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Clinical and microbiological benefits of systemic metronidazole and amoxicillin in the treatment of smokers with chronic periodontitis: a randomized placebo-controlled study

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Abstract

Aim: The aim of this study was to evaluate the clinical and microbiological effects of scaling and root planing (SRP) alone or combined with metronidazole (MTZ) or with MTZ and amoxicillin (AMX) in the treatment of smokers with chronic periodontitis.

Methods: A double-blind, placebo-controlled, randomized clinical trial was conducted in 43 subjects who received SRP alone ($n = 15$) or combined with MTZ (400 mg 3 × per day, $n = 14$) or with MTZ+AMX (500 mg 3 × per day, $n = 14$) for 14 days. Clinical and microbiological examinations were performed at baseline and 3 months post-therapy. Subgingival samples were analysed by checkerboard DNA–DNA hybridization.

Results: Subjects receiving MTZ+AMX showed the greatest improvements in mean probing depth and clinical attachment level. Both antibiotic therapies led to additional clinical benefits over SRP alone in initially shallow, intermediate, and deep sites. The SRP+MTZ+AMX therapy led to the most beneficial changes in the subgingival microbial profile. These subjects showed significant reductions in the mean counts and proportions of periodontal pathogens such as *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola*, and the greatest increase in proportions of host-compatible species.

Conclusion: Significant advantages are observed when systemic antibiotics are combined with SRP in the treatment of smokers with chronic periodontitis. The greatest benefits in clinical and microbiological parameters are achieved with the use of SRP+MTZ+AMX.

Key words: amoxicillin; chronic periodontitis; metronidazole; periodontal pathogens; periodontal therapy; scaling and root planing; smoking

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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The main goals of periodontal therapy are to reduce probing depth (PD), bleeding on probing (BOP) and suppuration and to gain attachment level. These clinical results are achieved when the levels, proportions and percentage of sites colonized by different periodontal pathogens are effectively reduced after therapy and a new microbial community with higher proportions of host-compatible microorganisms is established in the oral cavity (Socransky & Haffajee 2005, Teles et al. 2006). Scaling and root planing (SRP) is the most com-

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monly used periodontal therapy. However, this procedure does not frequently lead to the microbiological changes necessary for maintaining the long-term stability of the clinical benefits achieved initially (Cugini et al. 2000, Carvalho et al. 2005). Therefore, with the aim of potentiating the effects of SRP, other protocols, such as the association of mechanical debridement with systemic antibiotics, have been used successfully in the treatment of periodontal diseases (Herrera et al. 2002, Haffajee et al. 2003).

Smoker subjects are at a greater risk of developing periodontal disease than non-smokers (Bergström 2003, Hyman & Reid 2003, Kim et al. 2004, Thomson et al. 2007) and normally respond less favourably to periodontal treatment (for a review, see Heasman et al. 2006; Johnson & Guthmiller 2007). While some studies have showed that smokers may be more heavily colonized by certain periodontal pathogens than non-smokers (Zambon et al. 1996, Eggert et al. 2001, Haffajee & Socransky 2001, van Winkelhoff et al. 2001), others did not find this association (Boström et al. 2001, Apatzidou et al. 2005, Natto et al. 2005, Fritschi et al. 2008). However, most of the clinical trials that addressed post-therapy microbiological changes in smokers agree that it is more difficult to affect subgingival pathogens by mechanical periodontal therapy and to keep them under control in these subjects, in comparison with non-smokers (van Winkelhoff et al. 2001, van der Velden et al. 2003, Darby et al. 2005, Mascarenhas et al. 2005, Grossi et al. 2007). This is most probably due to their reduced capacity to combat periodontal pathogens (Barbour et al. 1997, Güntsch et al. 2006), as it has been demonstrated that smoking affects the human immune system and the cellular and humoral inflammatory responses (Kinane & Chestnutt 2000, Palmer et al. 2005, Ryder 2007). Therefore, the use of systemic antibiotics could be of special value to treat this group of patients.

Metronidazole (MTZ) alone or combined with amoxicillin (AMX) has been used successfully in the treatment of chronic periodontitis. MTZ is efficient against strictly anaerobic bacteria, characteristic of the main periodontal pathogens. Lindhe et al. (1983) and Loesche et al. (1984) were the first to point out the adjunctive benefits of this antibiotic in the periodontal therapy of adult patients. Afterwards, these findings were corroborated by various other studies that performed clinical and microbiological analyses (Loesche et al. 1991, 1992, Feres et al. 2001, Carvalho et al. 2004, 2005, Haffajee et al. 2007, 2008). Periodontal pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium* and *Prevotella* species are more effectively reduced by the use of systemic MTZ and SRP than by SRP alone (Loesche et al. 1984, 1992, Winkel et al. 1997, Carvalho et al. 2005).

In 1989, van Winkelhoff et al. showed that the combination of MTZ with AMX was effective in treating a group of individuals with localized aggressive or rapidly progressive periodontitis infected with *Aggregatibacter actinomycetemcomitans*. Subsequently, other investigations have suggested the advantages of this therapy in the treatment of generalized aggressive (Guerrero et al. 2005, Xajigeorgiou et al. 2006, Kaner et al. 2007, Moreira & Feres-Filho 2007) and chronic periodontitis (Berglundh et al. 1998, Winkel et al. 2001, Rooney et al. 2002, Pahkla et al. 2006, Moeintaghavi et al. 2007). Winkel et al. (2001) evaluated the effect of SRP combined with MTZ and AMX to treat a group of adult periodontitis subjects. This therapy led to a better clinical response than that observed in the SRP-only group, and was more effective in reducing the percentage of sites colonized by *P. gingivalis*, *T. forsythia* and *Parvimonas micra* at 3 months post-therapy.

Despite the substantial evidence of the adjunctive benefits of MTZ alone or combined with AMX in the treatment of periodontal diseases, few studies so far have evaluated the effect of these antibiotics on the periodontal treatment of smokers (Palmer et al. 1999, Söder et al. 1999, Winkel et al. 2001, Pahkla et al. 2006). In fact, no studies to date have thoroughly evaluated the changes that occur in the subgingival microbial profile when systemic antibiotics are used as part of the periodontal treatment of this risk group. Therefore, the aim of the present study was to evaluate and compare the clinical and microbiological effects of SRP combined with systemic MTZ alone or with AMX in the treatment of smokers with chronic periodontitis.

