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Change in subgingival microbial profiles in adult periodontitis subjects receiving either systemically-administered amoxicillin or metronidazole

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Abstract
Aim: The current investigation evaluated changes in levels and proportions of 40 bacterial species in subgingival plaque samples during, immediately after and up to 1 year after metronidazole or amoxicillin therapy combined with SRP.
Method: After baseline clinical and microbiological monitoring, 17 adult periodontitis subjects received full mouth SRP and 14 days systemic administration of either metronidazole (250 mg, TID, $n=8$) or amoxicillin (500 mg, TID, $n=9$). Clinical measurements including % of sites with plaque, gingival redness, bleeding on probing and suppuration, pocket depth (PD) and attachment level (AL) were made at baseline, 90, 180 and 360 days. Subgingival plaque samples were taken from the mesial surface of all teeth in each subject at baseline, 90, 180 and 360 days and from 2 randomly selected posterior teeth at 3, 7, and 14 days during and after antibiotic administration. Counts of 40 subgingival species were determined using checkerboard DNA-DNA hybridization. Significance of differences over time was determined using the Quade test and between groups using ANCOVA.
Results: Mean PD was reduced from 3.22±0.12 at baseline to 2.81±0.16 ($p<0.01$) at 360 days and from 3.38±0.23 mm to 2.80±0.14 mm ($p<0.01$) in the amoxicillin and metronidazole treated subjects respectively. Corresponding values for mean AL were 3.21±0.30 to 2.76±0.32 ($p<0.05$) and 3.23±0.28 mm to 2.94±0.23 mm ($p<0.01$). Levels and proportions of *Bacteroides forsythus, Porphyromonas gingivalis* and *Treponema denticola* were markedly reduced during antibiotic administration and were lower than baseline levels at 360 days. Counts ($\times 10^5$, ±SEM) of *B. forsythus* fell from baseline levels of 0.66±0.16 to 0.04±0.02, 0.13±0.04, 0.10±0.03 and 0.42±0.19 in the amoxicillin group at 14, 90, 180 and 360 days respectively ($p<0.001$). Corresponding values for metronidazole treated subjects were: 1.69±0.28 to 0.02±0.01, 0.20±0.08, 0.22±0.06 and 0.22±0.08 ($p<0.001$). Counts of *Campylobacter* species, *Eubacterium nodatum*, *Fusobacterium nucleatum* subspecies, *F. periodonticum* and *Prevotella nigrescens* were also detected at lower mean levels during and immediately after therapy, but gradually increased after withdrawal of the antibiotics. Members of the genera *Actinomyces, Streptococcus* and *Capnocytophaga* were minimally affected by metronidazole. However, amoxicillin decreased the counts and proportions of *Actinomyces* species during and after therapy.
Conclusions: The data suggest that metronidazole and amoxicillin are useful in rapidly lowering counts of putative periodontal pathogens, but must be accompanied by other procedures to bring about periodontal stability.

Key words: microbiology; bacteria; subgingival plaque; periodontal diseases; periodontitis; metronidazole; amoxicillin; antibiotics

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Systemically-administered antibiotics are widely used in the treatment of periodontal infections. For the most part, clinical experience and the literature suggest a beneficial effect resulting from the use of these agents (Van Winkelhoff et al. 1996). However, the clinician receives mixed signals from both personal experience and examination of the literature. On the one hand, remarkable successes can be observed in individual patients and positive results are reported in many clinical trials (Lindhe et al. 1983, Joyston-Bechal et al. 1984, Loesche et al. 1984, 1987, 1991, 1992, 1996, Van Oosten et al. 1987, Asikainen 1989, Jenkins et al. 1989, Hull et al. 1989, Soder et al. 1990, Eisenberg et al. 1991, Paquette et al. 1992, Noyan et al. 1997). On the other hand, failures can be observed for individual patients and in certain studies (Van Winkelhoff et al. 1996). Further, the clinician is informed that the biofilm structure of dental plaque confers remarkable resistance to species within the biofilm (Nichols et al. 1989, Nickel et al. 1985, Anwar et al. 1989a, b, Hoyle & Costerton 1989, Hoyle et al. 1990, Costerton et al. 1981, 1987, Brown et al. 1988). In addition, there is increasing concern expressed about development of antibiotic resistance (Levy 1997, Pallasch 2000).

The uncertainty about antibiotic usage arises in large part because of lack of knowledge about the microbial changes brought about in dental biofilms. Prior to the notion of biofilm-conferred antibiotic resistance, one might surmise that a systemically administered antibiotic reaching the subgingival microbiota would lay waste to virtually all sensitive species in that region. After the concept of increased resistance due to biofilm structure, one might entertain the possibility that few if any species are affected. The purpose of the present investigation was to attempt to clarify the nature of changes in the composition of the subgingival biofilm resulting from antibiotic usage. It was felt that the greatest observable effect would occur while the subject received the agent. Thus, samples were obtained while the patient was taking the agent, immediately thereafter and for 12 months post therapy. To augment these kinetic studies, 2 antibiotics, amoxicillin and metronidazole, with different mechanisms of action and different bacterial spectrums of activity were chosen for study.

Metronidazole is attractive for the treatment of adult periodontitis patients in part because the narrow spectrum of this agent is thought to work specifically on the anaerobic microbiota associated with periodontal diseases. Indeed, several investigations suggest that systemically administered metronidazole used as an adjunct to scaling and root planing (SRP) in the treatment of adult periodontitis offers a clinical benefit over SRP alone (Lindhe et al. 1983, Loesche et al. 1984, 1987, 1991, 1992, 1996, Van Oosten et al. 1987, Soder et al. 1990, Eisenberg et al. 1991, Noyan et al. 1997). In a series of studies by Loesche and co-workers (Loesche et al. 1987, 1991, 1992, 1996) treatment of adult periodontitis patients with metronidazole (750 mg to 1 g a day for 14 days) was associated with a reduced need for surgery.

Few studies have documented the microbiological changes associated with the systemic administration of metronidazole. Lindhe et al. (1983), used darkfield microscopy to demonstrate that systemically administered metronidazole (200 mg, 4× a day for 14 days) in combination with mechanical therapy was more effective in reducing spirochetes than SRP alone. Loesche et al. (1984, 1991, 1992) showed that the clinical improvements observed during metronidazole therapy were associated with significantly lower proportions of *Porphyromonas gingivalis*, spirochetes, selenomonads, motile rods and *Prevotella intermedia* compared with SRP alone. Winkel et al. (1997) found that 1.5 g of metronidazole a day for 7 days in conjunction with SRP was effective in reducing the levels of *Bacteroides forsythus*, *P. gingivalis* and *P. intermedia* in a group of 27 refractory periodontitis patients.

In vitro, the penicillins, especially amoxicillin, appear very effective against most periodontal pathogens (Sutter et al. 1983, Walker et al. 1983, 1985). However, very few studies in the literature have evaluated the clinical and the microbiological outcomes of these agents (Hclovno & Paunio 1989, Hclovno et al. 1993, Abu-Fanas et al. 1991, Hull et al. 1989). Hull et al. (1989) treated 8 patients with rapidly progressive periodontitis with a daily dose of 750 mg of amoxicillin combined with 375 mg of clavulanic acid, a β-lactamase inhibitor. The treatment reduced pocket depth, bleeding on probing, the total viable counts of anaerobic bacteria and the number and % of black pigmented *Bacteroides* species and *Fusobacterium nucleatum*. A recent series of studies carried out by Van Winkelhoff, Pavicic and co-workers have shown that the combined use of amoxicillin and metronidazole can be useful in the treatment of advanced periodontitis, especially in patients harboring *Actinobacillus actinomycetemcomitans* (Pavicic et al. 1994, Van Winkelhoff et al. 1989, 1992). Other studies have shown that the combination of these 2 agents was also effective in controlling the levels of other pathogens such as *P. gingivalis*, *B. forsythus* and *P. intermedia* (Berglundh et al. 1998, Lopez & Gamonal 1998, Winkel et al. 1997, 1998).

The studies described above indicated that adjunctive metronidazole, amoxicillin, or the combination of both agents was of clinical benefit to the patient and that reductions in the proportions of specific species or morphotypes could be observed. However, the effect of each of these agents individually on the composition of the subgingival microbiota has not been comprehensively described. Therefore, the current investigation was designed to evaluate changes in the levels and proportions of 40 bacterial species in subgingival plaque samples at multiple time points during, immediately after and at periods up to 1 year after metronidazole or amoxicillin therapy combined with SRP. In addition, the clinical changes brought about both therapies were also evaluated.

