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Change of antibiotic susceptibility following periodontal therapy

A pilot study in aggressive periodontal disease

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Abstract

Background: The hypothesis was tested that bacterial susceptibilities in aggressive periodontitis change upon administration of systemic antibiotics as adjuncts to periodontal therapy.

Methods: In 23 subjects (average age 38.9 ± 6.7 years) with aggressive periodontitis, microbial parameters were assessed prior to and 1 year after completion of comprehensive mechanical/surgical and systemic antimicrobial therapy. Following identification of five selected pathogens with the *Rapid ID 32 A* system, their susceptibilities towards amoxicillin/clavulanate potassium, metronidazole, and tetracycline were examined with the *E*-test. Antibiotics were administered according to the test results, and the minimal inhibitory concentrations (MIC_{90}) were reevaluated after 1 year. Statistical analysis was performed on a patient basis, with the site data used for evaluation of the MIC levels.

Results: Bacterial MIC levels remained constant among the three antibiotic treatment groups compared with baseline. Mean MIC_{90} values ranged from <0.02 to 0.11 μg/ml (amoxicillin/clavulanate potassium), <0.02 to 0.27 μg/ml (metronidazole), and <0.02 to 0.11 μg/ml (tetracycline). Observed changes in susceptibility were attributed to the elimination of single bacterial taxa in the subgingival environment after antibiotic therapy. There were no statistically significant differences in clinical parameters among the treatment groups. Single tetracycline MICs were 1.5- to 6-fold enhanced compared to amoxicillin/clavulanate potassium and metronidazole.

Conclusion: The periodontal pathogens investigated prior to and 1 year after periodontal therapy are tested sensitive to the antimicrobial agents. In aggressive periodontitis, changes in bacterial susceptibility upon the administration of systemic antibiotics are associated with the limited number of isolates tested following therapy.

*RF Müller died on 9 September 2000

Key words: antibiotic susceptibility; periodontal therapy, aggressive periodontitis; systemic antibiotics

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In a revised classification system for periodontal diseases (Armitage 1999), aggressive periodontitis was redefined to comprise a complex entity of microbial alterations and cellular dysfunctions that differentiate the underlying molecular mechanisms from chronic periodontal disease. The formation of microbial complexes in subgingival plaque samples (Socransky et al. 1998) with increased antibacterial resistances (Slavkin 1997, Handal & Olson 2000), and multilayer biofilms that act as physical barriers to antibiotics (Gander 1996, Costeron et al. 1999) play a pivotal role in triggering amplified host responses in aggressive periodontitis.

Aggressive periodontal disease is frequently associated with *Actinobacillus actinomycetemcomitans* (Van Winkelhoff et al. 1992, Buchmann et al. 2000, Winkel et al. 2001). However, in a subset of patients, the microorganism cannot be recovered from subgingival plaque samples, and antibiotic therapy with amoxicillin plus metronidazole is not justified. A number of issues have been raised concerning changes that occur in the subgingival microbiota of patients as a result of the administration of systemic antibiotics. There is evidence that the efficacy of antimicrobial agents is limited in suppressing or eliminating key periodontal pathogens due to the reappearance of toxic highly virulent strains following periodontal therapy (Saarela et al. 1999), or the clonal character of the infection (Ehmke et al. 1999). One of the major concerns is the rate at which changes of bacterial susceptibilities return to pretreatment levels, and whether altered susceptibility profiles were created as a result of antibiotic therapy. Most of the periodontal microorganisms harbor different

antimicrobial susceptibility profiles (Slots & Rams 1990), may change their susceptibility after antibiotic therapy, or acquire an altered susceptibility profile due to bacterial mutation (Hawkey 1998). When antibiotic therapy recommended for severe periodontal infections is aimed at eradication of the subgingival microbiota (Van Winkelhoff et al. 1992, Winkel et al. 2001), there is a risk of destroying the normal flora and impairing the restoration of sensitivity (Levy 1998).

Clinical trials have been conducted to evaluate the efficacy of systemic administration of amoxicillin clavulanate/potassium (Haffajee et al. 1995), metronidazole (Elter et al. 1997), and tetracycline (Feres et al. 1999, Ramberg et al. 2001) as adjuncts to treatment in severe periodontal disease. Most studies have not given sufficient consideration to antimicrobial susceptibility of the pathogens targeted. Thus, the pre- and post-treatment disease activities have not been adequately assessed (Slots & Rams 1990). Recently, the bacterial susceptibilities of periodontal microorganisms to amoxicillin clavulanate/potassium were tested revealing an insufficient efficacy of the drug against single targeted pathogens (Kleinfelder et al. 2000). The relevance of these findings has not yet been fully elucidated since clinical parameters have often not been adequately assessed.