Material and Methods

Sample size calculation

The ideal sample size to assure adequate power to this clinical trial was calculated considering differences of at least 1 mm for clinical attachment level (CAL) and a standard deviation of 0.94 mm between groups in initially deep periodontal pockets (>6 mm). Based on these calculations, it was defined that 14 subjects per group would be necessary to provide an 80% power with an α of 0.05.

Subject population

Forty-five subjects with a history of smoking and chronic periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University. A detailed medical, periodontal, dental and smoking history was obtained. Subjects who fulfilled the inclusion/exclusion criteria were invited to participate in the study. All eligible subjects were thoroughly informed of the nature, potential risks and benefits of their participation in the study and signed on an Informed Consent. This study protocol was approved previously by Guarulhos University's Ethics Committee in Clinical Research.

Inclusion and exclusion criteria

All subjects were >30 years old, had at least 15 teeth and at least six sites with PD between 5 and 7 mm and CAL between 5 and 10 mm at baseline. The subjects considered for the study had smoked at least 10 cigarettes per day for at least the past 5 years (Ammenheuser et al. 1997).

Exclusion criteria were aggressive periodontitis, pregnancy, lactation, periodontal or antibiotic therapy in the previous 6 months, any systemic condition that could affect the progression of periodontal disease (e.g. diabetes and immunological disorders), long-term administration of anti-inflammatory medication, need for antibiotic coverage for routine dental therapy and allergy to MTZ and/or penicillin.

Experimental design and treatment protocol

In this double-blinded, randomized and placebo-controlled clinical trial, subjects were randomly assigned to one

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control and two test groups ($n = 15$ per group), as follows:

(1) Control: SRP; (2) MTZ: SRP+systemic MTZ at the dosage of 400 mg; and (3) MTZ+AMX: SRP+systemic MTZ at the dosage of 400 mg and AMX at the dosage of 500 mg. Subjects in the control group received MTZ and AMX placebos and subjects from MTZ group received AMX placebo. Both antibiotics and placebo were administered 3 × per day for 14 days.

The study coordinator used a computer-generated table (in blocks of three) to randomly allocate the 45 subjects to one of the three therapeutic groups. Guarulhos University Pharmacy prepared the placebo and the antibiotics and sent them to the study coordinator, who marked the code number of each subject on a set of two tubes, according to the therapy assigned. The coded tubes were given to the examiner researcher (F. M.), who at no time during the study had any access to information about the contents of the tubes or the assignment of subjects to the two therapies. All study personnel, including the biostatisticians and participants, were blinded to treatment assignment for the duration of the study.

During the initial phase, subjects received clinical and microbiological monitoring, full-mouth supragingival scaling and oral hygiene instructions. Moreover, all volunteers received the same brand of toothpaste to use during the course of the study (Colgate Total®, Anakol Ind. Com. Ltda, Kolynos do Brasil, Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil). SRP was completed in four to six appointments lasting approximately 1 h each, performed under local anaesthesia. Treatment of the entire oral cavity was concluded in a maximum of 21 days. Antibiotic therapy was initiated at the first SRP visit. At the end of antibiotic/placebo therapy (day 14), all subjects answered a questionnaire about the side effects of the drugs. Clinical and microbiological monitoring was repeated at 3 months post-therapy.

Compliance

The subjects were asked to bring the tubes containing the medication at every SRP visit, when the pills were counted in order to check any inaccuracy in drug taking. In addition, two undergraduate students from the Guarulhos University Young Researcher program were responsible for calling the subjects every 4 days to monitor compliance.

Clinical monitoring

Clinical monitoring was performed by one examiner, calibrated according to the method described by Araujo et al. (2003). The intra-examiner variability was 0.19 mm for PD and 0.23 mm for CAL. This trained examiner was able to provide reproducible measurements of under 0.5 mm. Visible plaque (0/1), gingival bleeding (0/1), BOP (0/1), suppuration (0/1), PD (in mm) and CAL (in mm) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) in all teeth excluding third molars. The PD and CAL measurements were recorded to the nearest millimetre using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA).

Microbiological monitoring

Sample collection

After the clinical parameters had been recorded, the supragingival biofilm was removed and the subgingival samples were collected from nine non-contiguous inter-proximal sites per subject. The selected sites were randomized in different quadrants and subset according to baseline PDs, three samples in each of the following categories: shallow (PD $\leqslant$ 3 mm), intermediate (PD 4–6 mm) and deep (PD $\geqslant$ 7 mm). All samples were taken with individual sterile mini-Gracey curettes (11/12) and immediately placed in separate Eppendorf tubes containing 0.15 ml of TE (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). One hundred microlitres of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer.

Checkerboard DNA–DNA hybridization

Counts of 40 bacterial species were determined in each sample, using the Checkerboard DNA–DNA hybridization technique (Socransky et al. 1998). The microbiological analysis was entirely performed at the Laboratory of Microbiology of Guarulhos University. The samples were boiled for 10 min. and neutralized using 0.8 ml of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot 30 apparatus (Immunetics, Cambridge, MA, USA), concentrated on a 15 × 15 cm positively charged nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking it at 120°C for 20 min. 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 for 40 bacterial 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 the antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. The last two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. Signals were converted to absolute counts by comparison with the standard lanes on the membrane. The sensitivity of the assay was adjusted to permit the detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Statistical analysis

The percentage of sites with visible plaque, gingival bleeding, BOP and suppuration, as well as mean PD and CAL were computed for each subject and then averaged across subjects in the three therapeutic groups. Similarly, the changes in PD, CAL and BOP over time were examined in subsets of sites according to the initial PD of $\leqslant$3, 4–6 and $\geqslant$7 mm. Values for each clinical parameter were averaged separately within the three PD categories in each subject and then averaged across subjects in the treatment groups. Mean counts ($\times 10^5$) of individual bacterial species were averaged within each subject and then across subjects in the three therapeutic groups. The percentage of the total DNA probe counts was determined initially in each site, then per subject and averaged across subjects in the three groups. Prevalence was computed by determining the proportions of sites per subject colonized by the individual bacterial species at counts $\geqslant 10^6$ and then averaging these proportions across subjects in the different treatment groups. The significance of differences among the three groups for the clinical and microbiological parameters was sought using the Kruskal–Wallis test. If significance was achieved, the Mann–Whitney *U*-test was used to

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assess differences between two groups. The Wilcoxon test was used to detect statistically significant differences within each group between the two time points. Adjustments for multiple comparisons (Socransky et al. 1991) were performed when the 40 bacterial species were evaluated simultaneously. The level of significance was set at 5%.