Material and Methods

Subject Population

17 adult periodontitis subjects >20 years of age who had not received previous periodontal therapy were selected for study. Subjects had at least 8 pockets at posterior teeth with pocket depth and attachment level >5 mm. Subjects were excluded from the study if they were pregnant, lactating, required antibiotics for routine dental therapy or had any systemic condition which could affect the progress of periodontal disease. In addition, any subject with a known allergy to metronidazole or amoxicillin was excluded.

Experimental design and treatment

Subjects were clinically and microbiologically monitored at baseline, 90, 180 and 360 days (Fig. 1). Subgingival plaque samples were taken from the me-

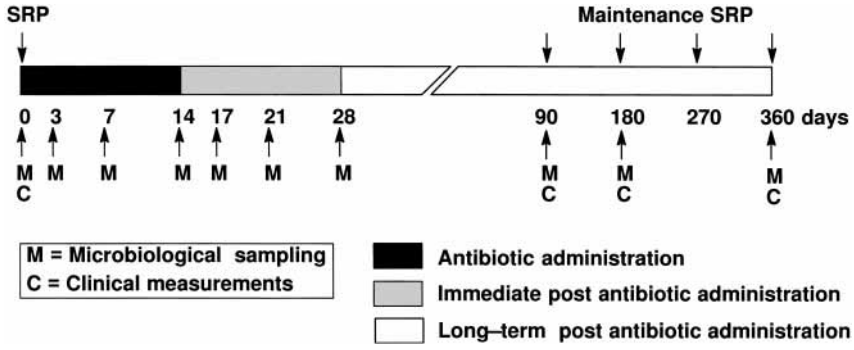

Fig. 1. Monitoring and treatment protocol. Subjects with adult periodontitis were monitored clinically at 6 sites per tooth and subgingival microbial samples were taken from the mesio-buccal aspect of all teeth excluding third molars at baseline. The subjects received full mouth SRP and systemically administered metronidazole or amoxicillin for 14 days. Subgingival plaque samples were taken from pairs of randomly selected teeth at 3, 7, 14 days during antibiotic administration and then at 3, 7, 14 days after withdrawal of the agent. Full mouth clinical monitoring and microbiological sampling were repeated at 90, 180 and 360 days. Subjects received full mouth maintenance SRP after each clinical monitoring visit and at 9 months.

sial aspect of all teeth, excluding third molars, at these time points. After the baseline monitoring visit all subjects received full mouth SRP. In addition, 8 subjects received systemically administered metronidazole (250 mg, 3× a day) and 9 subjects received systemically administered amoxicillin (500 mg, 3× a day) for 14 days. All subjects received full mouth maintenance SRP after the 90, 180 and 360 day monitoring visits. The antibiotic therapy was initiated at the first SRP visit.

6 posterior teeth with pocket depths >5 mm were chosen to be sampled while the patients were taking the antibiotics and immediately after withdrawal of the agents. Subgingival plaque samples were taken from a randomly selected pair of the 6 chosen teeth at 3, 7 and 14 days during antibiotic administration. The same pairs were sampled a second time at 3, 7, and 14 days after completion of antibiotic therapy. This sampling protocol facilitated the taking of representative samples for each time point by minimizing the number of times each tooth was sampled.

Clinical measurements

Subjects were screened and if accepted, were informed of the nature, potential risks and benefits of study participation. Upon signing an Institutional Review Board (IRB) approved informed consent, subjects were entered into the study. Subjects were clinically monitored prior to therapy and at 90, 180 and 360 days post therapy. Plaque accumulation (0/1), overt gingivitis (0/1), bleeding on probing (0/1), suppuration (0/1), probing pocket depth and probing attachment level were measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars. The attachment level measurements were repeated at all visits and the means of the pairs of measurements were used to determine change in attachment level (Haffajee et al. 1983). Such measurements were recorded to the nearest mm using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL). The baseline clinical parameters of the 17 subjects are presented in Table 1. There were no significant differences in the clinical parameters between the 2 groups at baseline. Measurements at all visits for a given subject were made by the same clinician. The clinician making the clinical measurements did not perform the therapy on that subject.

Microbiological assessment

Subgingival plaque samples were taken from the mesio-buccal aspect of all teeth excluding 3rd molars at baseline, 90, 180 and 360 days. In addition, pairs of selected posterior teeth were sampled subgingivally at 3, 7 and 14 days during and after antibiotic therapy as described above. Counts of 40 subgingival species were determined in each plaque sample using a modification (Haffajee et al. 1997) of the checkerboard DNA-DNA hybridization technique (Socransky et al. 1994). 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. The samples were lysed and the DNA placed in lanes on a nylon membrane using a Minislot device (Immunetics, Cambridge MA). 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-labeled 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 detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemifluorescence detection. The probes and their source strains were described in Ximenez-Fyvie et al. (2000). Signals were de-

Table 1. Mean (±SD) baseline clinical parameters of subjects in the amoxicillin and metronidazole groups

	Amoxicillin	Metronidazole
n	9	8
age (years)	46±15	42±10
no. missing teeth	2.4±2.6	3.3±2.4
% males	11	62
pocket depth (mm)	3.22±0.36	3.38±0.65
attachment level (mm)	3.21±0.90	3.23±0.80
% of sites with:		
plaque	64.8±15.2	72.3±22.8
gingival redness	48.8±20.7	62.0±31.6
bleeding on probing	34.8±15.9	44.7±20.5
suppuration	0.5±1.0	1.6±4.2

tected using AttoPhos substrate (Amersham Life Science, Arlington Heights, Illinois, USA) and a Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA, USA). 2 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. 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. A total of 1033 and 888 samples were examined in the amoxicillin and metronidazole groups respectively.

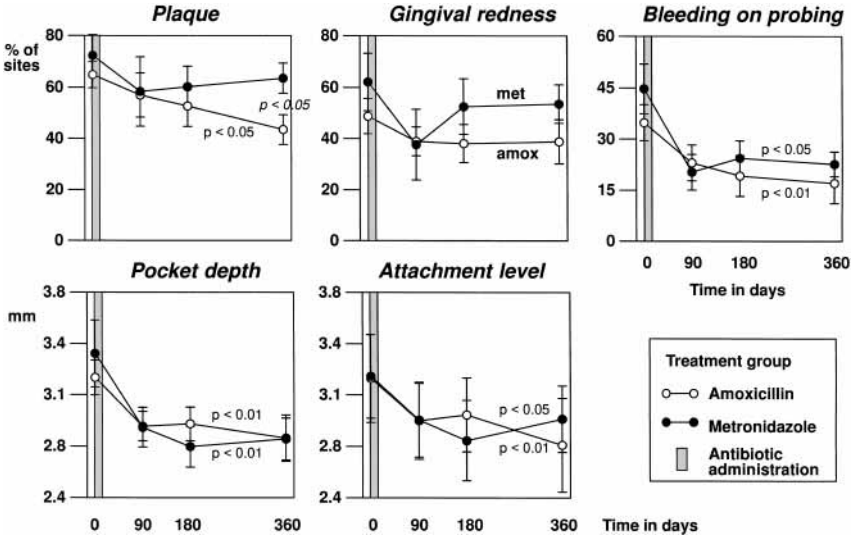

Fig. 2. Plots of the full mouth mean values (±SEM) for clinical parameters at baseline, 90, 180 and 360 days for subjects treated with SRP and metronidazole or SRP and amoxicillin. The circles represent the mean values and the whiskers represent the standard error of the mean. Values for each parameter 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. The shaded area represents the period of antibiotic administration. Significance of differences over time was tested using the Quade test. Significance of differences between groups at each time point was tested using ANCOVA.