Although systemic antibiotic therapy enhances the resolution of advanced adult periodontitis, data available in aggressive periodontal disease that demonstrate the treatment efficacy on the level of antimicrobial susceptibility are limited (Gordon & Walker 1993). The knowledge of microbial responses to antibiotics would advance our understanding to ensure the use of safe and efficacious antimicrobial treatments as well as elucidating the nature of progression and resolution of aggressive periodontal disease.

Thus, the hypothesis was tested that, in a long-term perspective, microbial susceptibilities in aggressive periodontitis may change upon the administration of systemic antibiotics used as adjuncts to periodontal therapy.

Materials and Methods

Subjects

The data for this study were taken from 23 subjects of the Department of Periodontology, University of Münster, with aggressive periodontal disease (Armitage 1999) and evidence of prior attachment loss. The average age of the patients, 10 women and 13 men, was 39.3 ± 6.7 years at the last visit (age range 32–49 years). Patients selected to enter into the study were screened for periodontal disease by radiographic evidence of intrabony defects exceeding more than 50% of the root length and probing depths >5 mm at least at eight sites within each quadrant. All subjects displayed generalized severe periodontal tissue destruction, loss of periodontal support inconsistent with age, persistent gingival inflammation, increasing probing depths >5 mm at a minimum of eight sites, and progressing tooth mobility (American Academy of Periodontology 2000). They were recruited from referring dentists since control of disease was not achievable in the past. All individuals were negative for *A. actinomycetemcomitans* at both visits, since they would have been otherwise subjected to amoxicillin plus metronidazole (Van Winkelhoff et al. 1992). Exclusion criteria included pregnancy, periodontal therapy, or antibiotics in the previous 6 months, allergies to any of the test antibiotics, and any systemic condition that might have affected the progression or treatment of periodontitis and the need for premedication for therapy. Patients were requested to report to the principal investigator any orofacial or medical infection where antibiotics were administered. Patients requiring additional antibiotic medications related to other acute infectious diseases during the 1-year observation period were exited from the study. No subject with localized juvenile periodontitis or acute necrotizing ulcerative gingivitis was included in the study. Subjects fulfilling the inclusion criteria above were asked to participate in the study and, if they accepted, to sign informed-consent forms.

Microbial analysis

Sampling

Subgingival plaque was sampled at four sites with probing pocket depths >5 mm (one in each quadrant) in each individual. After isolating the area with a cotton roll and gently air drying, supragingival plaque deposits were carefully removed with a Gracey 11/12-curet tip (Hawe Neos, Switzerland). Subgingival plaque samples were taken by inserting a sterile endodontic paper point (Roeko, Langenau, Germany, xxfine) to the bottom of the periodontal pocket for 10 s. The subgingival plaque samples were immediately transferred into an Eppendorf vial containing 500 μl one-fourth concentrated, ice-cold, filter-sterilized Ringer's solution. After resuspension for 10 s in an ultrasonic unit (Sonorex RK 82, Bandelin Electronic KG, Berlin, Germany), the samples were individually evaluated for the presence of five periodontal pathogens.

A. actinomycetemcomitans

For quantitative enumeration of *A. actinomycetemcomitans* in the subgingival plaque samples (detection limit: 100 cells/ml), 0.1 ml of the transport medium was diluted to 10^{-1} and 10^{-2} and spread on freshly prepared TSBV agar (Slots 1982). The TSBV agar consists of 4% Trypticase soya agar with 1g of yeast extract per liter at pH 7.2. The agar had been cooled to 56°C; then horse serum (10%), filter-sterilized bacitracin (75 mg/l), and vancomycin (5 mg/l) were added. The agar sheets were kept at 4°C and used within 7 days. To identify and determine the biotype, the sheets were incubated in a CO_2 (5%)-enriched incubator for 3 days at 35°C. Catalase-positive, small, convex colonies with star-shaped inner structures adhering to the agar were identified as *A. actinomycetemcomitans* colonies. The evaluation of *A. actinomycetemcomitans* on TSBV agar was expressed quantitatively in colony-forming units (CFU/ml).