Results

Subject retention

Figure 1 presents the flow chart of the study design. Two out of the 45 selected subjects (one from the MTZ group and one from the MTZ+AMX group) did not return for the 3-month follow-up visit and were excluded from the statistical analysis. Thus, a total of 43 subjects completed the study: 15 in the placebo group (C), 14 in the MTZ group and 14 in the MTZ+AMX group.

Adverse effects

All subjects who finished the study reported full adherence to the prescribed course of antibiotics/placebo. Two subjects, one from the MTZ group and one from the MTZ+AMX group, reported adverse events during the study, such as diarrhoea and vomiting (nausea). No adverse events were reported in the C group. All subjects affirmed that the medications did not cause any major disturbance in their daily routine and that they would start the treatment again if necessary.

Clinical findings

The demographic characteristics and full-mouth mean values of periodontal clinical parameters at baseline and at 3 months post-therapy for the treatment groups are presented in Table 1. No statistically significant differences were observed among groups for any parameter ($p > 0.05$) at baseline. All therapies led to a statistically significant decrease in mean PD, CAL and in the percentage of sites with visible plaque and suppuration. However, the percentage of sites exhibiting BOP was only significantly decreased in the test groups ($p < 0.01$). The MTZ-treated group also showed a significant reduction in the percentage of sites presenting gingival bleeding ($p < 0.01$).

The mean changes in PD, CAL and BOP between baseline and 3 months post-therapy are presented in Figs 2 and 3. The results are organized according to full-mouth values (Fig. 2) as well as different baseline PD categories in shallow ($\leqslant 3$ mm), intermediate (4–6 mm) and deep ($\geqslant 7$ mm) sites (Fig. 3). The three therapies led to improvements in these clinical parameters; however, differences among treatments were observed. Overall, subjects who received antibiotics as part of the periodontal treatment had the best clinical results, especially those from the MTZ+AMX group. When the full-mouth mean values were considered (Fig. 2), subjects receiving this combination of therapies showed the greatest reduction in mean PD and CAL compared with the other two groups ($p < 0.01$). Both antibiotic therapies were more effective in reducing the mean percentage of sites with BOP than SRP alone ($p < 0.01$). Figure 3 shows that the initially shallow sites of the MTZ+AMX group showed the greatest reduction in mean PD, followed by MTZ and C groups ($p < 0.01$). Although slight, there was an increase in mean CAL and in the percentage of sites with BOP in the C group at the shallow sites, while the test groups showed similar and more effective mean reductions in these parameters for this PD category. The antibiotic therapies also led to more effective reductions in mean PD and CAL in initially intermediate and deep sites compared with SRP alone ($p < 0.01$). The difference observed between the two test groups was a greater reduction in mean CAL in subjects receiving MTZ+AMX in initially intermediate sites ($p < 0.01$). Therapies with antibiotics were also more effective than SRP in reducing BOP in initially intermediate sites ($p < 0.01$).

Changes in mean full-mouth CAL for each subject at 3 months post-treatment are presented in Fig. 4. Overall, the greatest gains in this parameter were observed in subjects from the MTZ+AMX group, followed by those from the MTZ group. The majority of subjects who took both antibiotics gained more attachment than the median of change in CAL of the 43 subjects (0.61 mm).

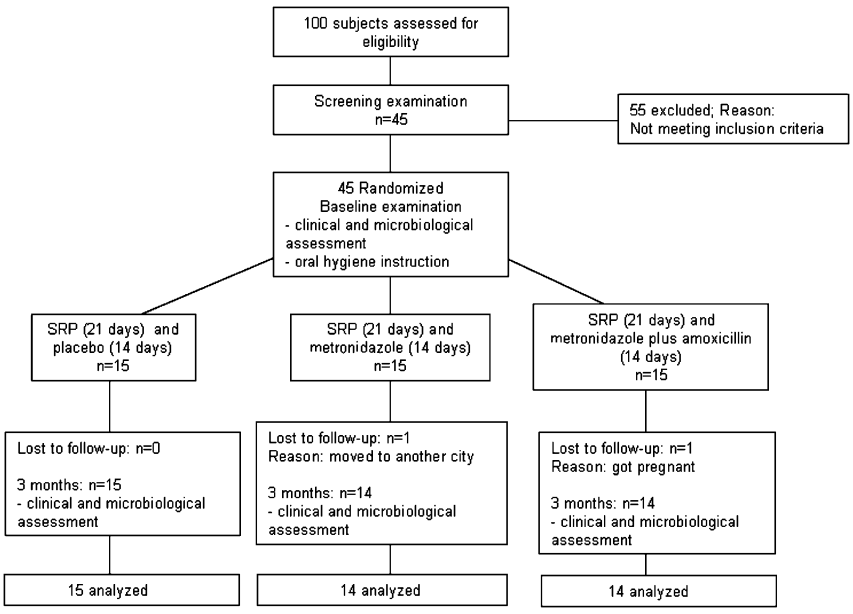

Fig. 1. Flow chart of the study design.

Microbiological findings

At baseline, no significant differences in mean counts, proportions and prevalence of the 40 bacterial species analysed were observed among the three treatment groups (data not shown). Figure 5 presents the mean counts ($\times 10^5$) of the species evaluated at baseline and at 3 months post-therapy in the three treatment groups. The species were grouped according to the microbial complexes described by Socransky et al. (1998). There was a reduction in the mean counts of the pathogens from the red complex, *T. forsythia*, *P. gingivalis* and *T. denticola*, in all groups.

