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Scaling and root planing, systemic metronidazole and professional plaque removal in the treatment of chronic periodontitis in a Brazilian population II – microbiological results

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Abstract

Objective: The current investigation evaluated changes in levels and proportions of 39 bacterial species in subgingival plaque samples after scaling and root planing (SRP) alone or in combination with systemic metronidazole and/or professional cleaning in subjects with chronic periodontitis.

Methods: Forty-four adult subjects (mean age 45 ± 6 years) with periodontitis were randomly assigned in four treatment groups, a control (C, $n = 10$) that received SRP and placebo and three test groups treated as follows: T1 ($n = 12$): SRP and metronidazole (M, 400 mg tid) for 10 days; T2 ($n = 12$): SRP, weekly professional supragingival plaque removal for 3 months (PC) and placebo; and T3 ($n = 10$): SRP, M and PC. Subgingival plaque samples were taken from seven sites per subject at baseline and 90 days post-therapy. Counts of 39 subgingival species were determined using checkerboard DNA–DNA hybridization. Significance of differences over time was determined using the Wilcoxon signed ranks test and among groups using ANCOVA.

Results: The mean counts of the majority of the species were reduced post-therapy in the 4 treatment groups. Counts ($\times 10^5 \pm$ SEM) of *Porphyromonas gingivalis*, *Tannerella forsythensis* and *Treponema denticola* were significantly reduced in groups T2 and T3. Levels of beneficial species, such as some *Actinomyces* species, *Veillonella parvula*, *Streptococcus sanguis*, *Streptococcus oralis* and *Streptococcus gordonii* were minimally affected in levels when the combined therapy was applied (T3). Mean proportions of red complex species decreased from 18.4% at baseline to 3% at 90 days post-therapy in group T3 ($p < 0.01$), from 25.8% to 2.3% in group T2 ($p < 0.01$), from 17.7% to 5.6% in group T1 ($p < 0.05$) and from 19.4% to 8.8% in group C (NS). Proportions of the suspected periodontal pathogens from the orange complex were also markedly reduced in groups T2 and T3.

Conclusions: All treatments reduced counts and proportions of red complex species. Adjunctive therapy appeared to have a greater effect and also affected members of the orange complex.

Key words: antibiotics; metronidazole; periodontal disease; periodontal therapy; professional cleaning; scaling and root planing; subgingival plaque; supragingival plaque; treatment

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The collective efforts of different groups of investigators in the past decades have provided significant data clarifying the etiology of periodontal diseases, including a description of the nature of bacterial species associated with periodontal health or different forms of periodontal disease (Newman et al. 1976, Slots et al. 1980, Loesche et al. 1982, Keyes & Rams 1983, Moore et al. 1985, Socransky et al. 1988, Haffajee & Socransky 1994, Riviere et al. 1997, Zambon 1996). This knowledge made it possible to define useful microbiological endpoints of periodontal therapy and re-enforced the concept that clinical efficacy is associated with suppression of periodontal pathogens and re-colonization by host-compatible species. Scaling and root planing (SRP) is the most common periodontal treatment and several studies have demonstrated an improvement in clinical parameters with the use of this therapy (Sbordone et al. 1990, Ali et al. 1992, Pedrazzoli et al. 1991, Haffajee et al. 1997, Cugini et al. 2000). Clinical benefits were associated with microbiological changes, including a decrease in total counts of microorganisms and in the percentages of certain pathogens, such as spirochetes, *Porphyromonas gingivalis* and *Tannerella forsythensis* (*Bacteroides forsythus*) (Slots et al. 1979, Hinrichs et al. 1985, Pedrazzoli et al. 1991, Haffajee et al. 1997, Cugini et al. 2000). Not all of these changes were sustained over time. Treated periodontal patients required enrollment in a maintenance program in order to keep counts of pathogens at lower levels and to maintain periodontal stability (Lindhe & Nyman 1975, Rosling et al. 1976, Nyman et al. 1977, Rosling 1983, Lindhe & Liljenberg 1984, Cugini et al. 2000).