Microbial isolation and identification

The undiluted suspension (0.1 ml) and 0.1 ml aliquots of the dilutions were spread on plates with nonselective blood agar base (CDC) containing 5% defibrinated sheep blood, supplemented with 5 mg/l hemin (Merck, Darmstadt, Germany) and 1 mg/l vitamin K_1. The plates were incubated for 7 days in an atmosphere containing 85% N_2, 10% H_2, and 5% CO_2. For each of the 23 subjects, the five most prevalent types of microbial colonies were selected on the incubated blood agar plate for identification. From each of these selected colonies, four subcultures were prepared also utilizing nonselective blood agar base. One was also incubated

and used for identification of the bacterial colonies with the detection system Rapid ID 32 A (Bio Merieux, Nuertingen, Germany). The remaining three subcultures were used for the susceptibility testing. The detection system Rapid ID 32 A is based on fermentative and biochemical properties of anaerobic microorganisms being identified by standard enzymatic reactions. Aliquots of bacterial suspensions prepared from the incubated subculture are given on a test panel consisting of 29 biochemical tests. The results of these tests are interpreted by comparing all the results obtained for the test microorganisms with the information in the computer-assisted database ATB 32 (Arzese et al. 1994).

Susceptibility testing

From each of the bacterial species identified, three subcultures were used for susceptibility testing of amoxicillin/ clavulanate potassium, metronidazole, and tetracycline utilizing the epsilometer *E*-test (AB Biodisk, Solna, Sweden). The *E*-test consists of strips containing gradients of different antibiotic concentrations that are placed directly on the agar plate. After 7 days of incubation in anaerobic conditions, the concentration of the drug that inhibits 90% of bacterial growth in vitro (minimal inhibitory concentration = MIC_{90}) was determined from the strip (Citron et al. 1991). The antimicrobial agent with the lowest MIC_{90} value was selected on an individual basis for prescription. For each subject, the number of resistant strains of the pathogens isolated on each of the agar plates was encountered and typed into a database for further statistical evaluation.

Periodontal examination and treatment procedures

All patients were monitored at baseline and 1 year after periodontal treatment. The clinical examination and the periodontal treatment protocols have been described in a previous investigation (Buchmann et al. 2000). Briefly, the probing depths (PD) and the clinical attachment levels (CAL) were assessed at four sites (m, v, d, and o) per tooth using a straight rigid periodontal probe (PCP 11, HuFriedy, Chicago, IL, USA). Bleeding on probing (BOP) was recorded dichotomously, and the PI (Silness & Löe 1964) and GI (Löe & Silness 1963) were registered at four sites per tooth. Subgingival scaling and root planing was performed at sites with PD > 4 mm. At periodontal defects with PD > 6 mm, surgical access to the periodontal defects was achieved according to the modified Widman flap technique. The patients were enrolled in a supportive periodontal maintenance program and monitored on a 3–6-month recall schedule, including repeated oral hygiene instructions and a full mouth tooth cleaning. Systemic antibiotics were selected according to the results of the *E*-test for each individual, and administered for 7 consecutive days during SRP and surgical therapy. The following dosages were used: amoxicillin/ clavulanate potassium, 3×500 mg/ day (Augmentan-Filmtabletten, 500 mg, SmithKline Beecham Pharma, Germany), metronidazole, 3×400 mg/day (Clont 400, Filmtabletten, Bayer Vital, Germany), tetracycline, 2×100 mg/day (Doxycyclin 100, Heumann, Germany).

Experimental protocol

At the outset, the full range of periodontal parameters was assessed as described above. At the first visit, all individuals were screened for *A. actinomycetemcomitans*. Prior to periodontal therapy after completion of the oral hygiene program, and at the periodontal reexamination after 1 year, subgingival plaque samples were harvested from interproximal sites with PD between 6 and 8 mm for microbial analysis and further antibiotic susceptibility testing. The reevaluation that included the recording of the clinical parameters, the microbial analysis and antibiotic susceptibility testing took place for all subjects 1 year after completion of active periodontal therapy.

Statistical analysis

Data analysis and statistical tests were performed on a patient basis with the site data used for evaluation of the MIC levels. For each individual, the means and standard deviations for the microbial and periodontal parameters were calculated. Microbial data were given as a percentage of the total cultivable flora. Due to the small sample size in each group, nonparametric test procedures were applied. The overall changes of periodontal pathogens, and the differences of clinical parameters in each antibiotic group after 1 year (intragroup comparison) were subjected to the Wilcoxon signed-rank test. Differences of change between the treatment groups (intergroup comparison) were analyzed by the Kruskal–Wallis test. Statistical significance was considered at an $\alpha = 0.05$.