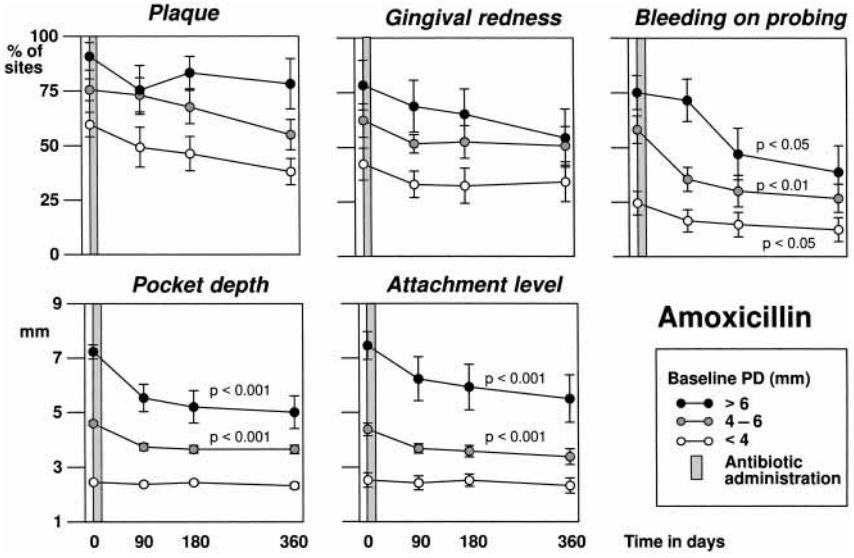

Fig. 3. Plots of the mean values (±SEM) for clinical parameters at baseline, 90, 180 and 360 days for sites with baseline pocket depths of <4, 4–6 and >6 mm in the amoxicillin group. The presentation of the clinical parameters and significance testing was as described in Fig. 2.

Data analysis

Changes in clinical and microbiological parameters over time

Clinical parameters including % of sites with plaque, gingival redness, bleeding on probing and suppuration as well as mean pocket depth and attachment level were computed for each subject and then averaged across subjects in the 2 groups at each time point. The significance of differences over time (baseline, 90, 180 and 360 days) in each group was sought using the Quade test (Conover, 1980). The total DNA probe count was computed at each sampled site in each subject and the proportion that each species comprised of that count determined. Proportions of each species were averaged across sites in each subject and then across subjects in each group at each time point. Up to 28 samples were averaged per subject at baseline, 90, 180 and 360 days and 2 samples at the short-term time points (days 3, 7 and 14 during and after antibiotic administration). For other analyses, the proportions of species within the different microbial complexes, described by Socransky et al. (1998), were summed and averaged for a subject and averaged across subjects in the 2 groups separately. In addition, mean levels of each bacterial species were computed at the sampled teeth for each subject and then averaged across subjects in the 2 groups. Significance of differences in mean levels of subgingival species and mean % of total DNA probe count over time for each species was sought using the Quade test in each treatment group separately.

In a similar fashion, the changes in clinical and microbiological parameters over time were examined in sites subset according to baseline pocket depths of <4, 4–6 and >6 mm. Values for each clinical parameter were averaged separately within the 3 pocket depth categories in each subject and then averaged across subjects in the 2 treatment groups. Significance of differences over time was sought using the Quade test. Proportions of each microbial complex were averaged within the 3 pocket depth categories in each subject and then averaged across subjects. Significance of differences between proportions was sought over time using the Quade test.

Differences in clinical and microbiological parameters between treatment groups
Differences at baseline and at 3, 6 and 12 months for all clinical parameters between the treatment groups were sought using ANCOVA with the baseline values for the subject as the co-variate. Differences in mean counts for 40 individual species from baseline values to each time point between the treatment groups were also determined using ANCOVA with baseline values for each species as the co-variate.

Results

Clinical findings

Fig. 2 presents the full mouth mean values for the clinical parameters at baseline, 90, 180 and 360 days for the 8 subjects in the metronidazole group and the 9 subjects in the amoxicillin group. There was a statistically significant decrease in the % of sites exhibiting bleeding on probing for both treatment groups. The amoxicillin-treated subjects also showed a significant reduction in the % of sites exhibiting plaque accumulation over the course of the study. Mean pocket depth values (±SEM) at baseline, 90, 180 and 360 days for the amoxicillin group were 3.22±0.12, 2.89±0.10, 2.90±0.12, 2.81±0.16 ($p<0.01$) and for the metronidazole group were 3.38±0.23, 2.88±0.13, 2.75±0.14, 2.80±0.14 ($p<0.01$). Corresponding mean attachment level values for amoxicillin were: 3.21±0.30, 2.92±0.26, 2.96±0.25, 2.76±0.32 ($p<0.05$) and for metronidazole 3.23±0.28, 2.93±0.25, 2.79±0.27, 2.94±0.23 ($p<0.01$). The only significant difference found between the 2 groups at the different time points was that amoxicillin-treated subjects had a lower % of sites exhibiting plaque accumulation at 360 days.

The sites were subset into initial pocket depth categories of <4, 4–6 and >6 mm and the analyses repeated (Figs. 3, 4). In both treatment groups there was a significant decrease in the % of sites that bled on probing for all initial pocket depth categories. Mean reductions from baseline to 360 days were 12±3, 31±8 and 36±19 for amoxicillin treated subjects at sites with initial pocket depths of <4, 4–6 and >6 mm. Corresponding values for the metronidazole treated subjects were 19±5, 30±5 and 26±13 respectively. A reduction in the % of sites exhibiting gingival redness and plaque accumulation was observed at 90 days for all pocket depth categories in both treatment groups; however, these changes were not statistically significant. The % of sites exhibiting gingival redness increased after 90 days in the subjects treated with metronidazole but remained at lowered levels in the amoxicillin group. Mean pocket depth and mean attachment level were significantly reduced in the initially deep (>6 mm) and intermediate sites (4–6 mm) over the course of the study in both treatment groups. Sites with baseline pocket depths <4 mm showed no significant change in mean attachment level or mean pocket

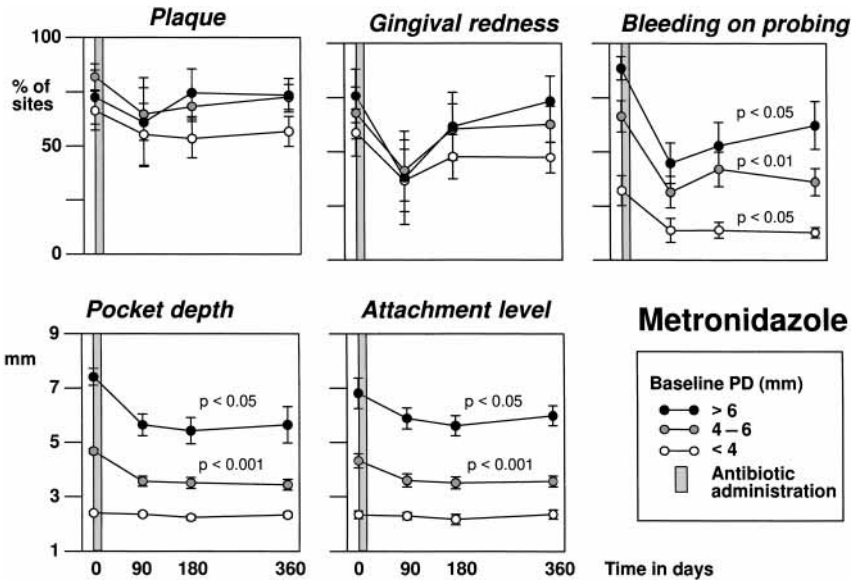

Fig. 4. Plots of the mean values (±SEM) for clinical parameters at baseline, 90, 180 and 360 days for sites with baseline pocket depths of <4, 4–6 and >6 mm in the metronidazole group. The presentation of the clinical parameters and significance testing was as described in Fig. 2.

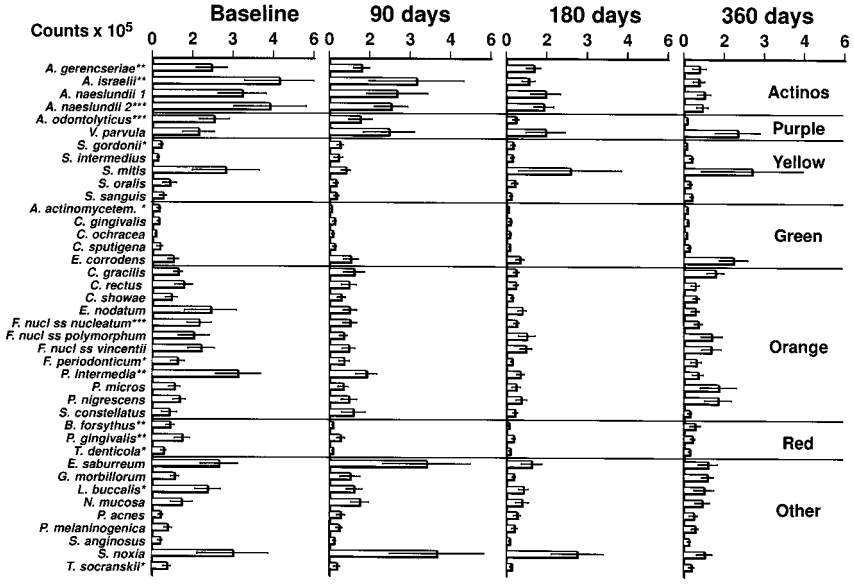

Fig. 5. Bar charts of the mean counts ($\times 10^5$, ±SEM) of 40 subgingival species at baseline, 90, 180 and 360 days for the amoxicillin treated subjects. The species are grouped according to the microbial complexes described by Socransky et al. (1998). Mean levels of each species were computed for each subject and then averaged across subjects at each time point. Significance of differences over time was tested using the Quade test (*$p<0.05$, **$p<0.01$, ***$p<0.001$). 13 species were significantly reduced.