Other forms of periodontal therapy have been proposed in conjunction with SRP with the objective of improving the clinical and microbiological results of therapy. Supragingival plaque removal represents a critical phase of periodontal therapy since patients with poor plaque control do not achieve satisfactory clinical results post-therapy (Nyman et al. 1977). Repeated professional supragingival plaque removal has been used in conjunction with SRP as part of periodontal therapy and has lead to beneficial changes in the subgingival microbiota (Magnusson et al. 1984, McNabb et al. 1992, Ximenez-Fyvie et al. 2000). Ximenez-Fyvie et al. (2000) treated 18 subjects, who were in a maintenance phase after treatment of moderate chronic periodontitis, with SRP and professional supragingival plaque removal every week for 3 months. Microbiologic changes for 40 bacterial species were determined using DNA probes and checkerboard DNA–DNA hybridization. This combined therapy decreased the subgingival levels and proportions of several periodontal pathogens, such as *Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis* and *Treponema denticola*. Surprisingly, the changes were maintained 9 months after cessation of weekly professional supragingival plaque removal. At the final monitoring visit, the composition of the subgingival microbiota of these subjects was similar to that of a group of 22 periodontally healthy subjects.

Systemically administered antibiotics have also been used in conjunction with SRP for the treatment of periodontitis (for review, see Slots & Ting 2002). Metronidazole seems to be a promising drug for the treatment of chronic periodontitis, in part, because of its narrow spectrum of activity, which is directed primarily to strict anaerobic bacteria. Some investigators showed beneficial changes in the composition of the subgingival microbiota when metronidazole was used in conjunction with SRP in the treatment of chronic periodontitis (Lindhe et al. 1983, Loesche et al. 1984, 1991, 1992, Winkel et al. 1997, Feres et al. 2001). Using whole genomic DNA probes for 40 bacterial taxa and the checkerboard DNA–DNA hybridization technique, Feres et al. (2001) observed that the combination of SRP and 250 mg of systemically administered metronidazole three times a day for 14 days led to beneficial changes in the composition of the subgingival microbiota in subjects with chronic periodontitis. There was a striking reduction in counts and proportions of the pathogens of the ''red complex'' (Socransky et al. 1998) and an increase in some host-compatible species, such as *Actinomyces* and *Capnocytophaga* species and *Veillonella parvula*. The microbiological changes observed in that study (Feres et al., 2001) were more profound than changes observed in a study involving the use of SRP only that used the same microbiological techniques and criteria for subject selection (Haffajee et al. 1997). In spite of the additional beneficial microbiological changes observed with the use of metronidazole or repeated professional removal of supragingival plaque, periodontal pathogens were not fully eliminated, and after 1 year of observation some re-colonization was observed. It was felt that an increase in antibiotic dosage and the combination of these therapies might be more effective in improving and maintaining these microbiological changes over time. Thus, the purpose of the present investigation was to evaluate the changes in the composition of the subgingival microbiota resulting from SRP alone or in combination with either systemically administered metronidazole, repeated professional supragingival plaque removal or both in subjects with chronic periodontitis.

Material and Methods

Subject population

Sixty Brazilian subjects >34 years of age with chronic periodontitis who had not previously received periodontal therapy were recruited for the study. Subjects had at least 15 teeth and a minimum of eight teeth with at least one site with pocket depth (PD) and attachment level (AL) between 6 and 10 mm and three sites with PD and AL <4 mm. Exclusion criteria included pregnancy, nursing, any systemic condition that could affect the progression of periodontal disease or required antibiotic coverage for routine dental therapy. In addition, subjects with a known allergy to metronidazole or who had received antibiotic therapy in the previous 6 months were excluded. Subjects who were unwilling to refrain from alcohol consumption during the antibiotic phase of therapy were also excluded.