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Table 1. Demographic characteristics and mean (± SD) full-mouth clinical parameters at baseline and 3 months post-therapy in the three treatment groups

Variable	Treatment groups					
	SRP ($n = 15$)		SRP+MTZ ($n = 14$)		SRP+MTZ+AMX ($n = 14$)	
	baseline	3 months	baseline	3 months	baseline	3 months
Gender (male/female)	7/8	7/8	7/8	6/8	7/8	6/8
Age (years)	40.5 ± 8.2	NA	40.8 ± 5.1	NA	42.8 ± 7.1	NA
Percentage of sites with PD⩽ mm	55.8 ± 20.4	NA	50.1 ± 22.2	NA	52.4 ± 25.9	NA
Percentage of sites with PD 4–6 mm	36.8 ± 29.3	NA	38.2 ± 27.1	NA	37.8 ± 30.2	NA
Percentage of sites with PD⩾7 mm	7.4 ± 5.5	NA	11.7 ± 8.4	NA	9.8 ± 5.9	NA
Probing depth (mm)	3.9 ± 0.6	3.3 ± 0.5***	3.7 ± 0.9	2.9 ± 0.6***	4.0 ± 0.7	3.0 ± 0.8***
Clinical attachment level (mm)	4.7 ± 1.2	4.2 ± 1.1***	4.5 ± 0.9	3.9 ± 0.9***	4.8 ± 0.8	3.9 ± 0.8***
Percentage of sites with						
Plaque accumulation	66.6 ± 22.9	42.6 ± 25.7***	75.3 ± 17.0	50.2 ± 15.7***	73.7 ± 18.8	43.5 ± 16.9***
Gingival bleeding	15.5 ± 19.1	16.7 ± 15.2	28.5 ± 29.9	15.9 ± 19.5**	15.1 ± 13.0	12.1 ± 11.6
Bleeding on probing	67.0 ± 18.2	65.3 ± 17.4	65.5 ± 25.8	55.1 ± 20.2**	75.8 ± 22.9	56.3 ± 16.5**
Suppuration	3.5 ± 5.4	0.9 ± 1.3***	1.6 ± 2.4	0.0 ± 0.0***	2.9 ± 1.7	0.0 ± 0.0***

$p > 0.05$, no significant differences among treatment groups at baseline (Kruskal–Wallis test).
Significance of differences between the two time points was assessed using the Wilcoxon test.
**$p < 0.01$, ***$p < 0.001$.
SRP, scaling and root planning; MTZ, metronidazole; AMX, amoxicillin; NA, data not analysed.

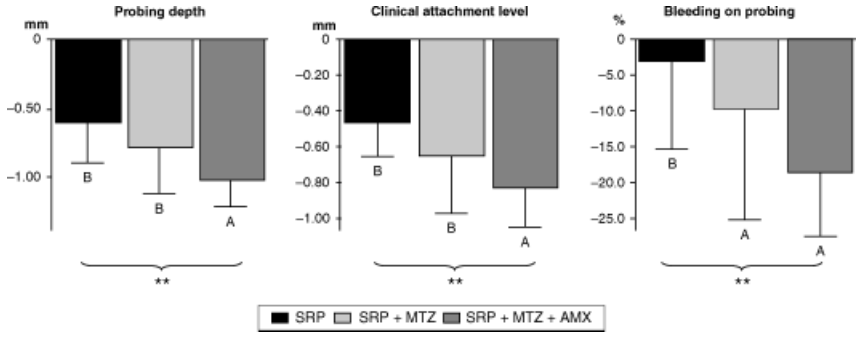

Fig. 2. Bar charts of the mean changes (± SD) in full-mouth probing depth, clinical attachment level and percentage of sites with bleeding on probing between baseline and 3 months post-therapy in the three treatment groups. The whiskers represent the SD. The significance of difference among the three treatment groups for each clinical parameter was assessed using the Kruskal–Wallis test (**$p < 0.01$). Subsequently, the significance of difference within pairs of groups was assessed using the Mann–Whitney *U*-test ($p < 0.05$; different letters indicate statistically significant differences). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

However, these reductions were not significant in the C group. Subjects treated with adjunctive MTZ or MTZ+AMX exhibited a significant decrease in the mean counts of all three species from this complex, while two species (*T. forsythia* and *P. gingivalis*) were significantly reduced in the MTZ group. The mean counts of some putative periodontal pathogens from the orange complex were also significantly reduced in the MTZ (*Campylobacter rectus*, *Eubacterium nodatum* and *P. micra*) and MTZ+AMX groups (*E. nodatum*, *Fusobacterium nucleatum polymorphum* and *P. micra*). Although not statistically significant, it was interesting to observe that all four *Fusobacterium* species evaluated increased in levels at 3 months after SRP alone (C group). Levels of the majority of host-compatible species from the purple, yellow, blue and green complexes, in addition to *Actinomyces* species, were minimally affected by the different therapies.

Figure 6 shows the mean proportions of DNA probe counts from the 40 individual bacterial species studied at baseline and at 3 months post-therapy. The proportions of the three red complex species were reduced after therapy in the MTZ+AMX group, two species in the MTZ group and one species in the C group. Furthermore, the proportions of one orange complex species, *E. nodatum*, were also reduced in the MTZ+AMX group. Several beneficial species, especially those from the blue and purple complexes, showed an increase in proportions after treatments. This was particularly noted in the two test groups. Although these changes were not statistically significant when the host-compatible species were considered individually, they were clearer when the species were grouped by complexes. These results are better represented in Fig. 7, which summarizes the proportions of the microbial complexes at baseline and at 3 months post-therapy. At baseline, the proportions of the different microbial complexes were similar among the three experimental groups ($p > 0.05$). All therapies led to a significant reduction in the red complex, with the most profound reduction occurring in the MTZ+AMX (from 32% to 2.01%, $p < 0.001$) and MTZ groups (from 25.51% to 4.51%, $p < 0.01$), followed by the C group (from 33.28% to 8.67%, $p < 0.05$). This complex was in a significantly lower proportion in the MTZ+AMX group than in the other two groups at 3 months post-treatment ($p < 0.05$). Interestingly, the proportions of orange complex were not affected by any of the three therapies. This microbial complex actually showed a slight increase at 3 months post-therapy in all