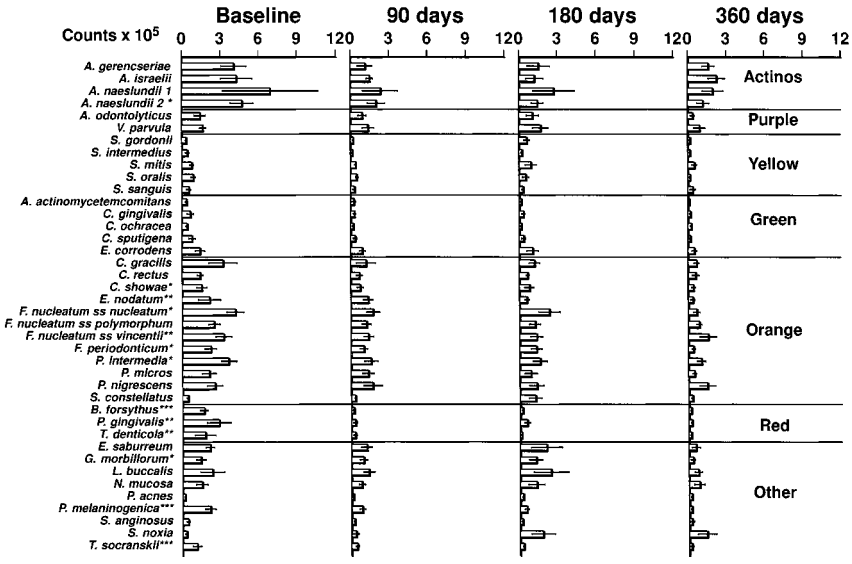

Fig. 6. Bar charts of the mean counts ($\times 10^5$, $\pm$SEM) of 40 subgingival species at baseline, 90, 180 and 360 days for the metronidazole treated subjects. The presentation of the data and significance testing was as described in Fig. 5. 13 species were significantly reduced.

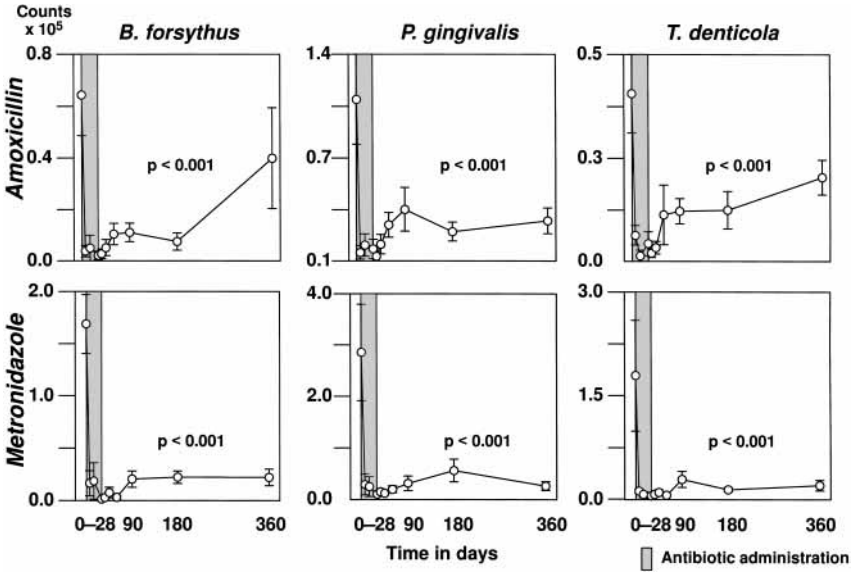

Fig. 7. Plots of the mean counts ($\times 10^5$, $\pm$SEM) of "red complex" species (*B. forsythus*, *P. gingivalis*, and *T. denticola*) at all time points in both treatment groups. The circles represent the mean and the whiskers the standard error of the mean. The shaded area represents the period of antibiotic administration. The computation of mean counts and significance testing was as described in Fig. 5.

depth over the course of the study. The only significant difference between treatment groups for any clinical parameter on the subset data was for BOP at sites with initial pocket depth >6 mm at 360 days. Amoxicillin treated subjects had significantly lower mean values than metronidazole treated subjects ($p<0.05$, ANCOVA).

Microbiological findings

Figs. 5, 6 present mean counts of the 40 subgingival species at baseline, 90, 180 and 360 days for the amoxicillin and metronidazole treated subjects respectively. Levels of the majority of species were reduced post therapy in both groups and the levels of 13 species in each group, including several periodontal pathogens, were significantly reduced. Mean total DNA probe counts ($\times 10^5$, $\pm$SEM) at baseline, 90, 180 and 360 days for amoxicillin were 54.1±9.8, 35.3±12.0, 21.6±5.6, 24.1±6.0 (NS) and for metronidazole were: 75.3±10.6, 33.9±7.5, 38.0±11.6, 24.0±6.1 ($p<0.01$). The only significant differences at 360 days between treatment groups was lower mean counts of *A. gerencseriae* and *A. israelii* in the amoxicillin treated subjects ($p<0.05$, ANCOVA). Metronidazole combined with SRP strikingly reduced mean counts of pathogens in the "red complex", *B. forsythus*, *P. gingivalis*, and *Treponema denticola* which were detected at reduced mean counts up to 360 days post therapy (Fig. 7). Amoxicillin and SRP also reduced the levels of these three species, however, at 360 days a re-growth was observed, especially for *B. forsythus* and *T. denticola* (Fig. 7). Fig. 8 presents the mean counts for the 3 "red complex" species at baseline and 360 days for individual subjects in the two treatment groups. All subjects treated with metronidazole showed a decrease in mean counts of "red complex" species at 360 days. However, 2, 1 and 4 subjects respectively, showed an increase in counts of *B. forsythus*, *P. gingivalis* and *T. denticola* at 360 days in the amoxicillin group. The mean increase in counts of *B. forsythus* at 360 days in the amoxicillin treated group (Fig. 7) was due to a marked increase in counts of this species in 2 of the 9 subjects (Fig. 8).

Mean counts of many suspected periodontal pathogens of the "orange complex" such as *Campylobacter rectus*, *Fusobacterium nucleatum* ss *vincentii* and *P. intermedia* were reduced after therapy (Fig. 9). These species were detected at very low levels 3 days after the start of antibiotic therapy but showed a regrowth after the withdrawal of the agents. After 360 days many of the species were still present in lowered levels compared with their baseline counts in both treatment groups. The individual subject data for these 3 species at baseline and 360 days are presented in Fig. 10. Similar to the "red complex" species, the majority of subjects showed a decrease in these species at 360 days in both groups with the exception of *F. nucleatum* ss *vincentii* in the amoxicillin treated subjects.

Figs. 11, 12 show changes in proportions of the microbial complexes in the amoxicillin and metronidazole

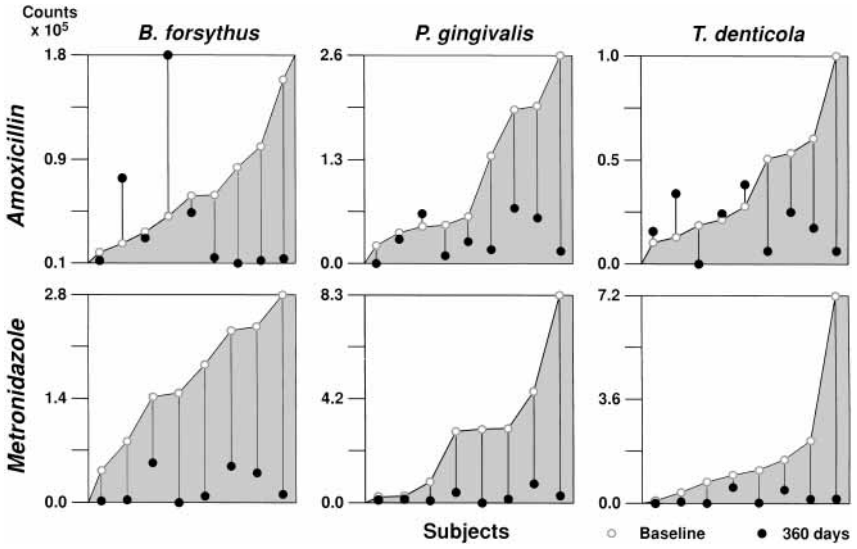

Fig. 8. Plots of the mean counts ($\times 10^5$) of "red complex" species (*B. forsythus*, *P. gingivalis*, and *T. denticola*) at baseline and 360 days in each subject in both groups. The open circles represent the mean values at baseline and the black circles represent the mean values for the same subject at 360 days. The black 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.