Experimental design and treatment

In this randomized, placebo-controlled, double-blind, clinical trial, subjects were screened for suitability, and if accepted, were informed of the nature, potential risks and benefits of study participation. Following the signing of an Ethics Committee approved informed consent, subjects were entered into the study. During the initial phase, subjects received clinical and microbiological monitoring, instructions in proper home care techniques and full-mouth SRP performed under local anesthesia (Haffajee et al. 1997). SRP was completed in at most six appointments of approximately 1 h each. Treatment of the entire oral cavity was completed in 14 days. Subsequently,

subjects were randomly assigned to one of the following therapeutic groups: Control (C) – SRP and placebo; Test 1 (T1) – SRP+systemically administered metronidazole (M); Test 2 (T2) – SRP+ weekly professional cleaning (PC) and placebo; Test 3 (T3) – SRP+M+PC. After the completion of SRP the adjunctive therapies were initiated (day 0). Subjects in groups T1 and T3 received 400 mg of systemically administered metronidazole 3 times a day for 10 days. Subjects in groups C and T2 received placebo capsules using the same regimen as the metronidazole therapy. The drug and placebo medications were specifically prepared for use in this study. Compliance was assessed by counting the remaining capsules at day 10 and calling the subject every 2 days during the antibiotic administration phase. Also at day 10 after baseline a questionnaire concerning adverse effects of metronidazole was filled out by each subject. No severe adverse effects were observed for any of the subjects. Subjects in groups T2 and T3 were seen weekly for professional supragingival plaque removal for 3 months. Supragingival plaque was revealed using disclosing solution. Visible supragingival plaque was removed using scalers on all accessible surfaces followed by polishing of the teeth using a rubber cup and dentifrice. Finally, all inter-proximal surfaces were cleaned using dental floss. When performing these procedures, care was taken to limit plaque removal to the supragingival area only. All subjects received clinical, microbiological monitoring and subgingival maintenance scaling at 3 months after therapy. The clinical results have been presented in a companion paper (Carvalho et al. 2004).

Microbiological monitoring

Subgingival plaque samples were taken at baseline and at 90 days post-therapy from seven sites per subject, four sites with PPD and AL between 6 and 10 mm and three sites with PD and AL <4 mm at baseline. Counts of 39 subgingival species were determined in each plaque sample using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994). After removal of supragingival plaque, subgingival plaque samples were taken with individual sterile Gracey curettes and were placed in separate Eppendorf tubes containing 0.15 ml TE (10mM Tris-HCl, 1 mM EDTA, pH 7.6). 0.15 ml of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer. 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 onto a 15×15 cm positively charged nylon membrane (Boehringer-Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking 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-labeled whole genomic DNA probes to 39 subgingival species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes

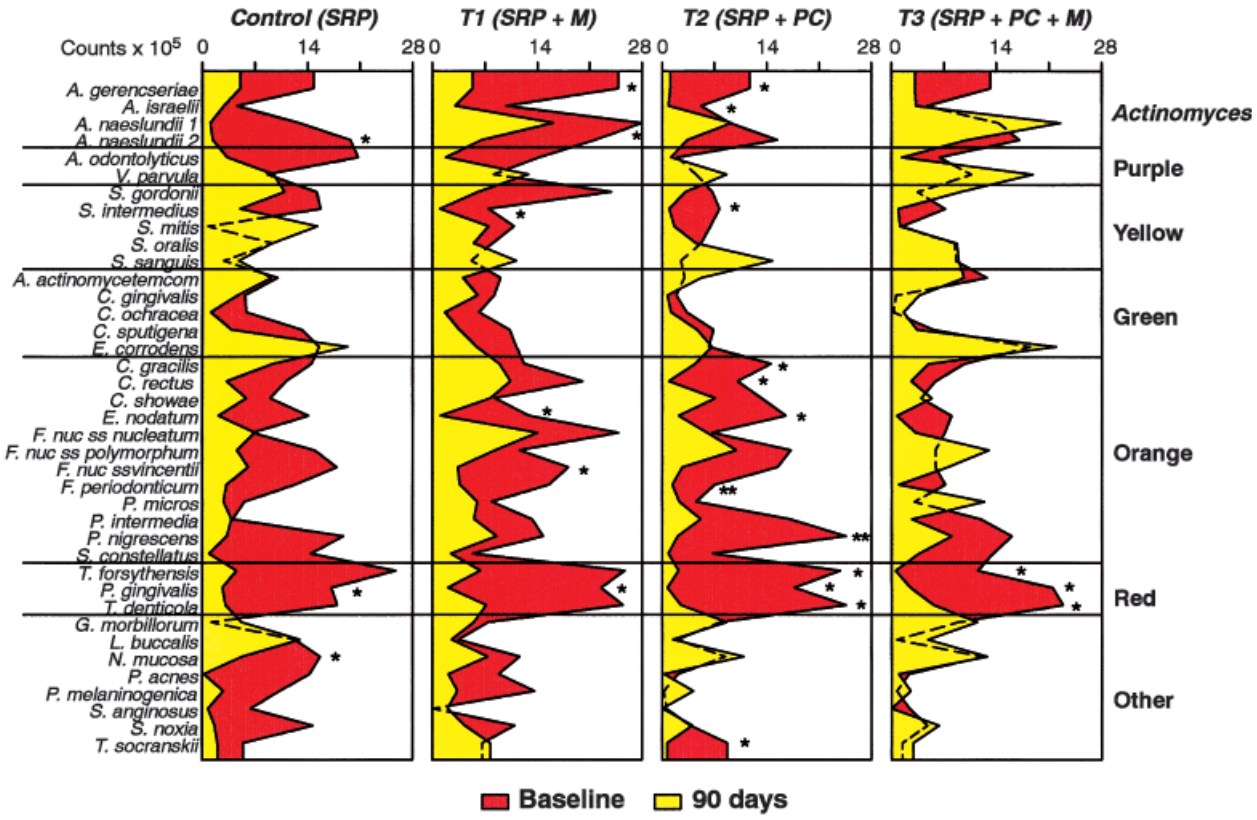

Fig. 1. Panel plots of the mean counts ($\times 10^5$) of the 39 test species in subgingival plaque samples taken at baseline and 90 days in the four treatment groups. Counts of individual species were averaged within a subject and then averaged across subjects in each treatment group at each time point. Significance of differences between baseline and 90 day mean values for each species was tested using the Wilcoxon signed ranks test; $^{*}p<0.05$, $^{**}p<.01$. The species were ordered according to the complexes described by Socransky et al. (1998). SRP, scaling and root planing; M, metronidazole; PC, professional cleaning.

detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. The 39 reference strains employed for the development of DNA probes are presented in Table 1. 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 detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Data analysis

Of the 60 subjects selected for the study, 16 were excluded from the data analysis, two had taken another drug during the course of the study (one in group T2 and the other in group T3) and the other 14 subjects failed to return for the follow-up appointment (five in the control group, three in group T1, two in group T2 and four in group T3). Therefore, microbiological data obtained from 44 subjects were available for data analysis.

Microbiological data available for each subject were the counts of each of the 39 species from seven subgingival plaque samples at baseline and at 90 days for each subject. A total of 616 subgingival plaque samples were examined, 140 in the control group, 168 in group T1, 168 in group T2 and 140 in group T3. In order to compare the counts of each of the bacterial species, the data were expressed as counts $\times$ 10^5, averaged within a subject and then averaged across subjects. Significance of differences in changes in mean counts of bacterial species from baseline to 90 days was computed using ANCOVA with the baseline count of the species as the co-variate. The significance of differences in the levels of each bacterial species over time (from baseline to 90 days) in each group was sought using the Wilcoxon signed ranks test. The total DNA probe count was also computed at each sampled site in each subject and the proportion that each species comprised of that count determined.