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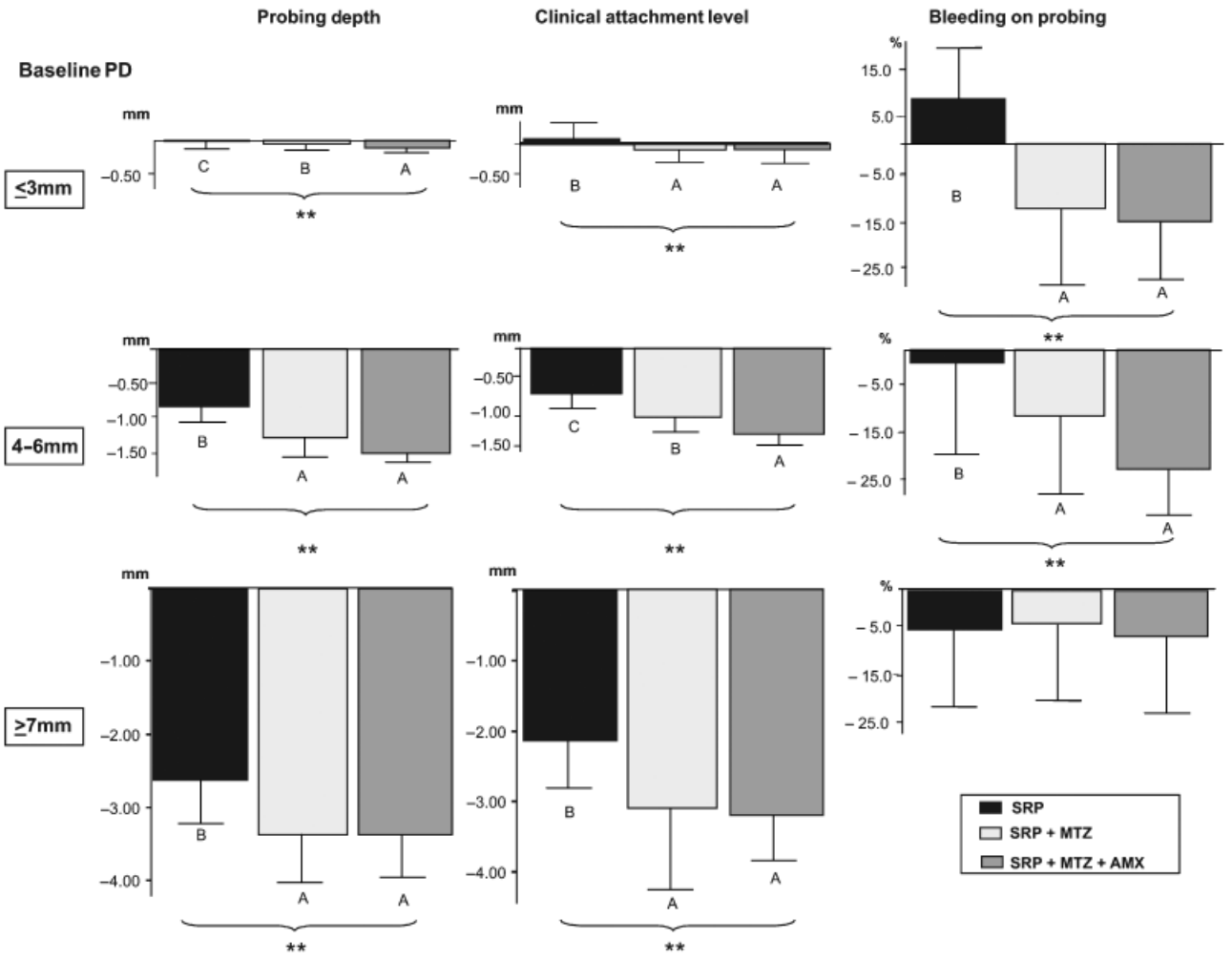

Fig. 3. Bar charts of the mean changes (± SD) in probing depth, clinical attachment level and percentage of sites with bleeding on probing at sites with initial probing depth ⩽3, 4–6 and ⩾7 mm between baseline and 3 months post-therapy in the three treatment groups. The whiskers represent the SD. The significance of difference among the three treatment groups for each clinical parameter was assessed using the Kruskal–Wallis test (**$p<0.01$). Subsequently, the significance of difference within pairs of groups was assessed using the Mann–Whitney *U*-test ($p<0.05$; different letters indicate statistically significant differences). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

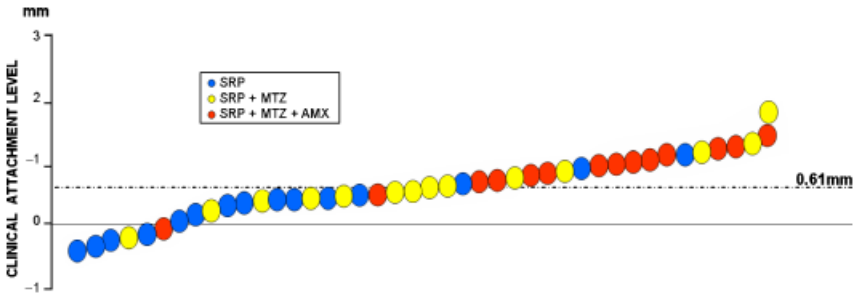

Fig. 4. Plots of the mean changes in individual full-mouth mean clinical attachment level between baseline and 3 months post-therapy of subjects in the three treatment groups. The circles represent the mean value of each subject. The dashed line represents the median of change of this clinical parameter in all 43 subjects. Positive values represent a gain in clinical attachment level (CAL), while negative values represent a loss in CAL at 3 months post-therapy.

groups, increasing from 29.11% to 36.73% in the C group, from 32.94% to 35.19% in the MTZ group and from 30.97% to 32.24% in the MTZ+AMX group.

The proportions of the beneficial blue complex were significantly increased after therapy in the MTZ+AMX group, as well as the purple complex in the MTZ and MTZ+AMX groups. Bacterial species that do not belong to any specific complex, represented by the grey colour, were also significantly decreased in the MTZ+AMX group. In Fig. 7, the areas of the pies were adjusted to reflect the total mean counts of the species evaluated at each time point in each group. All therapies

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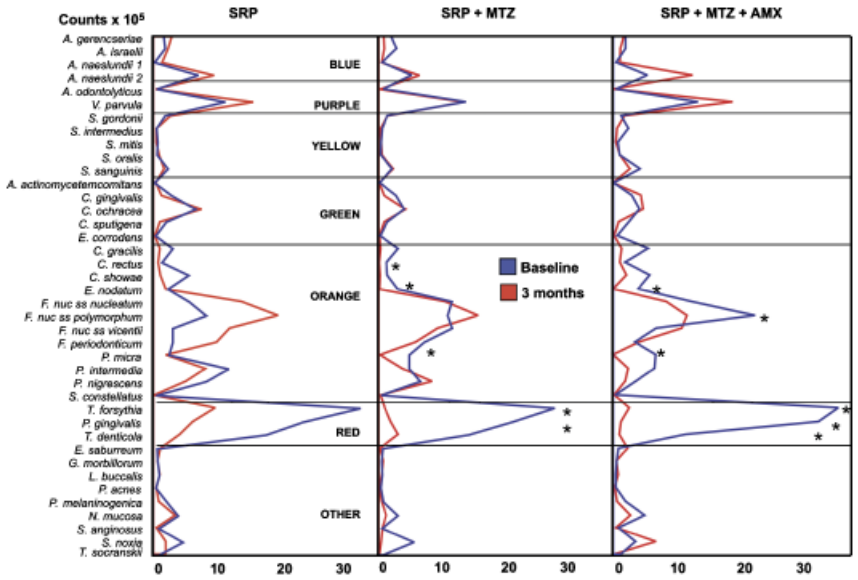

