Innovations in Periodontics

Specific Antibiotics in the Treatment of Periodontitis – A Proposed Strategy

Thomas Beikler,* Karola Prior,* Benjamin Ehmke,* and Thomas F. Flemmig*

Background: *The purpose of the present study was to propose a strategy for the selection of antibiotics that specifically target complexes of periodontal pathogens present in patients with periodontitis.*

Methods: *Seven hundred seventy-four (774) patients with various forms of periodontitis were included in the study. Subgingival plaque samples were taken from the deepest periodontal pockets in each quadrant using a sterile curet, and pooled.* Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Eikenella corrodens, Tannerella forsythensis, Prevotella intermedia, *and* Prevotella nigrescens *were identified by polymerase chain reaction, and the prevalence of combinations of these pathogens was determined. To each pathogen complex (PC), i.e., combination of pathogens, those antibiotics were assigned that were most specific according to the published minimum inhibitory concentration (MIC_{90}) values and the gingival crevicular fluid (GCF) concentrations achievable in vivo. Antibiotic GCF concentrations had to be at least 10 times the MIC_{90} values, and the narrowest spectrum was selected with respect to the assessed periodontal pathogens.*

Results: *Nine major PCs (each ≥3% of all patients) were found in 73.4% of all patients, whereas 38 minor PCs (each <3% of all patients) were distributed in 26.6% of all patients. Ten different antibiotic regimens were found to be specific for the total of 46 PCs; i.e., metronidazole and amoxicillin in 11 PCs (55.0% of all patients), metronidazole and amoxicillin/clavulanic acid or metronidazole and ciprofloxacin in 13 PCs (18.9%), amoxicillin in 4 PCs (8.3%), doxycycline in 2 PCs (6.1%), metronidazole in 8 PCs (4.1%), amoxicillin/clavulanic acid in 3 PCs (2.9%), clindamycin in 2 PCs (1.5%), ciprofloxacin in 2 PCs (0.4%), and tetracycline in 1 PC (0.3%).*

Conclusion: *The results of the study indicate that there are at least 46 different combinations of the assessed periodontal pathogens in subjects with periodontitis, and at least 10 different antibiotic regimens might be required to specifically target the various pathogen complexes.* J Periodontol 2004;75:169-175.

* Department of Periodontology, University of Münster, Münster, Germany.

KEY WORDS

Antibiotics, antibacterial/therapeutic use; antibiotics, combination/therapeutic use; periodontal diseases/epidemiology; periodontal diseases/microbiology; periodontal diseases/therapy; polymerase chain reaction.

Although the majority of species colonizing the gingival sulcus at or below the gingival margin are compatible with periodontal health, a subset of species may initiate or contribute to the progression of periodontitis: *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Eikenella corrodens, Tannerella forsythensis, Prevotella intermedia*, and *Prevotella nigrescens*.[1-3] The prevalence of each of these bacteria in patients with various forms of periodontitis and in healthy subjects has been extensively studied, and their occurrence together with a variety of other species is well documented.[4-8] These studies have resulted in the identification of specific bacterial complexes that appear to be strongly associated with periodontitis.[6-10] High interindividual variability in intraoral colonization with periodontal pathogens has further been demonstrated,[8] indicating that an adjunctive antibiotic regimen might have varying efficacy in different patients. Therefore, there is general consensus that microbiological testing should be performed prior to systemic antibiotic therapy to aid in the selection of a specific antibiotic regimen.[11,12]

However, the identification of periodontal pathogens by microbial testing in a clinical setting is generally limited to the main putative pathogens. Moreover, the clinician has to translate the microbiological information into a treatment decision, i.e., selection of the most specific antibiotic therapy, a process that is not yet supported by guidelines taking account of the variety of combinations of periodontal pathogens in patients with periodontitis.

Therefore, the aim of the present study was to propose a more specific strategy for the selection of antibiotics by assigning published MIC_{90} values and gingival crevicular fluid concentrations achievable in vivo via oral administration of antibi-

otics to the treatment of various periodontal infections.