Results

Periodontal pathogens

We observed a high prevalence of the pathogens at baseline (26.1–86.9%) that was reduced after antibiotic therapy. The prevalence declined for all species, except for *Prevotella buccae*, which increased slightly from 26.1 to 34.8%. *Fusobacterium nucleatum* and *Peptostreptococcus micros* occurred less frequently at the 1-year visit, but their mean proportions did not change statistically. Overall, antibiotic therapy resulted in a significant decrease in the proportions of *Porphyromonas gingivalis* and *Bacteroides forsythus* ($p < 0.05$). At baseline, the mean proportions of the test species were between 3.5% and 35.2%, and between 2.6% and 16.9% after antibiotic therapy (Table 1).

Minimal inhibitory concentrations

After amoxicillin clavulanate/potassium therapy, susceptibilities of *P. gingivalis* and *B. forsythus* were less frequently encountered. Four strains of *P. gingivalis* yielded a lower sensitivity after antibiotic therapy. The encountered MICs for *P. buccae* increased after therapy (4 to 11). Amoxicillin clavulanate/potassium was less effective against *F. nucleatum* and *P. micros,* and four amoxicillin clavulanate/potassium resistant *F. nucleatum* strains were detected pretherapy, and eight after antibiotic treatment (Table 2).

Administration of metronidazole lowered the number of susceptibilities of *P. gingivalis* and *B. forsythus.* Metronidazole was less effective against *P. buccae.* The number of susceptible strains of *F. nucleatum* and *P. micros* was reduced after antibiotic therapy. Metronidazole might be useful when these taxa are targeted. Some clinical strains of *P. micros* yielded a lower susceptibility towards metronidazole that disappeared after therapy (Table 3).

Table 1. Proportions of selected periodontal pathogens at baseline and at the 1-year reappointment

Species	Visit	*n*	Prevalence (%)	Mean (%)	SD	Min–Max	*P*[a]
Pg	pre	19	82.6	35.2	21.4	0–72.5	
	1 year	11	47.8	10.6	13.3	0–35.0	0.008
Bf	pre	15	65.2	10.0	8.9	0–27.5	
	1 year	6	26.1	2.6	5.1	0–14.0	0.025
Pb	pre	6	26.1	3.5	6.7	0–22.5	
	1 year	8	34.8	7.4	12.7	0–35.0	0.310
Fn	pre	20	86.9	22.8	16.4	0–50.0	
	1 year	8	34.8	11.7	17.6	0–45.0	0.083
Pm	pre	15	65.2	10.2	9.6	0–27.5	
	1 year	12	52.2	16.9	18.4	0–45.0	0.345

Pg = *Porphyromonas gingivalis*, Bf = *Bacteroides forsythus*, Pb = *Prevotella buccae*, Fn = *Fusobacterium nucleatum*, Pm = *Peptostreptococcus micros*. *n* = subjects.

[a]Wilcoxon signed-rank test.

Table 2. Distribution of amoxicillin clavulanate/potassium MIC_{90} values (μg/ml), baseline and 1 year post-therapy

Species	Visit	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.250	0.282	0.500	0.800	Resistant
Pg	pre	26[a]	—	—	—	—	—	—	—	—	—	—	—
	1 year	13	4	—	—	—	—	—	—	—	—	—	—
Bf	pre	20	—	—	—	—	—	—	—	—	—	—	—
	1 year	5	—	—	—	—	—	—	—	—	—	—	—
Pb	pre	4	—	—	—	—	—	—	—	—	—	—	—
	1 year	6	—	5	—	—	—	—	—	—	—	—	—
Fn	pre	12	5	2	4	—	—	—	—	—	—	—	4
	1 year	6	—	13	—	—	—	—	—	—	—	—	8
Pm	pre	10	8	5	—	—	—	—	—	—	—	—	—
	1 year	9	4	4	—	—	—	—	—	—	4	—	—

Pg = *Porphyromonas gingivalis*, Bf = *Bacteroides forsythus*, Pb = *Prevotella buccae*, Fn = *Fusobacterium nucleatum*, Pm = *Peptostreptococcus micros*.

[a]Number of isolates tested.