Fig. 5. Mean counts ($\times 10^5$) of the 40 test species at baseline and 3 months post-therapy in the three treatment groups. The species were ordered according to the microbial complexes described by Socransky et al. (1998). Counts of individual species were averaged within a subject and then across subjects in each treatment group at each time point. The significance of differences between the two time points was assessed using the Wilcoxon test ($^*p<0.05$), and adjusted for 40 comparisons (Socransky et al. 1991). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

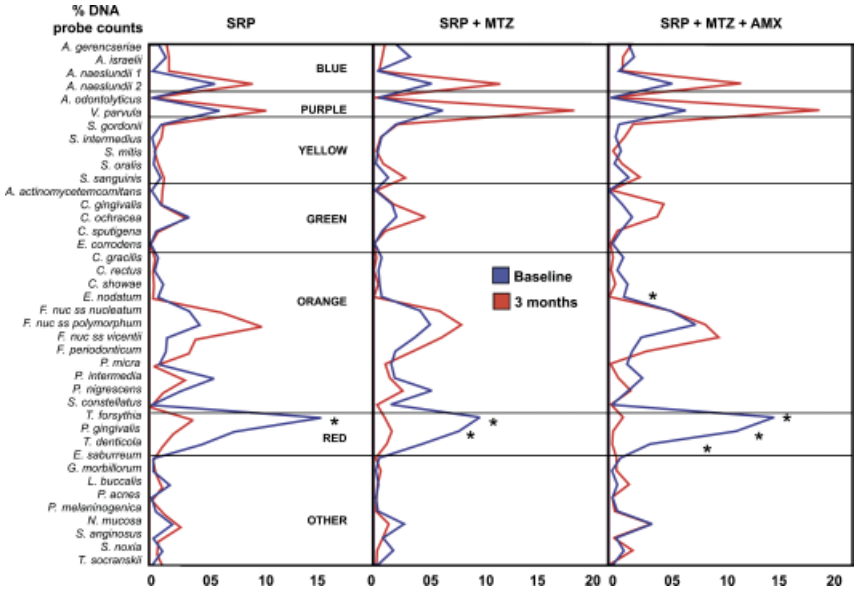

Fig. 6. Mean percentage DNA probe counts of the 40 test species at baseline and 3 months post-therapy in the three treatment groups. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The proportion that each species comprised the total DNA probe count was determined at each site, and then averaged within and across subjects in each treatment group at each time point. The significance of differences between the two time points was assessed using the Wilcoxon test ($^*p<0.05$), and adjusted for 40 comparisons (Socransky et al. 1991). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

reduced the total bacterial counts, and no significant differences were observed among groups at baseline or at 3 months post-treatments.

The comparison among treatments as regards reducing the proportions of periodontal pathogens and increasing beneficial bacterial species is summarized in Fig. 8. The microbial species were divided into two groups: those compatible with the host (blue, purple, yellow and green complexes; not including *A. actinomycetemcomitans*) and periodontal pathogens (orange and red complexes, *A. actinomycetemcomitans* and *Treponema socranskii*). Both therapies including antibiotics were more effective in reducing periodontal pathogens than SRP alone ($p<0.05$), while the MTZ+AMX treatment had the most striking effect on increasing host-compatible species ($p<0.05$).

The percentage of sites colonized by *T. forsythia*, *P. gingivalis*, *T. denticola*, *P. micra* and *E. nodatum* at levels $\geqslant 10^6$ cells was significantly decreased in the MTZ+AMX group and by *P. gingivalis* in the MTZ group. SRP alone did not change the percentage of sites colonized by any of the species evaluated (data not shown).

Discussion

This study evaluated the clinical and microbiological effects of SRP in combination with MTZ or with MTZ+AMX in the treatment of smokers with chronic periodontitis. The growing interest in defining more effective periodontal therapies for smokers is related to the fact that this risk group may respond poorly to mechanical therapies such as SRP (Winkel et al. 2001, van der Velden et al. 2003, Darby et al. 2005, Mascarenhas et al. 2005, Heasman et al. 2006, Hughes et al. 2006, Pahkla et al. 2006, Grossi et al. 2007). The adjunctive use of MTZ or MTZ+AMX was selected based on earlier studies that suggested the clinical and microbiological benefits of these antibiotics, alone or combined, in the treatment of chronic periodontitis in non-smokers (Lindhe et al. 1983, Loesche et al. 1984, 1991, 1992, 2005, van Winkelhoff et al. 1996, Elter et al. 1997, Feres et al. 2001, Winkel et al. 2001, Rooney et al. 2002, Carvalho et al. 2004, 2005, Haffajee et al. 2007, 2008) or in smokers (Söder et al. 1999, Winkel et al. 2001, Pahkla et al. 2006).

The optimal dose of MTZ and AMX for the treatment of periodontal diseases has not yet been appraised directly; however, most of the studies evaluating the effects of systemically administered AMX on the periodontal treatment, including the present study, have used 1,500 mg/day of this antibiotic (López & Gamonal 1998, López et al. 2000,