MATERIALS AND METHODS

Study Population

Seven hundred seventy-four (774) patients with different forms of periodontitis were enrolled in the study. The patients were previously untreated or had not received any periodontal therapy in the year preceding enrollment into the study. Patients were recruited from the Department of Periodontology, University of Münster, Germany. Disease severity and extent were determined according to the current classification of periodontitis.[13]

Sample Collection

In generalized forms of periodontitis, subgingival plaque samples were obtained with a sterile curet from the four deepest periodontal pockets, each representing the most severely affected site per quadrant. This sampling procedure was chosen according to the recommendation of the American Academy of Periodontology.[11] In localized forms, the plaque samples were drawn with sterile curets from all affected sites. The plaque samples were pooled, placed in 200 µl of sterile distilled water, and then placed in an ultrasonic cleaning bath† for 5 minutes at 37°C to disperse the plaque. In addition, the samples were vortexed and centrifuged at 12,000 × g for 1 minute to pellet the bacterial cells, and then kept at –70°C for further study. Total DNA was isolated with a blood mini kit‡ according to the manufacturer's instructions.

Identification of Periodontal Pathogens

Periodontal pathogen-specific primers amplifying a part of the *lktA* gene as described by Tonjum and Haas[14] were used for the identification of *A. actinomycetemcomitans*. Collagenase gene *prtC* was used for the identification of *P. gingivalis*. A 548 bp fragment from the central portion of the *prtC* gene was amplified using primers coll-1 (5′-ACA ATC CAC GAC ACC ATC-3′) and coll-2 (5′-GAT TCC CTT GCC TAC ATA-3′).[15] *T. forsythensis, E. corrodens, P. intermedia*, and *P. nigrescens* were identified with 16S rRNA specific primers designed by Slots et al.[4] and Ashimoto et al.[3]

Three microliters of the isolated DNA was added to the polymerase chain reaction (PCR) samples containing 30 pmol of each specific forward and reverse primer; 200 µM of each of the 4 dNTPs, 2.5 µl of polymerase synthesis buffer,‡ 1.5 mM $MgCl_2$, and 2.0 U of Taq-DNA polymerase were added for a final volume of 25 µl. As positive controls, isolated DNA from *A. actinomycetemcomitans* ATCC 33384, *P. gingivalis* ATCC 53977, *E. corrodens* BCMG 00232, *T. forsythensis* ATCC 43037, *P. intermedia* ATCC 25611, and *P. nigrescens* ATCC 33563 were used. Water was used as a negative control. Each sample was amplified by 35 cycles of 30 seconds at 95°C, 30 seconds at the primer-specific annealing temperature stated in the original papers[3,4,14,15] (for *A. actinomycetemcomitans*, 65°C; *P. gingivalis*, 53°C; *P. intermedia* and *P. nigrescens*, 55°C; *E. corrodens* and *T. forsythensis*, 60°C), and 60 seconds at 72°C. Eighteen microliters of the PCR product was subjected to agarose gel electrophoresis on 1.8% agarose gels. The gels were stained with ethidium bromide (1 µg/ml) and assessed under ultraviolet light. Precautions as described by Kwok and Higuchi[16] were taken to prevent contamination.

Determination of Antibiotic Efficacy

A review of the published studies concerning the MIC_{90} and GCF concentrations of the corresponding antibiotics was conducted. The following databases were searched: MEDLINE and Cochrane library. The search key words for determination of the gingival crevicular fluid concentrations in English were "antibiotic" to be analyzed, "gingival crevicular fluid," and "GCF," and for the MIC_{90} "minimal inhibitory concentration," "MIC," and "pathogen" to be analyzed. Additionally, references from the literature lists of the articles reviewed were included. All antibiotics for which the gingival crevicular fluid concentrations by oral application as well as the MIC_{90} of the tested periodontal pathogens were available were included in the study (GCF: references 17-21; MIC_{90}: references 1, 17, 22-29). From all articles selected, the GCF concentrations and MIC_{90} values were extracted and compared. To account for the variability and to ensure the greatest margin of safety for antimicrobial efficacy, the lowest GCF concentration and the highest MIC_{90} values for the assessed antibiotics and periodontal pathogens, respectively, were used. To minimize the effect on the overall microbiota, antibiotics with the narrowest spectrum covering the assessed pathogens were selected. The antibiotics were further classified with regard to their GCF concentrations reached by oral application that were at least 10-fold above the in vitro MIC_{90} values of the tested periodontal pathogens.