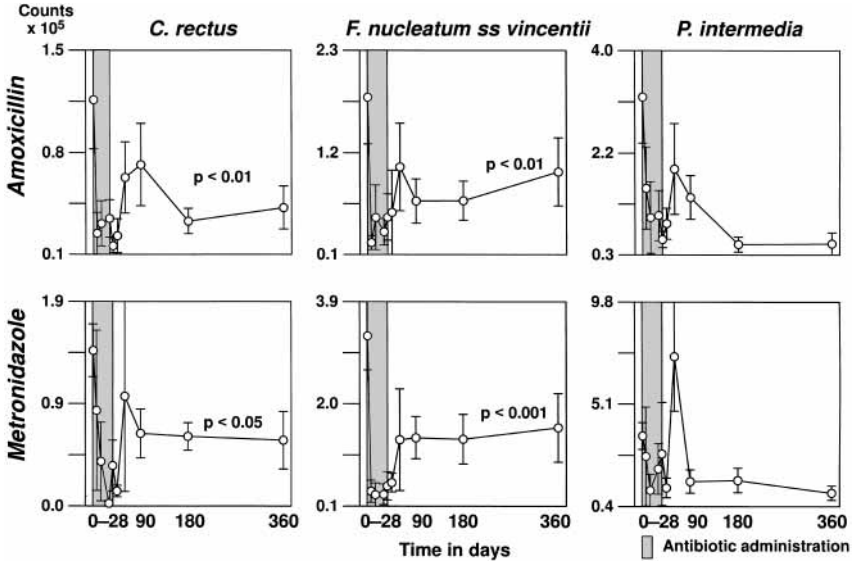

Fig. 9. Plots of the mean counts ($\times 10^5$, $\pm$SEM) of 3 "orange complex" species (*C. rectus*, *F. nucleatum* ss *vincentii* and *P. intermedia*) at all time points in both treatment groups. The circles represent the mean and the whiskers the standard error of the mean. The shaded area represents the period of antibiotic administration. The computation of mean counts and significance testing was as described in Fig. 5.

treated groups respectively. The areas of the pies reflect the relative total bacterial counts at each time point. Striking changes took place in the proportions of "red" and "orange complex" species as well as *Actinomyces* species during the course of treatment. The "red complex" species, *B. forsythus*, *P. gingivalis* and *T. denticola* constituted a mean of 8.2% of the subgingival microbiota of the 8 subjects in the metronidazole group at baseline and 0.7% after 2 weeks of metronidazole therapy. Although a gradual regrowth of these pathogens was observed over time, at 360 days post therapy the species remained at lower proportions (3.7%) when compared to baseline (Fig. 12). Amoxicillin-treated subjects also showed a reduction in the proportions of the "red complex" species during antibiotic administration; however, they showed an increase in proportion after the antibiotic was withdrawn (Fig. 11). Species of the "orange complex" decreased in proportion soon after the subjects started taking the antibiotics. In the amoxicillin group the proportions of this microbial complex increased immediately after withdrawal of the drug. At 1 week after the antibiotic had been discontinued the proportions were similar to baseline. In the metronidazole group proportions of "orange complex" species continued to decrease up to 28 days but at 90 days the proportions of species in this complex were comparable to baseline values.

The *Streptococcus* ("yellow complex") and *Actinomyces species* showed a different pattern of change in the two treatment groups. In the metronidazole group, the proportions of *Actinomyces* and *Streptococcus* species increased while the patients were taking the antibiotic and remained elevated up to day 28, returning to baseline proportions at 90 days (Fig. 12). Subjects receiving amoxicillin showed a striking decrease in the proportions of *Actinomyces* accompanied by a marked increase in the proportions of *Streptococcus* species during the 2 weeks of antibiotic administration. At 360 days the reduction in the proportion of *Actinomyces* was even more evident. The proportions of *Actinomyces* were significantly lower in the amoxicillin group than the metronidazole group at 360 days ($p<0.01$, ANCOVA). In contrast, the proportion of "green complex" species was higher in the amoxicillin group ($p<0.01$, ANCOVA). The proportions of *Streptococcus* species returned to baseline values at 90 days (Fig. 11).

Of interest was the change in the total counts of the organisms during and after treatment (Figs. 11, 12). The total bacterial counts were reduced during the 14 days of metronidazole or amoxicillin administration and gradually increased after withdrawal of the agents. However, at 90 days post therapy, total counts were reduced below baseline levels and the lowered levels were maintained at 180 and 360 days post therapy in both groups.

Changes in proportion of microbial complexes from baseline to 360 days at sites with baseline pockets of <4, 4–6

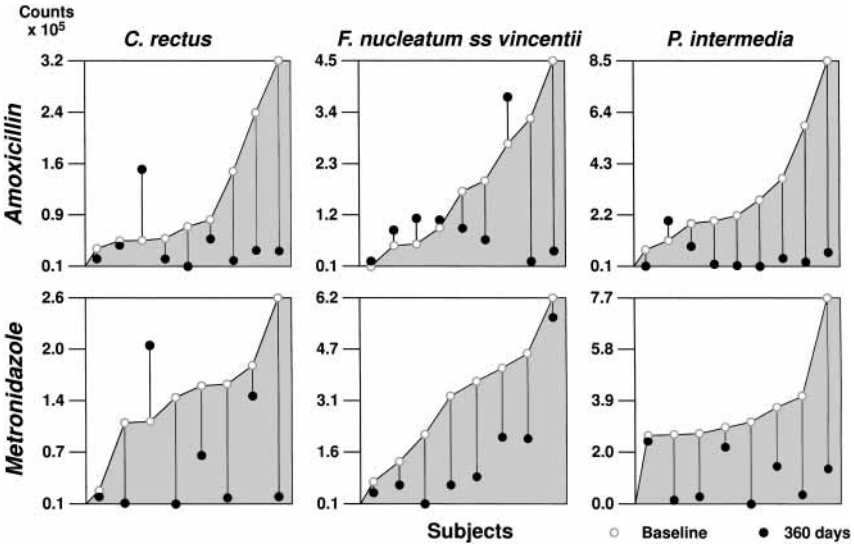

Fig. 10. Plots of the mean counts ($\times 10^5$) of 3 "orange complex" species (*C. rectus*, *F. nucleatum* ss *vincentii* and *P. intermedia*) at baseline and 360 days in each subject in both groups. The presentation of the data was as described in Fig. 8.

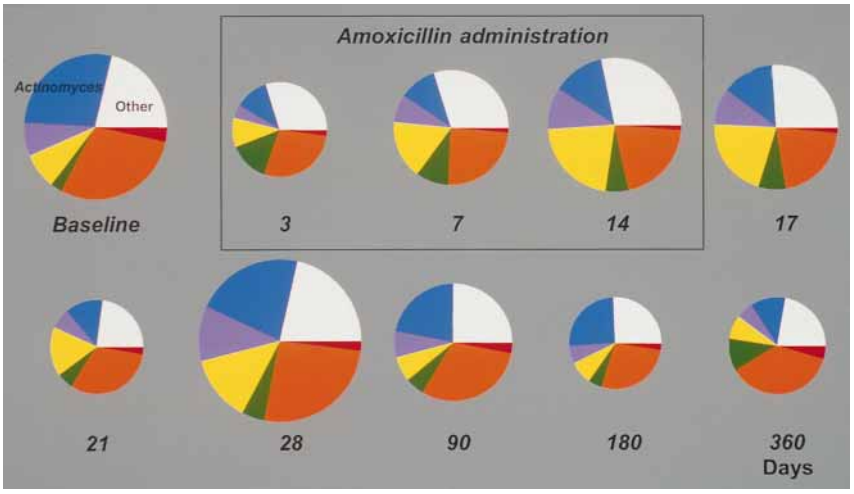

Fig. 11. Pie charts describing the mean proportion of microbial complexes at different time points for the 9 subjects in the amoxicillin group. The % DNA probe counts for each species were determined at each site, averaged within a subject and then averaged across subjects at each time point. Species in the complexes were summed and the proportions that each complex comprised was determined. The area of each pie was adjusted to reflect its size relative to the baseline total count. The boxed area represents the period of antibiotic therapy. The "other" group was composed of species that did not fit any complex or new probes whose relationship to existing complexes have not yet been determined.