Table 3. Distribution of metronidazole MIC_{90} values (μg/ml), baseline and 1 year post-therapy

Species	Visit	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.250	0.282	0.500	0.800	Resistant
Pg	pre	20[a]	—	—	—	—	—	—	—	—	—	—	—
	1 year	12	—	—	—	—	—	—	—	—	—	—	—
Bf	pre	16	—	—	—	—	—	—	—	—	—	—	—
	1 year	4	—	—	—	—	—	—	—	—	—	—	—
Pb	pre	10	—	—	—	—	—	—	—	—	—	—	—
	1 year	8	—	—	—	—	—	—	—	—	—	—	—
Fn	pre	20	—	—	—	—	—	—	—	—	—	—	—
	1 year	16	—	—	—	—	—	—	—	—	—	—	—
Pm	pre	—	—	4	—	—	—	8	—	—	—	4	—
	1 year	—	—	—	—	—	—	—	—	—	—	—	—

Pg = *Porphyromonas gingivalis*, Bf = *Bacteroides forsythus*, Pb = *Prevotella buccae*, Fn = *Fusobacterium nucleatum*, Pm = *Peptostreptococcus micros*.

[a]Number of isolates tested.

After tetracycline therapy, MICs of *P. gingivalis* decreased. The susceptibility change of *B. forsythus* was less evident. The MICs of *F. nucleatum* decreased after therapy. Postantibiotic treatment, some strains of *P. micros* displayed enhanced MICs. Compared to amoxicillin clavulanate/potassium and metronidazole, all pathogens tended to be slightly less sensitive against tetracycline (Table 4). However, the range of the mean MICs was similar to that of the other antibiotics. All test species were sensitive to metronidazole and tetracycline, while 12.9% and 25.8% of strains were resistant to amoxicillin clavulanate/potassium pre- and post-therapy, respectively.

Clinical parameters

All subjects benefited from antibiotic therapy as evidenced by the treatment induced changes of PD and CAL in the three antibiotic groups (intragroup comparison), Wilcoxon signed-rank test, $p<0.05$. The mean decreases in PD were 2.8 ± 1.7 mm (aug), 3.5 ± 1.9 mm (met), and 2.4 ± 1.2 mm (tet). The average decreases in CAL were 2.2 ± 1.0 mm (aug), 1.7 ± 1.2 mm (met), and 3.1 ± 1.4 mm (tet), respectively. No statistically significant differences could be observed for all periodontal measures between the three antibiotic groups (intergroup comparison), Kruskal–Wallis test, $p<0.05$ (Table 5).

Table 4. Distribution of tetracycline MIC_{90} values (μg/ml) baseline and 1 year post-therapy

Species	Visit	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.250	0.282	0.500	0.800	Resistant
Pg	pre	—	—	—	10[a]	—	10	—	—	—	—	—	—
	1 year	3	—	—	—	—	—	—	—	—	—	—	—
Bf	pre	10	10	—	—	—	—	—	—	—	—	—	—
	1 year	20	—	—	—	—	—	—	—	—	—	—	—
Pb	pre	—	—	—	10	—	—	—	—	—	—	—	—
	1 year	—	—	—	10	—	—	—	—	—	—	—	—
Fn	pre	—	—	10	—	—	10	—	—	—	—	—	—
	1 year	10	—	—	10	—	—	—	—	—	—	—	—
Pm	pre	—	10	—	—	10	—	—	—	—	—	—	—
	1 year	20	—	—	—	—	—	—	—	10	—	—	—

Pg = *Porphyromonas gingivalis*, Bf = *Bacteroides forsythus*, Pb = *Prevotella buccae*, Fn = *Fusobacterium nucleatum*, Pm = *Peptostreptococcus micros*.

[a]Number of isolates tested.

Table 5. Baseline and 1-year results of clinical parameters for subjects treated with amoxicillin clavulanate/potassium, metronidazole, and tetracycline