RESULTS

Demographics and Periodontal Condition

Seven hundred seventy-four (774) patients were enrolled in the study. Their demographics and periodontal conditions are summarized in Table 1. The majority of patients (588 subjects, 75.9%) were diagnosed with generalized moderate to severe chronic periodontitis (412 patients), or aggressive periodontitis (176 patients). One hundred thirty-nine (139) patients were diagnosed with moderate to severe

† Bransonic 1510E, Branson, Dietzenbach, Germany.
‡ gDNA, Eppendorf, Hamburg, Germany.

Table 1.

Demographics and Periodontal Conditions of Study Subjects

Study Population		
N		774
Females		270
Mean age ± SD		49.2 ± 12.2
Periodontal Conditions		
Chronic periodontitis	Localized	Generalized
Mild	1	10
Moderate	37	137
Severe	65	275
Aggressive periodontitis	Localized	Generalized
Mild	9	4
Moderate	9	25
Severe	28	151
Periodontal abscess	3	
Periodontal disease as a manifestation of systemic disease	4	
Others	16	

forms of localized chronic (102 patients) or aggressive (37 patients) periodontitis. Only 24 patients were diagnosed with mild chronic or aggressive periodontitis, and 23 patients with other forms of periodontitis.

Prevalence of Pathogen Complexes in Study Population

Forty-seven different pathogen complexes (PCs) were identified in the 774 patients (Fig. 1). Nine major PCs occurring in ≥3% of the study population were identified. These comprised *P. intermedia, P. nigrescens, P. gingivalis, E. corrodens*, and *T. forsythensis* (20.8% of all patients); *P. nigrescens, P. gingivalis, E. corrodens*, and *T. forsythensis* (12.5%); *P. nigrescens, E. corrodens*, and *T. forsythensis* (8.8%); *A. actinomycetemcomitans, P. intermedia, P. nigrescens, P. gingivalis, E. corrodens*, and *T. forsythensis* (8.1%); *P. intermedia, P. gingivalis, E. corrodens*, and *T. forsythensis* (6.5%); *P. gingivalis, E. corrodens*, and *T. forsythensis* (5.0%); *E. corrodens* and *T. forsythensis* (4.5%); *A. actinomycetemcomitans, P. nigrescens, P. gingivalis, E. corrodens*, and *T. forsythensis* (3.8%); and *P. intermedia, P. nigrescens, E. corrodens*, and *T. forsythensis* (3.4%). Thirty-eight minor PCs (each <3% of all patients) were distributed among 26.6% of all patients. In only 2.5% of the study population could none of the subgingival pathogens tested be identified.

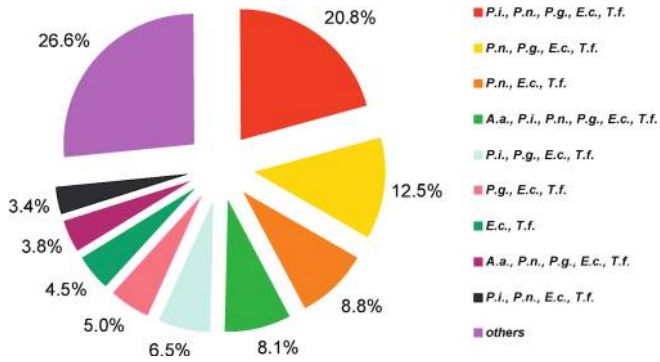

Figure 1.
Prevalence (%) of bacterial complexes (≥3%) in the study population.

MIC_{90} Values in Correlation to Gingival Crevicular Concentrations

Table 2 lists those antibiotics whose systemic administration exhibits a gingival crevicular concentration that is at least 10 times the in vitro MIC_{90} of the periodontal pathogens tested. Application of the described analytical model revealed that 55.0% of the patients would have been specifically treated with a combination of metronidazole and amoxicillin as an adjunctive antibiotic regimen, and 18.9% with a combination of metronidazole and amoxicillin/clavulanic acid or metronidazole and ciprofloxacin, respectively. The administration of tetracycline would have been specific in 0.3% of patients, ciprofloxacin in 0.4%, clindamycin in 1.5%, amoxicillin/clavulanic acid in 2.9%, metronidazole in 4.1%, doxycycline in 6.1%, and amoxicillin in 8.3%, respectively.