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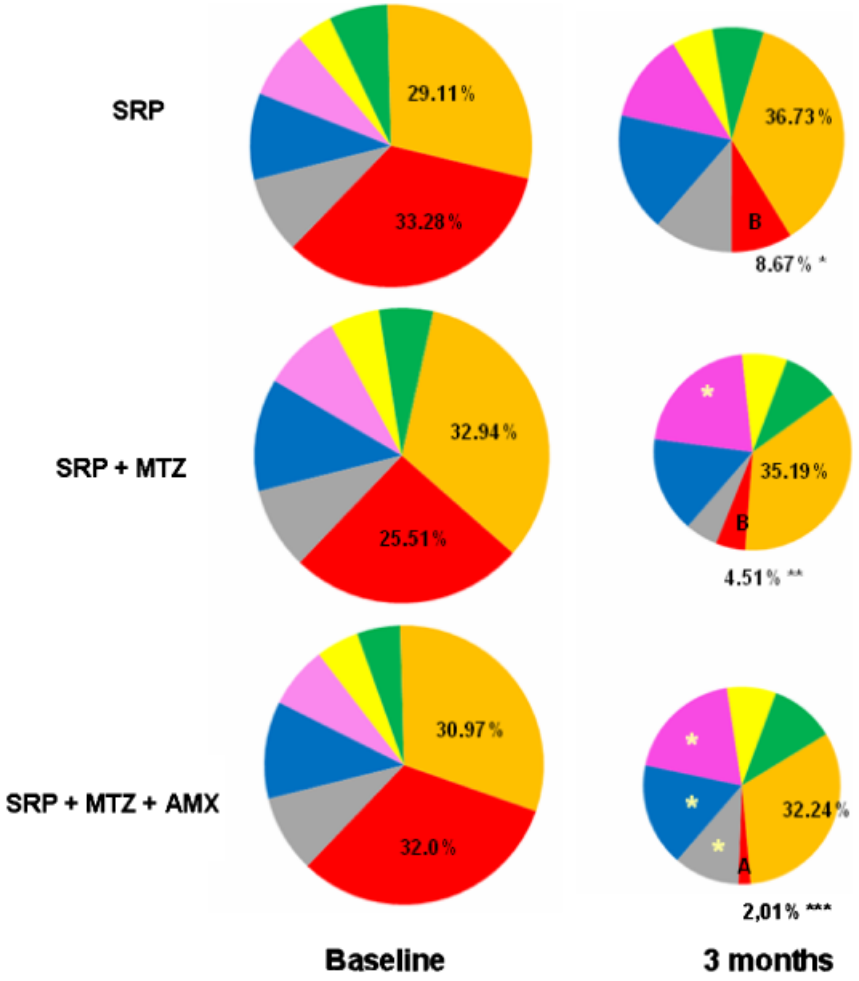

Fig. 7. Pie charts of the mean proportion of each microbial complex at baseline and 3 months post-therapy in the three treatment groups. The colours represent different microbial complexes (Socransky et al. 1998). The areas of the pies were adjusted to reflect the mean total counts at each time point. The significance of differences between the two time points was assessed using the Wilcoxon test (***$p<0.001$, **$p<0.01$, *$p<0.05$). The significance of differences among treatment groups at baseline and at 3 months post-therapy was assessed using the Kruskal–Wallis and the Mann–Whitney *U*-test ($p<0.05$; different letters indicate statistically significant differences). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

2006, Feres et al. 2001, Guerrero et al. 2005, Xajigeorgiou et al. 2006). The ideal MTZ dose is somewhat more controversial. Early studies used 750 mg/day of this antibiotic to treat periodontal infections (Lindhe et al. 1983, Loesche et al. 1984, 1987, 1991). However, the knowledge that lower antibiotic dosages may limit the clinical and microbiological effects of systemically administered agents (van Winkelhoff et al. 1999) has led to an overall increase in the MTZ dosage by different groups of investigators. Therefore, the 1,200 mg/day of MTZ used in the present investigation is in agreement with previous studies from our (Carvalho et al. 2004, 2005, D'avila et al. 2005) and other groups (Guerrero et al. 2005, Xajigeorgiou et al. 2006).

Clinical data

All three therapies used in the present study improved the majority of the clinical parameters evaluated. However, both antibiotic therapies had significant clinical benefits over SRP alone, and the greatest improvements were observed in the MTZ+AMX-treated subjects.

SRP combined with either MTZ or with MTZ+AMX was more effective in improving PD and CAL in initially shallow (<3 mm), intermediate (4–6 mm) and deep (⩾7 mm) sites than SRP alone. The same pattern was observed for the BOP parameter. These results are in agreement with previous studies in the literature that showed an enhanced clinical response when these two antibiotics were combined with SRP on the treatment of chronic periodontitis (Berglundh et al. 1998, Feres et al. 2001, Carvalho et al. 2004, Haffajee et al. 2003b, 2007). Although few clinical trials have addressed the effects of systemic antibiotics on smokers, the benefits of MTZ (Söder et al. 1999) or MTZ+AMX (Winkel et al. 2001, Pahkla et al. 2006) in the periodontal treatment of this risk group have been suggested previously. Conversely, Palmer et al. (1999) showed no additional benefits of systemic MTZ when treating a group of smokers with periodontal disease. The low antibiotic dose used in that study (600 mg/day) may have contributed to the lack of clinical efficacy (van Winkelhoff et al. 1999).

Subjects in the MTZ+AMX group showed the greatest improvements in mean PD and CAL after therapy, in comparison with the other two groups. The individual change in mean CAL is also an important finding (Fig. 4). Twelve out of the 14 subjects receiving MTZ+AMX presented a mean gain in CAL above the median of change of this parameter for the 43 subjects (0.61 mm). Conversely, 13 out of the 15 subjects in the C group showed attachment change within or below this value. This is an important observation, as it indicates a constant beneficial response at the subject level with the use of this combined antimicrobial therapy.

The better clinical response with the use of SRP+MTZ+AMX over SRP+MTZ observed in the present study is in agreement with the controlled clinical trial of Rooney et al. (2002), which, to our knowledge, is the only study to date that has compared these two therapies in the periodontal treatment of chronic periodontitis in a non-smoking population.

Microbiological data

Very few studies to date have evaluated the microbiological changes that occur with the use of MTZ or MTZ+AMX in the treatment of smokers with periodontal disease, or have directly compared these two treatment protocols.

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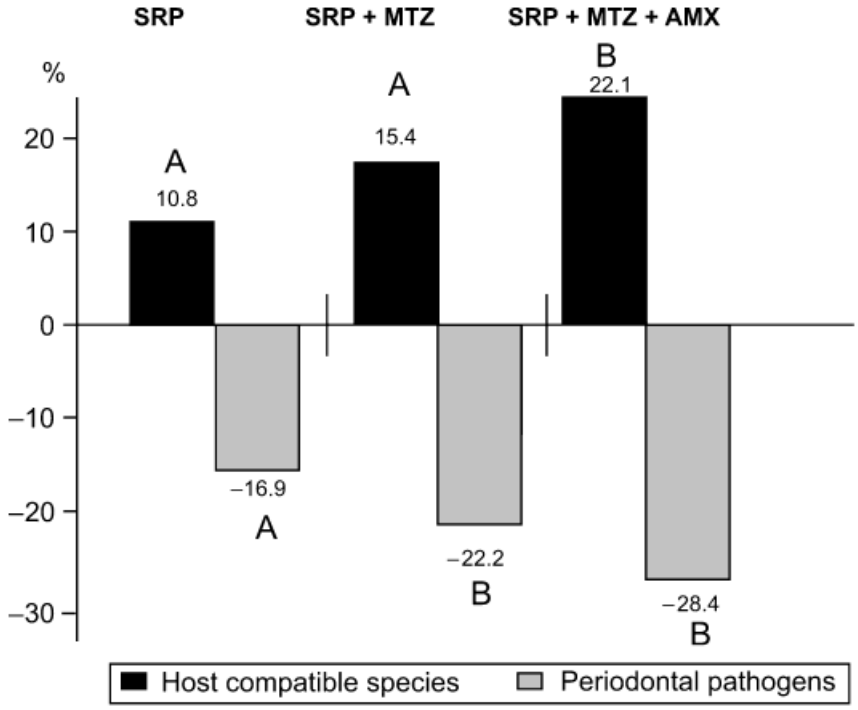

