodontal pocket or have employed ultrasonic devices with characteristics very different from those used for subgingival scaling (Thilo & Baehni 1987). Otherwise, the results of the Schenk et al. (2000) study showed that the primary effect of ultrasonic scalers is mechanical plaque removal, and found that the ultrasonic scaler did not show any bactericidal effect on some periodontal pathogens. As the primary effect of ultrasonic scalers is mechanical plaque removal, it is highly improbable that supragingival scaling, as performed in the current study, was the cause of spirochete elimination in the patients in the test group.

The present investigation had two clinically relevant findings. It demonstrated that systemically administered M+A as the sole treatment provided clinical and microbiological improvements similar to those observed as a result of SRP alone in previously untreated subjects with chronic periodontitis. This is not in accord with the notion that has developed in the field of periodontology, that it is essential to mechanically disrupt biofilms in order for antibiotics to be effective. This notion may have developed because of the recognition that species living in biofilms are far more resistant to an antibiotic(s) than the same species living in a planktonic (single cell) state. The susceptibility of the organisms that were reduced in this study might be due in part to the possibility of a lessened amount of intercellular glycocalyx in the species that are epithelial cell associated or in the loosely adherent biofilm between the epithelial-associated and tooth-associated biofilms (Socransky & Haffajee 2002). The effectiveness of M+A as the sole therapy may also be of importance in designing approaches to control periodontal infections in underserved populations with limited access to dental care as metronidazole and amoxicillin are comparatively inexpensive agents. However, it is essential to be cautious in extending these findings to subject populations who have received prior periodontal therapy. As described earlier, a better treatment response would be expected, using most therapies, in subjects whose subgingival microbiota has been minimally altered by periodontal therapy. It remains to be tested whether the subgingival microbiota in subjects who have had periodontal treatment can be affected by systemic antibiotics when used as the only form of therapy. The second clinically relevant finding of the current investigation was that reduction in the counts of subgingival species, particularly periodontal pathogens, resulted in clinical improvement. It did not matter whether the reduction occurred as a result of a mechanical debridement procedure or systemic administration of antibiotics. This reinforces the long-held notion that control of the subgingival microbiota is the critical step in periodontal therapy.

The prevailing concept for the use of antibiotics in periodontal therapy is that systemic antimicrobial drugs should be restricted to certain patients and administered as an adjunct to mechanical therapy. One concern in the use of antibiotics is the potential emergence of antibiotic-resistant bacteria strains. Studies by Feres et al. (1999, 2002) indicated that the percentage of resistant isolates increases during antibiotic administration of such agents. In addition, the use of antibiotics might be considered in subjects where these agents provide the only practical means of treating their infections. It should be pointed out, however, that there are risks associated with not treating periodontal infections adequately.

The acquisition of antibiotic resistance in the periodontal microflora has been recently reported. In a study comparing the antibiotic resistance of periodontal pathogens isolated in the Netherlands and Spain (van Winkelhoff et al. 2000), it was found that microbial isolates from Spain required higher minimal concentrations to inhibit microbial isolates than isolates from the Netherlands. The authors suggested that the more widespread use of antibiotics and the higher non-compliance rates in Spain than in the Netherlands was the main cause of the increased resistance to antibiotics. However, to date there are no models to aid the periodontist to determine under which circumstances the benefit of systemic antibiotics outweighs the costs and risks for the unwanted effects (Mombelli 2005). Therefore, additional studies in a large number of subjects are needed to determine, in the long term, whether the therapeutic modality used in the current study can produce bacterial resistance, and whether the non-removal of subgingival calculus can increase the risk for recurrence of periodontitis.

Acknowledgements

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Clinical relevance

Scientific rationale: There is a need to find cost-effective measures to control periodontal infections in populations without access to conventional periodontal therapy. It has been shown in several studies that metronidazole plus amoxicillin (M+A) produces beneficial effects on clinical parameters of periodontal diseases, but the data are limited regarding the effect of M+A on the subgingival microbiota.

Principal findings: Systemic administration of M+A, as the only treatment, in previously untreated chronic periodontitis subjects provided clinical and microbiological improvements similar to those found when SRP only was employed.

Practical implications: The effectiveness of M+A in reducing the counts of subgingival periodontal pathogens may be useful in controlling periodontal infections in populations without access to dental care.