and >6 mm are presented in Fig. 13. For all pocket depth categories, there was a significant decrease in total counts at 360 days in both treatment groups. The overall reduction in the proportion of *Actinomyces* species observed in the amoxicillin group (Fig. 11) was due primarily to a significant decrease in these species at shallow and intermediate pocket depth sites. Indeed, at 360 days, these species were in significantly lower proportion in the initially shallow ($p<0.05$) and intermediate pockets ($p<0.01$) when compared with similar pockets in the metronidazole treated subjects. The proportion of "green complex" species increased significantly at the same sites in the amoxicillin treated subjects and were significantly higher at 360 days than in similar sites in the metronidazole treated subjects. In the metronidazole treated subjects, the most striking and significant reduction was seen in the proportion of "red complex" species at initially deep and intermediate periodontal pockets.

Fig. 14 summarizes the mean % reduction or increase in the mean counts of each species in each treatment group from baseline to 360 days. Greater than 50% reductions were observed for 17 species in the amoxicillin group and 29 species in the metronidazole group, while 2 and 1 species increased by >50% in these groups respectively. All 3 members of the "red complex" were reduced by >50% in the metronidazole treated subjects after 360 days. Noteworthy was the striking reduction in counts of *Actinomyces* species in the amoxicillin treated subjects.

Discussion

The rationale for the use of a systemic antibiotic in the treatment of periodontal infections is to rapidly suppress target microbial species and foster the establishment of a host compatible microbiota. This goal is sometimes difficult to achieve because of the spectrum of activity of different antibiotic agents, the difficulty in achieving high levels of the antibiotic at the site of infection and the protective effect of the so-called biofilm community, dental plaque. Periodontal investigators and therapists have empirically employed a series of antibiotics in attempts to control periodontal infections. These agents have largely, but not always, led to clinical and microbial beneficial changes (Van Winkelhoff et al. 1996, Feres et al. 1999). In examining the literature amoxicillin and metronidazole were of particular interest, in part, because of reported beneficial changes and in part because of their very different mechanisms of action and spectrum of activity. The present investigation examined the clinical and microbiological effects of these 2 agents in 17 patients with adult periodontitis during and after systemic administration. An important aspect of the study design was the evaluation of the effects of the systemic antibiotic while the patient was taking the agent and immediately after its withdrawal. This strategy facilitated an understanding of the agents' peak efficacy during administration as well as the kinetics of microbial recolonization after cessation of therapy. Such data might suggest more appro-

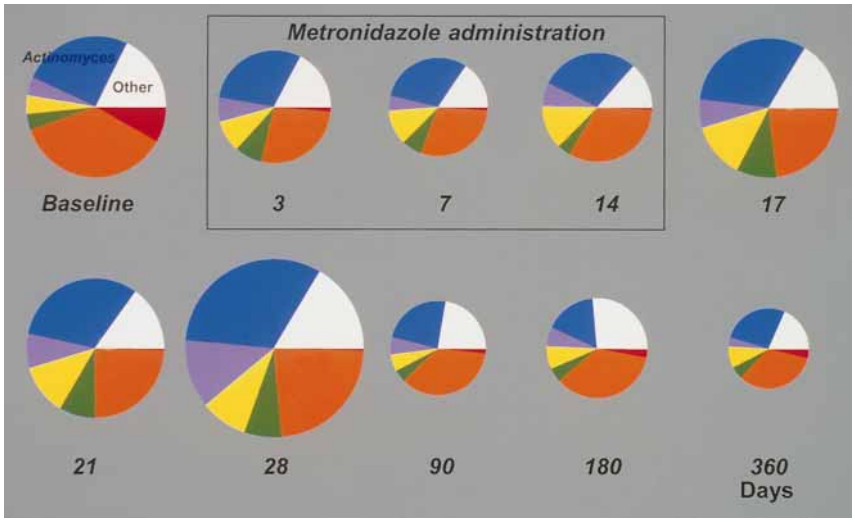

Fig. 12. Pie charts describing the mean proportion of microbial complexes at different time points for the 8 subjects in the metronidazole group. The computation of proportions and the sizing of the pies was as described for Fig. 11.

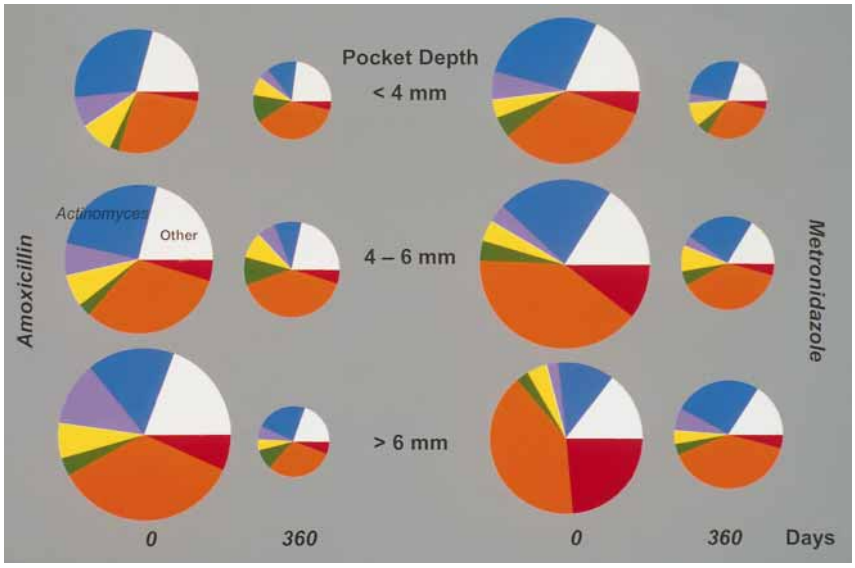

Fig. 13. Pie charts describing the mean proportion of microbial complexes at baseline and 360 days at sampled sites subset according to baseline pocket depth categories of <4, 4–6 and >6 mm in the 2 treatment groups. The computation of proportions and the sizing of the pies was as described for Fig. 11.

priate antibiotic usage in the treatment of periodontal infections.

The major effect of the two antibiotic treatments was a reduction in mean counts of the pathogens of the "red complex", *B. forsythus*, *P. gingivalis* and *T. denticola*. However, there was a tendency for counts of *B. forsythus* and *T. denticola* to increase in some but not all amoxicillin treated subjects by 360 days. Indeed, the mean % reduction in counts at 360 days for the "red complex" species ranged from 37% for *B. forsythus* to 71% for *P. gingivalis* in the amoxicillin group, but from 87% for *B. forsythus* to 91% for *P. gingivalis* in the metronidazole group (Fig. 14). The proportion of "red complex" species was markedly decreased in both treatment groups during the 2 weeks of antibiotic administration. Once the antibiotic was withdrawn, "red complex" species started to rebound in the amoxicillin group (Fig. 11). In the metronidazole-treated subjects, the combined proportions of these pathogens remained at low levels immediately after cessation of therapy and at 90 to 360 days these species were still in much lower proportions compared with baseline values (Fig. 12).

Several putative periodontal pathogens from the "orange complex" were also reduced by one or both treatments. When all ten visits were evaluated, significant changes were observed for *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter showae*, *Eubacterium nodatum*, all subspecies of *Fusobacterium nucleatum*, *Fusobacterium periodonticum* and *Prevotella nigrescens*. The counts of these species were markedly decreased during antibiotic administration but tended to increase once the agents had been withdrawn. However, at 360 days counts of all and 7 of 12 "orange complex" species were below baseline values in the metronidazole and amoxicillin groups respectively (Fig. 14). When the proportions of the "orange complex" species were considered together, the results showed a decrease in this microbial complex in both the metronidazole and amoxicillin-treated groups. However, at 90 days the proportions of the "orange complex" species were comparable to baseline values in both antibiotic treatment groups (Figs. 11, 12).