Fig. 8. Bar charts of the mean changes in the proportion of host-compatible species (blue, purple, yellow and green complexes – not including *Aggregatibacter actinomycetemcomitans*) and periodontal pathogens (orange and red complexes, *A. actinomycetemcomitans* and *Treponema socranskii*) between baseline and 3 months post-therapy in the three treatment groups. The significance of differences among treatment groups was assessed using the Kruskal–Wallis and the Mann–Whitney *U*-test ($p < 0.05$; different letters indicate statistically significant differences). SRP, scaling and root planing; MTZ, metronidazole; AMX, amoxicillin.

Overall, these investigations have targeted at most six bacterial species and used dark-field microscopy or culture techniques (Palmer et al. 1999, Söder et al. 1999, Winkel et al. 2001, Pahkla et al. 2006). Therefore, to our knowledge, this is the first controlled clinical trial that systematically evaluated the effects of MTZ and MTZ+AMX on the subgingival microbial composition of smokers. The use of a checkerboard DNA–DNA hybridization assay for 40 bacterial species allowed a thorough evaluation of the effects of the three different treatments on the subgingival microbial profile.

In agreement with the clinical results, the MTZ+AMX group presented the most favourable changes in the subgingival microbial profile after treatment, even superior to those of the MTZ group. These subjects showed a striking reduction in the mean counts, proportions and prevalence (percentage of sites colonized at levels $\geqslant 10^6$) of the three red complex pathogens, *T. forsythia*, *P. gingivalis* and *T. denticola*, followed by the MTZ group, which also demonstrated a significant reduction in the counts and proportions of *T. forsythia* and *P. gingivalis*. In addition, both antibiotic therapies were more effective in reducing the overall proportions of periodontal pathogens than SRP alone (Fig. 8). These results are in agreement with other studies that also demonstrated the adjunctive effects of these two antibiotics, alone or combined, in reducing red complex species in populations mainly composed of smokers (Söder et al. 1999, Winkel et al. 2001) or of non-smokers with chronic periodontitis (Berglundh et al. 1998, Winkel et al. 1998, Feres et al. 2001, Haffajee et al. 2003, 2008, Carvalho et al. 2005, Moeintaghavi et al. 2007). SRP alone had a limited effect on red complex species, leading to a significant decrease only in the individual mean proportions of *T. forsythia*. The reduced effect of the mechanical therapy in suppressing individual levels or proportions of these pathogens in smokers has been reported by other authors (Haffajee et al. 1997, Söder et al. 1999, Winkel et al. 2001, Van der Velden et al. 2003, Darby et al. 2005, Mascarenhas et al. 2005, Grossi et al. 2007).

The majority of putative periodontal pathogens from the orange complex were not affected by mechanical therapy. In fact, the counts, proportions and prevalence of these species were increased after SRP alone (from 29% to 37%). This was particularly observed for the *Fusobacterium* species. Only subjects who received systemic antibiotics exhibited significant reductions in some orange complex microorganisms, such as *E. nodatum*, *P. micra*, *C. rectus* and *F. nucleatum* ssp. *polymorphum*. *E. nodatum*, whose counts, proportions and prevalence were effectively decreased in the present study in the MTZ+AMX group, was recently associated with the aetiopathogenesis of chronic periodontitis in American (Haffajee et al. 2006) and Brazilian subjects (Colombo et al. 2002).

This trend of increasing levels of orange complex species after SRP alone in the treatment of smokers has been reported recently by Grossi et al. (2007). van Winkelhoff et al. (2001) also showed that certain species from the orange complex may persist after non-surgical mechanical periodontal treatment in smokers. The authors observed that treated smoker subjects had higher prevalence and/or levels of *P. micra*, *C. rectus* and *F. nucleatum* than non-smokers. The lack of efficacy of SRP on pathogens of the orange complex, or even the overgrowth of this species, as observed in the control group, could be one of the reasons for the inefficiency of this therapy for treating and maintaining the long-term periodontal stability of smoker subjects. It is worth noting that putative pathogens from the orange complex precede the colonization of the red complex (Socransky & Haffajee 2005, Kolenbrander et al. 2006) and therefore suppressing these species is one of the important endpoints of periodontal therapy (Teles et al. 2006).

It was also interesting to observe that the proportions of several host-compatible species from the blue, purple, yellow and green complexes tended to increase after the antibiotic treatments, especially in the MTZ+AMX group (Figs 7 and 8).

Taken together, the microbiological data suggest a clear benefit of the combined use of MTZ, and especially of MTZ+AMX in the subgingival

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microbial profile of smoker subjects at 3 months after periodontal therapy. This is a very important finding, as recent knowledge about oral biofilm ecology suggests that the microbiological profile obtained in the short term would probably determine the long-term periodontal stability (Winkel et al. 2001, Haffajee et al. 2007, 2008). The longitudinal follow-up of these subjects will help to clarify this hypothesis.

Overall, the results of this study indicate that systemic antibiotics are useful tools in the treatment of smoker subjects with chronic periodontitis and lead to additional beneficial effects when compared with SRP alone. Moreover, the greatest benefits in clinical parameters and in the composition of the subgingival microbiota are obtained with the use of SRP+MTZ+AMX.

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Clinical Relevance

Scientific rationale for the study: Previous studies have suggested the adjunctive benefits of MTZ and AMX in the treatment of chronic periodontitis, but little is known about the value of these agents in the treatment of smokers.

Principal findings: Subjects receiving a combination of SRP with MTZ or MTZ+AMX showed a better clinical response and the most beneficial changes in the subgingival microbial profile, especially those receiving both antibiotics.

Practical implications: There are adjunctive clinical benefits in using MTZ+AMX combined with SRP in the treatment of smokers. The improved microbiological changes obtained might help to maintain long-term clinical stability.

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