DISCUSSION

Specific associations among bacteria in dental biofilms and the presence of specific microbial groups within dental plaque have been demonstrated.[5,8] Furthermore, there is a stronger association of certain bacterial complexes with periodontitis.[5,8] These studies have contributed significantly to our understanding of the infectious etiology of periodontitis, i.e., the assembling of different bacteria, the interrelationship between putative pathogens and non-pathogenic bystanders, and their association with different forms of periodontitis.[5,6,8-10,30] Two findings common to these studies are that the subgingival ecosystem represents a vast heterogeneity of subgingival colonization patterns, and the main periodontal pathogens can be detected frequently in the different bacterial complexes, a finding that is corroborated in the present study, where 46 different complexes of the assessed pathogens were detected in the 774 patients analyzed.

It has been demonstrated repeatedly that adjunctive antibiotic therapy may enhance the effect of subgingival debridement in severe forms of periodontitis (for

Table 2.

Depiction of Most Specific Antibiotics in the 47 Bacterial Complexes Found

	A.a.	*T.f.*	*E.c.*	*P.g.*	*P.i.*	*P.n.*	Specific Tx (%)
Tetracycline	I	I		I			
A.a., P.g., T.f.	+	+		+			0.3
						Total	0.3
Ciprofloxacin	I		I				
A.a., E.c.	+		+				0.3
A.a.	+						0.1
						Total	0.4
Clindamycin		2				I	
P.n., T.f.		+				+	0.9
T.f.		+					0.6
						Total	1.5
Amoxicillin/clavulanic acid	I	I	I	3			
A.a., P.g., E.c., T.f.	+	+	+	+			2.2
A.a., E.c., T.f.	+	+	+				0.4
A.a., P.g., E.c.	+		+	+			0.3
						Total	2.9
Metronidazole		2		I	I	2	
P.i., P.n., P.g., T.f.		+		+	+	+	1.7
P.n., P.g., T.f.		+		+		+	1.6
P.n., P.g.				+		+	0.3
P.n.						+	0.1
P.i., T.f.		+			+		0.1
P.i., P.g., T.f.		+		+	+		0.1
P.i., P.n.					+	+	0.1
P.i., P.n., T.f.		+			+	+	0.1
						Total	4.1
Doxycycline		I	I				
E.c., T.f.		+	+				4.4
E.c.			+				1.7
						Total	6.1
Amoxicillin		I	I	2			
P.g., E.c., T.f.		+	+	+			5.0
P.g., T.f.		+		+			1.8
P.g.				+			1.2
P.g., E.c., T.f.			+	+			0.3
						Total	8.3

review, see reference 31). However, some clinical trials found no improvement in clinical results following adjunctive antibiotic treatment,[32-39] or even negative effects on disease progression.[40] In most of these randomized clinical studies, the clinical efficacy of one single adjunctive antibiotic regimen was tested versus a debridement alone control group. Since both the present and other studies have shown that periodontitis is a polymicrobial infection with highly heterogeneous colonization patterns, it may be assumed that the antibiotics selected in those studies failed in some cases to address the individual composition of the subgingival biofilm, resulting in an individually inadequate clinical therapy outcome.

Table 2. (continued)

Depiction of Most Specific Antibiotics in the 47 Bacterial Complexes Found

	A.a.	*T.f.*	*E.c.*	*P.g.*	*P.i.*	*P.n.*	Specific Tx (%)
Metronidazole & clprofloxacin or	1	2	1	1	1	2	
Metronidazole & amoxicillin/ clavulanic acid (GCF)	1	2	1	3	1	2	
A.a., P.i., P.n., P.g., E.c., T.f.	+	+	+	+	+	+	8.1
A.a., P.n., P.g., E.c., T.f.	+	+	+	+		+	3.7
A.a., P.i., P.g., E.c., T.f.	+	+	+	+	+		2.8
A.a., P.n., E.c., T.f.	+	+	+			+	2.1
A.a., P.i., P.n., E.c., T.f.	+	+	+		+	+	1.2
A.a., P.g., P.i., P.n., E.c.	+		+	+	+	+	0.3
A.a., P.g., P.i., P.n., T.f.	+	+		+	+	+	0.1
A.a., P.i., P.g., E.c.	+		+	+	+		0.1
A.a., P.i., P.g., T.f.	+	+		+	+		0.1
A.a., P.n., P.g., T.f.	+	+		+		+	0.1
A.a., P.i., E.c., T.f.	+	+	+		+		0.1
A.a., P.n., T.f.	+	+				+	0.1
A.a., P.n.	+					+	0.1
						Total	18.9
Metronidacole & amoxicillin		2	1	2	1	2	
P.i., P.n., P.g., E.c., T.f.		+	+	+	+	+	20.9
P.n., P.g., E.c., T.f.		+	+	+		+	12.4
P.n., E.c., T.f.		+	+			+	8.8
P.i., P.g., E.c., T.f.		+	+	+	+		6.5
P.i., P.n., E.c., T.f.		+	+		+	+	3.4
P.n., E.c.			+			+	0.9
P.i., E.c., T.f.		+	+		+		0.8
P.n., P.g., E.c.			+	+		+	0.5
P.i., P.n., E.c.			+		+	+	0.4
P.i., P.g., E.c.			+	+	+		0.3
P.i., P.n., P.g., E.c.			+	+	+	+	0.1
						Total	55.0
None							2.5