In general, the counts and proportions of members of the genera *Actinomyces* and *Streptococcus*, and most of the members of the "purple" and "green complexes" such as *Capnocytophaga* species and *Veillonella parvula* tended to increase during and immediately after administration of metronidazole, but decreased in mean counts from 90 to 360 days. This increase in the proportions of "host compatible" species may have been due to the intrinsic resistance of many of these species to this agent. In contrast, amoxicillin caused a marked short-term reduction in the proportions of *Actinomyces* species accompanied by a dramatic increase in the *Streptococcus* species. Of concern was the fact that even at 360 days post treatment the proportions of *Actinomyces* in this group of subjects were still at much lower levels than baseline (Fig. 11).

The microbial findings in the present investigation are in accord with and extend the data presented by Winkel et al. (1997). In their study, 27 "refractory" periodontitis patients were monitored for a period of 6 months. After a combined treatment of metronidazole at

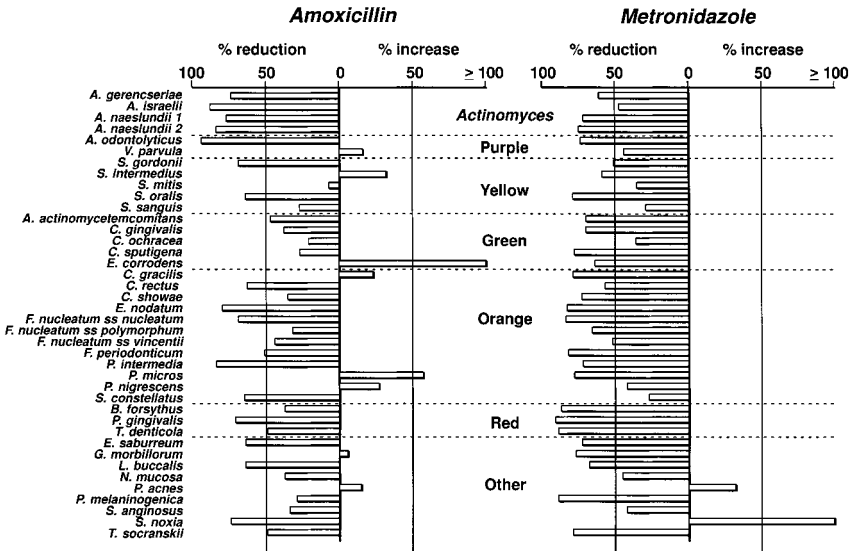

Fig. 14. Bar charts of the mean % change in counts of bacterial species from baseline to 360 days. The mean count was computed for each species at baseline and 360 days in all subjects in the 2 groups separately and the proportional increase or decrease calculated. Note that the % increase indicated on the right side of each panel has been truncated to ≥100%.

500 mg 3 times a day for 7 days and supra- and subgingival debridement, *B. forsythus* was suppressed below detection levels in 17 of the 27 patients and *P. gingivalis* in 9 of 15 patients. Other studies also suggested a role of metronidazole in controlling periodontal pathogens such as spirochetes, *P. intermedia*, *B. forsythus* and *P. gingivalis* (Loesche et al. 1991, 1992, Paquette et al. 1992). No comprehensive studies have evaluated the effect of amoxicillin alone on the composition of the subgingival microbiota; however, using a daily dose of 750 mg of amoxicillin combined with 375 mg of clavulanic acid, Hull et al. (1989) showed a reduction in the total viable counts of anaerobic bacteria and in the number and percentage of black pigmented *Bacteroides* species and *F. nucleatum* in 8 patients with rapidly progressive periodontitis. Many recent studies have attempted to determine the effect of the combination of amoxicillin with metronidazole on the composition of the subgingival microbiota of patients with adult periodontitis. This therapy was initially used to decrease the levels of *A. actinomycetemcomitans* (Van Winkelhoff et al. 1989), but subsequent studies have shown a beneficial effect of the combined drug regimen in decreasing the levels and proportions of other pathogens such as *P. gingivalis*, *B. forsythus* and *P. intermedia* (Berglundh et al. 1998, Lopez & Gamonal, 1998, Pavicic et al. 1994, Van Winkelhoff et al. 1992, Winkel et al. 1998).

The microbiological changes brought about by the systemic administration of metronidazole or amoxicillin in conjunction with SRP led to improvements in clinical parameters. Full mouth mean pocket depth was significantly reduced and there was a significant gain in full mouth mean attachment level in both treatment groups. The subjects also showed a significant decrease in the % of sites exhibiting bleeding on probing.

In agreement with previous studies (Pihlström et al. 1983, Ramfjord et al. 1987, Haffajee et al. 1995, 1997), the data in the present investigation showed that the therapies had little effect on attachment level at shallow pockets (<4 mm) while the deep pockets (>6 mm) showed the most attachment gain in both groups. An interesting and clinically important finding in this study was the effect of both therapies on the intermediate pockets. Sites with initial probing pocket depth between 4 and 6 mm showed a highly significant gain in mean probing attachment level (4.33±0.26 to 3.57±0.20 in the metronidazole group and 4.60±0.24 to 3.66±0.47 in the amoxicillin group). Even shallow sites that usually tend to lose attachment after SRP (Hill et al. 1981, Pihlstrom et al. 1981, Lindhe et al. 1982a, b, c, Badersten et al. 1987) showed minimal change in attachment level post therapy in both treatment groups. Sites in all pocket depth categories showed an improvement in the parameters used to measure inflammation, bleeding on probing and gingival redness. The % of sites exhibiting either of these parameters was reduced at 3 months post therapy and the reduction in bleeding on probing was statistically significant. The majority of clinical changes were maintained up to 1 year.

The results of the present investigation are in accord with many studies that showed a clinical benefit when metronidazole was used in conjunction with SRP (Lindhe et al. 1983, Joyston-Bechal et al. 1984, Loesche et al. 1984, 1987, 1991, 1992, 1996, Van Oosten et al. 1987, Asikainen 1989, Jenkins et al. 1989, Soder et al. 1990, Eisenberg et al. 1991, Paquette et al. 1992, Noyan et al. 1997). In contrast to our data, some studies have suggested that the efficacy of metronidazole may be limited to deep pockets (Clark et al. 1983, Lekovic et al. 1983, Joyston-Bechal et al. 1984, 1986, Loesche et al. 1984, Sterry et al. 1985). However, Asikainen (1989) showed that a single course of SRP with systemically administered metronidazole (200 mg, 3×/day for 14 days) in 30 patients with advanced periodontitis was equally effective in reducing pocket depth in both intermediate (4–6 mm) and deep (>6 mm) periodontal pockets. No studies in the literature have evaluated the clinical effects of amoxicillin with SRP in adult periodontitis patients. Nevertheless, clinical improvements in terms of reducing pocket depth and bleeding on probing have been reported with the use of amoxicillin and clavulanic acid in combination with SRP (Abu-Fanas et al. 1991, Hull et al. 1989). Further, the combination of metronidazole and amoxicillin has also been shown to improve clinical parameters in adult periodontitis subjects (Berglundh et al. 1998, Lopez & Gamonal, 1998, Pavicic et al. 1994, Van Winkelhoff et al.1989, 1992, Winkel et al. 1998).

The recent recognition that bacteria residing in biofilms are far more resistant to antibiotics than the same species in a planktonic state gave rise to the concern that systemically administered antibiotics may have little or no effect on the subgingival microbiota. This notion seems unlikely given the reported usefulness of these agents as adjuncts to periodontal treatment described above. Thus, the goal of this investigation was

to determine whether the subgingival microbiota was changed at the presumed peak of antibiotic activity (while the subject was taking the agent), immediately after its withdrawal and for a period of one year after treatment.

The results of the study were encouraging in that the "red complex" species and most members of the "orange complex" were markedly decreased in counts and proportions during the administration of the antibiotics. Thus, despite the concerns about resistance of organisms in biofilms, the antibiotics did have a profound effect on specific subsets of organisms including many thought to be periodontal pathogens. The changes between 28 and 90 days are unclear. The counts of the organisms actually diminished (Figs. 5, 6), while the proportions, aside from "red complex" species, tended to revert to those observed in the baseline climax community. One might speculate that the rapid reduction in pathogenic species may have led to tissue changes that in turn affected the numbers of organisms that could colonize and to some extent the proportional distribution of different species. This appears to be a clear case of bacterial-habitat interaction. SRP plus an antimicrobial agent reduced specific subsets of the subgingival microbiota which affected local inflammatory status. This reduction in inflammation in turn affected the numbers and composition of the adjacent microbiota. It might be surmised that additional therapy, perhaps some form of mechanical debridement, imposed when pathogens were at their lowest level might have helped to prolong this beneficial change. The data suggest that the antibiotics "did their job", that is they rapidly lowered the number of putative periodontal pathogens. They did not eliminate the organisms, nor was the effect permanent. This suggests that these tools are useful in rapidly lowering target species, but like any tool, they cannot be expected to perform beyond their capabilities. Thus, this study suggests that antibiotics are useful in achieving a limited goal, but must be accompanied by other therapeutic and maintenance procedures to bring about the desired end point of long-term periodontal stability.