Antibiotic		Amoxicillin clavulanate/ potassium		Metronidazole		Tetracycline		P[a]
Subjects		8		7		8		
Sites		32	31	28	31	31	27	
Visit		pre	1 year	pre	1 year	pre	1 year	
PD	mean	6.4	3.6	7.0	3.5	6.0	3.6	0.693
	SD	1.1	0.8	0.8	0.7	1.3	0.5	
	min	4.8	2.5	6.0	3.0	5.5	2.7	
	max	7.5	4.7	8.0	4.3	6.5	5.5	
	p[b]	0.012		0.068		0.048		
CAL	mean	7.7	5.5	7.3	5.6	7.6	4.5	0.459
	SD	1.6	1.4	1.1	0.3	0.9	0.4	
	min	5.0	4.3	6.0	5.3	7.3	3.3	
	max	10.3	7.8	8.5	5.8	8.0	5.5	
	p	0.012		0.068		0.047		
GI	mean	1.5	0.5	1.8	0.3	1.1	0.3	0.212
	SD	0.5	0.5	0.7	0.4	0.5	0.3	
	min	0.8	0	1.0	0	0.5	0	
	max	2.3	1.3	2.5	0.8	1.5	0.5	
	p	0.018		0.068		0.109		
BOP	mean	0.5	0.3	0.7	0.3	0.3	0.1	0.299
	SD	0.3	0.4	0.2	0.3	0.4	0.3	
	min	0.2	0	0.5	0	0	0	
	max	1.0	1.0	1.0	0.5	0.8	0.5	
	p	0.306		0.109		0.564		
PI	mean	0.4	0.6	0.4	0.3	0.8	0.3	0.501
	SD	0.5	0.6	0.4	0.6	0.3	0.1	
	min	0	0	0	0	0.5	0.3	
	max	1.3	1.3	1.0	1.0	1.0	0.5	
	p	0.180		0.357		0.652		

PD = probing depths, CAL = clinical attachment level, GI = gingival index, BOP = bleeding on probing, PI = plaque index.

[a]Kruskal–Wallis test.

[b]Wilcoxon signed-rank test.

Discussion

We examined antimicrobial susceptibilities of periodontal pathogens towards three antibiotics prior to and after periodontal therapy to enhance knowledge about the benefits and drawbacks of antibiotic therapy in aggressive periodontal disease. Two major findings were noted. First, all pathogens recovered from untreated and treated individuals were sensitive to the antimicrobial agents tested. Second, long-term susceptibility changes were attributed to a limited number of microorganisms after antibiotic therapy.

Our patient population group presented with high proportions of *P. gingivalis* and *F. nucleatum*. The baseline proportions of microorganisms exceeded those documented in a cross-sectional study on 61 patients with chronic periodontitis at approximately one-third (Van Winkelhoff et al. 2000).

For example, proportions of the total cultivable flora for *P. gingivalis* reached 35.2% in our study, but only 21.7–28.6% in chronic periodontitis. The initial proportions of *F. nucleatum* were 22.8% versus 6.9–9.3% in chronic periodontitis, while *P. micros* was 10.2% in the current study versus 4.0–7.2% in the Van Winkelhoff study. Interestingly, the clinical disease severity measured by PD and CAL scores in both patient population groups was almost identical. This recognition supports the concept of a transition from chronic to aggressive periodontal disease bridged by a microflora that becomes more heterogenous and complex (Haffajee et al. 1988, Walker et al. 1993).

Our findings agree with recent data reported in refractory patients where baseline proportions of *P. gingivalis* were about 31% (Van Winkelhoff et al. 1992), and are similar to refractory subjects culture negative for *A. actinomycetemcomitans* with *P. gingivalis* levels of 31.8%, and *B. forsythus* levels of 16.1% (Winkel et al. 1997). When SRP with antibiotic therapy based on susceptibility testing was performed in refractory periodontitis, amoxicillin clavulanate/potassium was effective since clavulanate/potassium is a potent blocker of microbial beta-lactamases (Walker et al. 1993, Magnusson et al. 1994). Recently, the drug yielded insufficient efficacy eliminating *F. nucleatum* and *P. micros* from the subgingval environment of patients not responding to mechanical therapy (Kleinfelder et al. 2000). After 6 weeks, the authors reported MIC_{90} values of *F. nucleatum* and *P. micros* exceeding more than 4 μg/ml. Our data support this hypothesis since we observed post-antibiotic treatment MIC_{90} levels of *P. micros* reaching 0.5 μg/ml. The increment in MIC values detected for *P. micros* 6 weeks following antibiotic therapy might be due to a post-treatment overgrowth of less sensitive taxa present pretherapy that disappeared in a long-term perspective after 1 year. As some strains of *P. micros* became less sensitive after antibiotic therapy, our observation in a limited number of patients suggested that amoxicillin clavulanate/potassium selected for some pathogens with a low susceptibility. Moreover, our population group elicited percentage levels between 12.9% and 25.8%, while in chronic periodontitis the occurrence of amoxicillin clavulanate/potassium resistant *F. nucleatum* strains was reported to reach 6.5% (Van Winkelhoff et al. 2000).