Specific Tx (%) = percentage of patients who would have been treated with the most specific antibiotic therapy.
1, 2, and 3 = antibiotic GCF concentrations are 10-, 100-, and 1,000-fold higher, respectively, than the MIC_{90} of the tested periodontal pathogen.
+ = antibiotic has a GCF concentration above the MIC_{90} of the tested periodontal pathogen.

However, the selection of a systemic antibiotic regimen according to the MIC values assessed in vitro does not necessarily imply clinical effectiveness, since biofilm-grown microorganisms exhibit a reduced antibiotic susceptibility compared to planktonic cultures of the same organism.[41,42] In this regard, the MICs were found to be 10-, 100-, and 1,000-fold higher in biofilm-grown bacteria than in planktonic-grown bacteria.[41] The difference in antibiotic susceptibility between planktonic and biofilm populations of the same organism may result from differences in the diffusion of antibiotics[43] or from much more complex changes in the microbial physiology of the biofilm.[42,44] Systemic antibiotic treatment in periodontal therapy is, however, an adjunct to mechanical therapy that disrupts the biofilm and thus makes the bacteria more susceptible to antibiotics by reducing the MIC values.[45] Therefore, the selection of antibiotics whose GCF concentrations were found to be at least 10 times higher than the in vitro MIC_{90} of the analyzed bacteria should have the potential to generate in vivo antibiotic efficacy. However, it needs to be noted that the antibiotic susceptibility of periodontal pathogens might vary in different populations.[46] The present study focused on pathogens most strongly associated with periodontitis. There are, however, numerous other microorganisms in the oral cavity that were not taken into account in this study. About 500

bacterial species have been detected in the oral cavity so far.[47] In addition, yeasts[48] and viruses[49,50] have been found in subjects with periodontitis. Therefore, the information presented in our study is limited to the assessed putative periodontal pathogens.

Besides microbiological factors, pharmacokinetic and pharmacodynamic properties of an antibacterial agent can also influence antibiotic effectiveness in vivo.[51-53] In this regard, it has been shown that the additive synergistic effects between metronidazole and its hydroxymetabolite might increase the in vivo antibiotic effectiveness of this drug.[54,55] Moreover, it has been found that the GCF concentration of an antibiotic exhibits high interindividual variations,[20,56] resulting in individual GCF concentrations below antibiotic effects. Therefore, it needs to be stressed that the findings of the present study represent a proposed strategy for the selection of specific antibiotic regimens. The efficacy of the proposed antibiotic regimens for the various pathogen complexes needs to be determined clinically.

The results of the present study indicate that periodontal pathogens occur in a great variety of complexes in patients with periodontitis. With regard to the known MIC value required and the GCF concentrations achieved by systemic administration, different antibiotic regimens may be required to specifically target the periodontal pathogens present in individuals with periodontitis. The presented antibiotic regimens for the bacterial complexes found in patients with periodontitis represent a proposal for a specific antibiotic treatment regimen that needs to be validated in clinical trials.

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Correspondence: Thomas Beikler, Dr. med. Dr. med. dent, Department of Periodontology, Waldeyerstrasse 30, 48149 Münster, Germany. Fax: 49-251-83-47134; e-mail: beikler@uni-muenster.de.

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