Acknowledgments

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Zusammenfassung

Veränderung der subgingivalen mikrobiellen Profile bei der Erwachsenen-Parodontitis, die entweder systemisch Amoxicillin oder Metronidazol erhielten

Ziel: Die gegenwärtige Untersuchung evaluiert die Veränderungen in den Niveaus und Proportionen von 40 bakteriellen Spezies in subgingivalen Plaqueproben während, sofort nach und bis zu 1 Jahr nach Metronidazol- oder Amoxicillintherapie in Kombination mit SRP.

Methoden: Nach der klinischen und mikrobiologischen Basisuntersuchung erhielten 17 erwachsene Personen mit Parodontitis eine vollständige SRP und 14 Tage eine systemische Gabe von entweder Metronidazol (250 mg, TID, $n=8$) oder Amoxicillin (500 mg, TID, $n=9$). Die klinischen Messungen schlossen die Prozentwerte der Flächen mit Plaque, der gingivalen Rötung, der Provokationsblutung und Suppuration, der Sondierungstiefe (PD) und des Stützgewebeniveaus (AL) ein. Die Messungen wurden zur Basis, am 90., am 180. und 360. Tag gemacht. Die subgingivalen Plaqueproben wurden von der mesialen Oberfläche aller Zähne zur Basis, zum 90., zum 180. und 360. Tag von jedem Probanden genommen sowie von 2 zufällig ausgesuchten posterioren Zähnen am Tag 3, 7 und 14 während und nach der Antibiotikaverordnung. Die Mengen von 40 subgingivalen Spezies wurden unter Nutzung einer checkerboard DNA-DNA Hybridisation bestimmt. Die Signifikanzen der Differenzen über die Zeit wurden mit dem Quade-Test und zwischen den Gruppen mit der ANCOVA überprüft.

Ergebnisse: Die mittleren PD reduzierten sich von 3.22 ± 0.12 mm zur Basis zu 2.81 ± 0.16 mm ($p<0.01$) zum 360. Tag und von 3.38 ± 0.23 mm zu 2.80 ± 0.14 mm ($p<0.01$) bei den mit Amoxicillin bzw. mit Metronidazol behandelten Patienten. Korrespondierende Werte für die mittleren AL waren 3.21 ± 0.30 zu 2.76 ± 0.32 ($p<0.05$) und 3.23 ± 0.28 mm zu 2.94 ± 0.23 mm ($p<0.01$). Die Niveaus und die Verteilung von *Bacteroides forsythus*, *Porphyromonas gingivalis* und *Treponema denticola* wurden während der Antibiotikabehandlung deutlich reduziert und waren am 360. Tag niedriger als zur Basis. Die Mengen ($\times10^5$, $\pm$SEM) von *B. forsythus* fielen von der Basis von 0.66 ± 0.16 auf 0.04 ± 0.02, 0.13 ± 0.04, 0.10 ± 0.03 und 0.42 ± 0.19 in der Amoxicillin Gruppe an den Tagen 14, 90, 180 und 360 ($p<0.001$). Korrespondierende Werte für die mit Metronidazol behandelten Personen waren: 1.69 ± 0.28 zu 0.02 ± 0.01, 0.20 ± 0.08, 0.22 ± 0.06 und 0.22 ± 0.08 ($p<0.001$). Die Mengen von *Campylobacter* sp., *Eubacterium nodatum*, *Fusobacterium nucleatum* subspecies, *F. peridonticum* und *Prevotella nigrescens* waren in den mittleren Niveaus während und sofort nach der Therapie auch niedriger, aber graduell erhöht nach Absetzen der Antibiotika. Mitglieder der Klassen *Actinomyces*, *Streptococcus* und *Capnocytophaga* wurden durch Metronidazol minimal beeinflußt. Jedoch verringerte Amoxicillin die Mengen und Verhältnisse von *Actinomyces* sp. während und nach der Therapie.

Zusammenfassung: Die Daten suggerieren, daß Metronidazol und Amoxicillin in der schnellen Verringerung der Mengen von putativen parodontalen Pathogenen nützlich sind, daß dies aber durch andere Prozeduren begleitet wurden muß, um parodontale Stabilität zu erbringen.

Résumé

Modifications des profils microbiologiques sous gingivaux chez des sujets atteints de parodontite de l'aulte ayant reçu soit de l'amoxicilline ou du métronidazole par voie systèmique

But: La présente recherche a évalué les modifications de niveaux et de proportions de 40 espèces bactériennes dans des prélèvements de plaque sous gingivale pendant, immédiatement après, et jusqu'à un an après un traitement par métronidazole ou amoxicilline associè avec le détartrage/surfaçage radiculaire.

Méthode: Après avoir relevé les paramètres cliniques et microbiologiques initiaux, 17 sujets atteints de parodontite de l'adulte ont subi un détartrage/surfaçage radiculaire de toute la bouche et l'administration systémique pendant 14 jours de métronidazole (250 mg, 3× fois par jour, $n=8$) ou d'amoxicilline (500 mg, 3× par jour, $n=9$). Les mesures cliniques relevées initialement, à 90 jours, à 180 jours, et à 360 jours, étaient: le % de sites avec de la plaque, la rougeur gingivale, le saignement au sondage et la suppuration, la profondeur de poche (PD) et le niveau d'attache (AL). Des échantillons de plaque sous gingivale étaient prélevés sur la surface mésiale de toutes les dents, chez chaque sujet, initialement, à 90 jours, à 180 jours, et á 360 jours, et sur 2 dents postérieures choisies au hasard à 3, 7, et 14 jours pendant et après l'administration d'antibiotique. Le comptage de 40 expèces sous gingivales fut déterminé par la technique de l'hybridisation en damier DNA-DNA. La signification des différences au cours du temps fut déterminée par le test de Quade et entre les groupes par ANCOVA.

Résultats: La profondeur moyenne des poches a étê réduite de 3.22 ± 0.12 mm initialement à 2.81 ± 0.16 mm ($p<0.01$) à 360 jours et de 3.38 ± 0.28 mm à 2.80 ± 0.14 mm ($p<0.01$) dans les groupes amoxicilline et metronidazole, respectivement. Les valeurs correspondantes pour AL étaient 3.21 ± 0.30 à 2.76 ± 0.32 ($p<0.05$) et 3.23 ± 0.28 à 2.94 ± 0.23 ($p<0.01$). Les niveau de *B. forsythus*, *P. gingivalis* et *T. denticola*, étaient fortement réduits pendant l'administration d'antibiotique et restaient plus bas à 360 jours qu'initialement. Les comptages ($\times10^5$, $\pm$SEM) de *B. forsythus* tombaient de niveaux initiaux de 0.66 ± 0.16 à 0.04 ± 0.02, 0.13 ± 0.04, 0.10 ± 0.03 et 0.42 ± 0.19 dans le

groupe amoxicilline à 14 jours, 90 jours, 180 jours, et 360 jours, respectivement ($p<0.001$). Les valeurs correspondantes pour les sujets traits par métronidazole étaient de: 1.69±0.28 à 0.02±0.01, 0.20±0.08, 0.22±0.06 et 0.22±0.08 ($p<0.001$). Les comptages des espèces *Camopylobacter*, *Eubacterium nodatum*, des espèces *Fusobacterium nodatum*, *F. periodonticum* et *Prevotella nigrescens* étaient également détectés à des niveaux moyens plus bas pendant, et immédiatement après traitement, mais augmentaient graduellement après cessation des antibiotiques. Les membres des genres *Actinomyces*, *Streptococcus* et *Capnocytophaga* étaient très peu affectés par le métronidazole. Par contre, l'amoxicilline diminuait les comptage et les proportions des Actinomyces pendant et après le traitement.

Conclusions: Ces données suggèrent que le métronidazole et l'amoxicilline sont utiles pour diminuer rapidement les comptages des pathogènes parodontaux putatifs, mais qu'ils doivent être accompagnés d'autres procédés pour apporter une stabilité parodontale.

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