The efficacy of metronidazole is due to a reduction in the anaerobic flora, especially *Bacteroides* species and spirochetes (Gusberti et al. 1988). In a comparative in vitro study utilizing the *E*-test, metronidazole demonstrated excellent activity towards *P. gingivalis*, *P. intermedia*, and *Fusobacterium* spp. with a limited efficacy against *P. micros*. Clinical strains from 15 RPP patients, 13 LJP and 13 CP subjects displayed MICs of 0.097 μg/ml for *P. gingivalis*, and 0.236 μg/ml for *F. nucleatum. P. micros* revealed enhanced MICs of 11.89 μg/ml (Poulet et al. 1999). Based on the in vitro susceptibility data, metronidazole is very effective when strict anaerobes make up most of the subgingival flora. Since the elimination of facultative and capnophilic anaerobes, such as *A. actinomycetemcomitans*, is unlikely (Gordon & Walker 1993), the drug is efficacious only when *A. actinomycetemcomitans* cannot be recovered. One study used metronidazole in combination with flap surgery in advanced periodontitis (Mahmood & Dolby 1987). As *A. actinomycetemcomitans* was not screened prior to administration of metronidazole, and no further susceptibility test was applied to the subgingival microbiota, no attributable benefits of the drug were documented. It is important to recognize that a known susceptible flora is a prerequisite for an efficacious treatment protocol using metronidazole in aggressive periodontitis as outlined by the low MIC levels prior to and after periodontal therapy. The drug should be restricted to treating patients with a known susceptible flora, as otherwise the disease may worsen due to the presence of intrinsic taxa normally resistant to metronidazole (Gordon & Walker 1993).

Beside the effects on the metabolism of host tissues, one of the unique feature of tetracyclines is the attachment to the root surface creating a reservoir of antibiotic activity that is not easily cleared out of its environment. It is not clear whether the lower susceptibilities of single strains compared to other antimicrobial agents reported in our study and by several other investigators (Slots & Rams 1990, Gordon & Walker 1993, Piccolomini et al. 1997) are due to the protection in biofilms (Feres et al. 1999) or the development of intrinsic or novel tetracycline resistant taxa (Olsvik et al. 1996). We observed MIC_{90} levels towards doxycycline ranging from 0.02 to 0.11 μg/ml. No major susceptibility change against tetracycline was noted after therapy. The bacterial susceptibility to minocycline of 55 strains in chronic periodontitis patients was determined revealing a MIC range from 0.03 to 32 μg/ml. The MIC levels amounted to 0.06 μg/ml for *P. gingivalis*, 0.25–0.5 μg/ml for *Prevotella* species, and 0.12–0.25 for *F. nucleatum* (O'Connor et al. 1990). *P. gingivalis* was not killed by routine systemic doses of minocycline. In contrast, *P. gingivalis* was successfully targeted in our study. However, as evidenced by the frequency distribution of tetracycline MIC_{90} levels compared to amoxicillin clavulanate/potassium and metronidazole, our findings support the hypothesis that systemic minocycline in a dosage of 2×100 mg/day has low beneficial microbial effects. Limited doxycycline efficacy was recently documented even after 14 days of systemic administration of 100 mg/day (Feres et al. 1999). Before tetracycline is used as adjunctive therapy for refractory periodontitis, the subgingival flora should be tested for susceptibility (Olsvik & Tenover 1993).

In our investigation, the MICs were far from peak serum concentrations. Since the *E*-test ensures a precise degree of susceptibility in all those cases where the MICs are very close to the peak serum concentration (Nachnani et al. 1992), MIC values as reference parameter for defining antimicrobial susceptibility are relatively imprecise when the microflora is highly sensitive. One should recall that results of in vitro susceptibility tests do not always correlate with in vivo findings. Pathogens might select to be less susceptible after antibiotic therapy (Van Winkelhoff et al. 2000), and multilayer biofilms that act as physical barriers to antibiotics (Costerton et al. 1999) limit the routine use of MIC values in clinical pratice. However, in aggressive periodontitis where the microbial infection results in amplified host responses with severe damage of periodontal tissues, the use of microbiologic information can assist in selecting the most optimal antibiotic regimen based on the presence and levels of selected periodontal pathogens.

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Zusammenfassung

Veränderung der Antibiotikaampfindlichkeit nach Parodontalbehandlung. Eine Pilotstudie von Fällen, die wegen Aggressiver Parodontitis behandelt wurden.

Hintergrund: Es wurde die Hypothese getestet, dass sich die bakterielle Empfindlichkeit bei Aggressiver Parodontitis durch Gabe von systemischen Antibiotika als Adjuvans der Parodontalbehandlung verändert.