Results

The mean counts at baseline and 90 days in the four treatment groups are presented in Fig. 1. There was a reduction in mean counts of a number of bacterial species particularly those of the red complex, *T. forsythensis, P. gingivalis* and *T. denticola*. All three species in this complex were significantly reduced in the subjects in groups T2 and T3. Subjects in group T2 also showed a reduction in mean counts of five of the orange complex species, *C. gracilis, C. rectus, E. nodatum, F. periodonticum* and *P. nigrescens*. Of interest was the minimal change in mean counts of *Actinomyces,* purple, yellow and green complex species in group T3. There were no significant differences in changes in mean counts of each species among treatment groups as determined by ANCOVA adjusting for the baseline counts of the species. The proportions of the different microbial complexes at baseline and 90 days in the four treatment groups are presented in Fig. 2. The proportions of red complex species were significantly reduced in the subjects receiving any of the adjunctive therapies, T1, T2 or T3. The mean proportion of this complex was also reduced in the subjects receiving SRP only, but the difference was not statistically significant. Although there were some changes in proportions of other complexes as a result of therapy, these changes were not statistically significantly different. There were no significant differences in changes in proportions of the complexes among treatment groups as determined by ANCOVA.

Table 1. Strains employed for the development of DNA probes

Actinomyces species		*Campylobacter rectus*	33238
Actinomyces gerencseriae	23860	*Campylobacter showae*	51146
Actinomyces israelii	12102	*Eubacterium nodatum*	33099
Actinomyces naeslundii genospecies 1	12104	*Fusobacterium nucleatum* ss. *nucleatum*	25586
Actinomyces naeslundii genospecies 2	43146	*Fusobacterium nucleatum* ss. *polymorphum*	10953
		Fusobacterium nucleatum ss. *vincentii*	49256
''Purple'' complex		*Fusobacterium periodonticum*	33693
Actinomyces odontolyticus	17929	*Peptostreptococcus micros*	33270
Veillonella parvula	10790	*Prevotella intermedia*	25611
		Prevotella nigrescens	33563
''Yellow'' complex		*Streptococcus constellatus*	27823
Streptococcus gordonii	10558		
Streptococcus intermedius	27335	**''Red'' complex**	
Streptococcus mitis	49456	*Tannerella Forsythensis*	43037
Streptococcus oralis	35037	*Porphyromonas gingivalis*	33277
Streptococcus sanguis	10556	*Treponema denticola*	B1
''Green'' complex		Other species and new DNA probes	
*Actinobacillus actinomycetemcomitans**		*Gemella morbillorum*	27824
Capnocytophaga gingivalis	33624	*Leptotrichia buccalis*	14201
Capnocytophaga ochracea	33596	*Neisseria mucosa*	19696
Capnocytophaga sputigena	33612	*Prevotella melaninogenica*	25845
Eikenella corrodens	23834	*Propionibacterium acnes***	
		Selenomonas noxia	43541
''Orange'' complex		*Streptococcus anginosus*	33397
Campylobacter gracilis	33236	*Treponema socranskii*	S1

All strains were obtained from the American Type Culture Collection (ATCC) except *Treponema denticola* B1 and *Treponema socranskii* S1 which were obtained from the Forsyth Institute. Microbial ''complexes'' were described by Socransky et al. (1998).

*ATCC strains 43718 and 29523;