Methoden: Bei 23 Patienten (Durchschnittsalter 38,9 ± 6,7 Jahre) mit Aggressiver Parodontitis wurden die mikrobiologischen Parameter vor und 1 Jahr nach dem Abschluss der umfassenden mechanischen/chirurgischen uns systemisch antibiotischen Behandlung bestimmt. Nach der Identifikation von 5 ausgewählten Pathogenen mit dem *Rapid ID 32 A*-System wurde ihre Empfindlichkeit gegen Amoxicillin/Kalium-Clavulanat, Metronidazol und Tetracyclin mit dem *E*-test untersucht. Die Antibiotika wurden entsprechend der Testergebnisse verabreicht und die minimalen Hemmkonzentrationen (MIC_{90}) nach einem Jahr reevaluiert. Die statistische Analyse wurde mit dem Patienten als Untersuchungeinheit durchgeführt, wobei die Taschendaten zur Evaluation der MIC-Level verwendet wurden.

Ergebnisse: Die bakteriellen MIC-Level blieben innerhalb der 3 Antibiotika-Behandlungsgruppen im Vergleich zum Ausgangswert konstant. Die Mittelwerte für MIC_{90} lagen im Bereich von < 0,02 bis 0,11 µg/ml (Amoxicillin/Kalium-Clavulanat), < 0,02 to 0,27 µg/ml (Metronidazol) und < 0,02 to 0,11 µg/ml (Tetracyclin). Die beobachteten Veränderungen in der Empfindlichkeit wurden der Elimination eines einzelnen Bakterientaxa des subgingivalen Milieus nach Antibiotikatherapie zugeschrieben. Zwischen den Behandlungsgruppen gab es keine statistisch signifikanten Unterschiede bei den klinischen Parametern. Einzelne Tetracyclin-MICs waren im Vergleich zu Amoxicillin/Kalium-Clavulanat und Metronidazol 1,5- bis 6-fach erhöht.

Schlussfolgerung: Die Parodontalpathogene, die vor und 1 Jahr nach der Parodontalbehandlung untersucht wurden, sind empfindlich für antimikrobielle Wirkstoffe. Die bei Aggressiver Parodontitis nach Gabe von systemischen Antibiotika beobachteten Veränderungen, in der nach der Therapie getesteten bakteriellen Empfindlichkeit, sind assoziiert mit einer begrenzten Anzahl von Isolaten.

Résumé

Variations de la susceptibilité aux antibiotiques à la suite du traitement parodontal. Une étude pilote de cas traités pour maladie parodontale agressive

L'hypothèse analysée portait sur les variations de la susceptibilité bactérienne dans la parodontite agressive après administration systémique d'antibiotiques utilisés comme traitement parodontal. Chez 23 sujets (de 39 ± 7 ans) avec parodontite aggressive, des paramètres microbiens ont été relevés avant et un an après l'achèvement du traitement mécanique/chirurgical et l'utilisation d'antimicrobiens par voie systémique. Suivant l'identification de cinq pathogènes sélectionnés par le système Rapid ID 32A leurs susceptibilités envers l'amoxicilline/potassium clavulanate, le métrodinazole, et la tétracycline ont été examinées avec le test E. Les antibiotiques ont été administrés suivant les résultats des tests et les concentrations inhibitrices minimales (MIC90) réévaluées après une année. L'analyse statistique a été effectuée en prenant le patient comme unité de base, avec les données du site utilisées pour l'évaluation des niveaux MIC. Les niveaux MIC bactériens restaient constants parmi les trois groupes de traitement par antibiotique comparés au départ. Les valeurs MIC90 moyennes étaient < 0,02 à 0,11 µg/ml (l'amoxicilline/potassium clavulanate,) < 0,02 à 0,27 µg/ml (métrodinazole) et < 0,02 à 0,11 µg/ml (tétracycline). Les variations observées de la susceptibilité étaient attribuées à l'élimination d'un groupe taxinomique bactérien simple dans l'environement sous-gingival après le traitement par antibiotiques. Il n'y avait aucune différence statistique dans les paramètres cliniques parmi les groupes traités. Les MIC de la tétracycline étaient 1,5 à 6 fois plus élevés comparés à ceux de l'amoxicilline/potassium clavulanate et du métrodinazole. Les pathogènes parodontaux étudiés avant et une année après le traitement parodontal ont été définis sensibles aux agents microbiens. Dans la parodontite agressive les variations de la susceptibilité bactérienne suite à l'administration d'antibiotiques systémiques sont associées avec le nombre limité d'isolats testés après le traitement.

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