**ATCC strains 11827 and 11828.

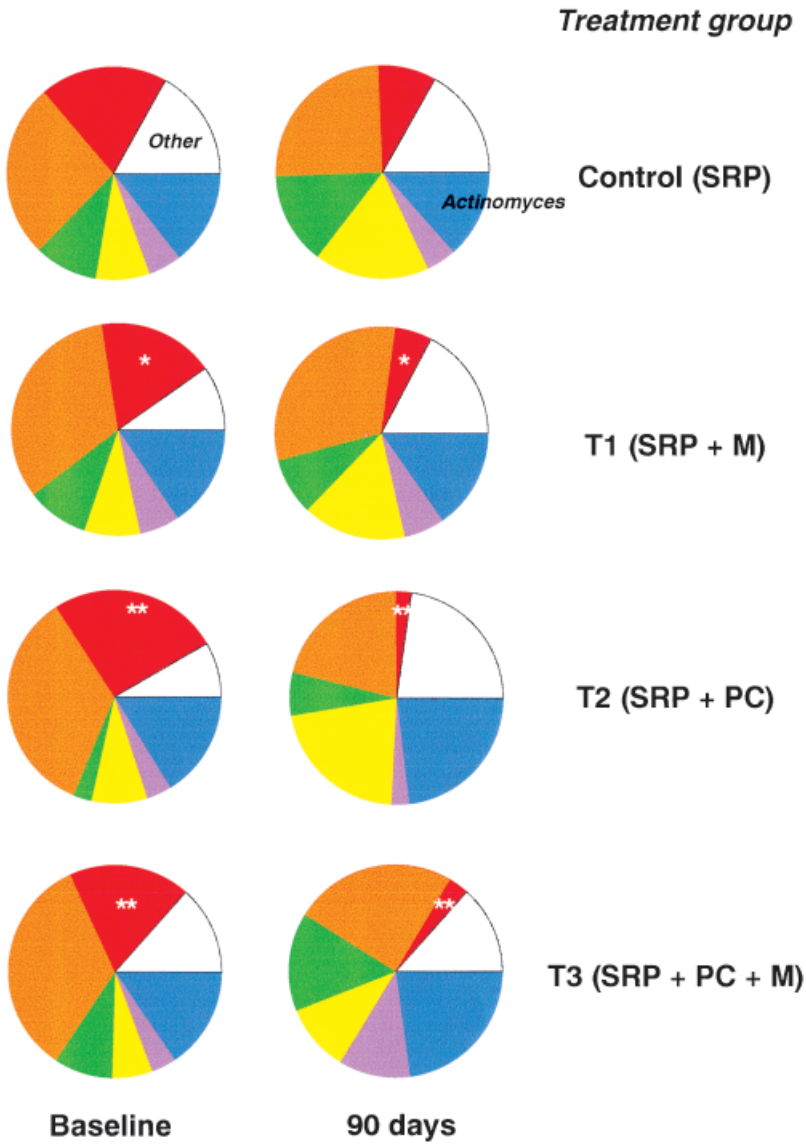

Fig. 2. Pie charts of the mean proportions of each microbial complex at baseline and 90 days in subgingival plaque samples from subjects in the 4 treatment groups. The colours represent the different complexes. Significance of differences in mean proportions between baseline and 90-day mean values for each complex was tested using the Wilcoxon signed ranks test; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. SRP, scaling and root planing; M, metronidazole; PC, professional cleaning.

Discussion

The present study demonstrated a reduction in mean total counts of microorganisms in the subgingival microbiota following the different test therapies in accord with previous studies (Listgarten et al. 1978, Socransky & Haffajee 1993, Haffajee et al. 1997, Cugini et al. 2000). The data suggest that the microbial shifts were more striking in the groups receiving the adjunctive therapies particularly groups T2 and T3. Notable in all three adjunctive groups was a reduction in mean counts and proportions of red complex species and in groups T2 and T3 a reduction of orange complex species, which have also been associated with the pathogenesis of periodontal diseases. Because of the limited numbers of subjects in this study, it could not be determined whether the adjunctive therapies provided a ''statistically significant'' added effect to the beneficial effects demonstrated by SRP alone (Figs 1 and 2). However, the data are suggestive that the adjuncts may provide a better clinical outcome (Haffajee et al. 2003, Carvalho et al. 2004) and microbiological outcome. The greater reduction in counts of red and orange complex species was similar to that observed in a 3-month study on 54 subjects described by Haffajee et al. (2003). In that study, the greatest reduction in red and orange complex species also occurred in the subjects receiving adjunctive therapy, with the most striking effects in the subjects receiving SRP, systemically administered metronidazole and repeated professional supragingival plaque removal.

Both studies, however, did provide data beyond 3 months which will be critical in determining whether the improved clinical and microbiological outcomes observed for the adjunctive therapies, particularly the combined adjunctive therapies, will be sustained over time. In addition, larger numbers of subjects would be helpful in distinguishing additive or even synergistic effects of combining two different forms of therapy to SRP. Thus, the data in the present investigation and that of Haffajee et al. (2003) provide encouraging, but not conclusive evidence that combining therapies with different modes of action may be beneficial in controlling periodontal infections.

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