Summary and conclusions

Summary

In this thesis a number of clinical trials have been described in which the additional effects of systemic antimicrobial therapies were investigated in adult patients with periodontitis. The effects of therapeutic regimes with metronidazole and amoxicillin and the combination of both drugs were clinically and microbiologically further substantiated. Attempts were made to link the clinical outcome of the periodontal therapy changes in the subgingival microflora.

Effects of metronidazole in patients with "refractory" periodontitis associated with Bacteroides forsythus

Subjects refractory to initial periodontal therapy are prime candidates for continued periodontal treatment in conjunction with systemic antimicrobial therapy. In Chapter 2 the microbiological and clinical effects of renewed supra- and subgingival debridement followed by systemic metronidazole (500 mg TID for 7 days) were monitored in 27 adult refractory periodontitis patients, culture positive for Bacteroides forsythus. Patients culture positive for Actinobacillus actinomyctemcomitans were excluded from this study since the combination of systemic amoxicillin and metronidazole is advocated in this patient category. Clinical evaluation included assessment of plaque index (PI), bleeding index (BI), probing pocket depth (PPD) and clinical attachment level (CAL) at the deepest, bleeding site in each quadrant. Microbiological evaluation was carried out by anaerobic cultivation of subgingival plaque samples from the same sites. Six months after renewed debridement and systemic metronidazole (RD+M) a mean reduction of the bleeding index of 0.9, in the probing pocket depth of 1.5 mm and a mean gain in clinical attachment level of 1.0 mm was observed. There was no change in the plaque index shown. After RD+M, B. forsythus was suppressed below detection level in 17 of the 27 patients, Porphyromonas gingivalis in 9 out of 15 patients and Prevotella intermedia in 14 of the 21 patients. Before RD+M, 12 patients harboured simultaneously B. forsythus, P. gingivalis as well as P. intermedia. Six of these subjects were culture negative for the 3 species after therapy and showed the greatest reduction in probing pocket depth (3.1 mm) and gain of clinical attachment (2.5 mm). It was concluded that, in the treatment of refractory periodontitis, patients culture positive for B. forsythus and negative for A. actinomyctemcomitans, renewed debridement and subsequent systemic metronidazole can significantly improve the microbiological and clinical parameters.
A number of authors have used amoxicillin and clavulanic acid (Augmentin®) or amoxicillin and metronidazole in refractory periodontitis patients. Since it is extremely difficult to select refractory periodontitis patients to perform double-blind placebo-controlled studies, adult periodontitis patients were used as a model to study the additional effect of the most commonly used antibiotics, *i.e.* amoxicillin plus clavulanic acid and the combination of amoxicillin and metronidazole.

**$\beta$-Lactamase producing bacteria in adult periodontitis**

The rational to use systemic amoxicillin and clavulanic acid in the treatment of periodontitis is the presence of $\beta$-lactamase producing bacteria in the deepened periodontal pocket. Therefore, in Chapter 3, the occurrence of $\beta$-lactamase producing periodontal bacteria was determined in 23 untreated adult periodontitis patients. In addition to non-selective isolation media, selective isolation and growth of $\beta$-lactamase positive subgingival bacterial species was carried out on blood agar plates supplemented with amoxicillin and plates with amoxicillin + clavulanic acid. Isolates from the amoxicillin plates that were absent on the amoxicillin/clavulanic acid plates were identified and tested for $\beta$-lactamase production. *P. gingivalis, P. intermedia, A. actinomycetemcomitans, Peptostreptococcus micros, Fusobacterium nucleatum, B. forsythus* and *Campylobacter rectus* isolates from the selective and non-selective medium were tested for $\beta$-lactamase activity by a nitrocefin disk method (DrySlide®) and by a laboratory chromogenic nitrocefin-based test. Based on the non-selective plates, 6 of 23 *P. intermedia* isolates, 2 of 19 *B. forsythus* isolates and 3 of 23 *F. nucleatum* isolates were $\beta$-lactamase positive. Moreover, from the selective amoxicillin plates, the $\beta$-lactamase positive species *Prevotella loescheii, Prevotella buccae, Prevotella buccalis* and *Actinomyces* spp were recovered. $\beta$-Lactamase positive subgingival species were recovered from 17 of 23 patients (74%) but usually comprised low proportions of the subgingival microflora (range < 0.01-15%). Comparison of the DrySlide® test and the nitrocefin-based laboratory test revealed full agreement of test results. The majority of $\beta$-lactamase positive strains were found among species of the *Prevotella* genus. In addition to culture isolates, whole subgingival plaque samples were tested using the rapid chromogenic nitrocefin method. The results showed that in 12 patients of the 23 patients samples (52%) $\beta$-lactamase activity was found. There was a lack of correlation between the isolation of $\beta$-lactamase positive species on the selective- and non-selective plates and the outcome of the whole plaque sample. It was concluded that $\beta$-lactamase activity in subgingival bacteria in adult periodontitis is a common feature.
Clinical and microbiological effects of initial periodontal therapy in conjunction with amoxicillin and clavulanic acid in patients with adult periodontitis

The use of amoxicillin protected by clavulanic acid against $\beta$-lactamase activity in the treatment of adult periodontitis was studied in Chapter 4. In a randomised double-blind, placebo-controlled study, the clinical and microbiological effects of initial periodontal therapy followed by amoxicillin and clavulanic acid were investigated. Twenty-one patients with a clinical diagnosis of generalized adult periodontitis were recruited. Clinical measurements and microbiological assessments were carried out at baseline, 3, and 12 months post-treatment. Approximately 6 weeks after initial periodontal treatment (3-6 h), patients were randomly assigned to receive coded study medication of 500 mg amoxicillin plus 125 mg clavulanic acid or placebo, every 8 h for 10 days. Patients returned for follow-up visits 3, 6, 9, and 12 months after completion of the medication. The mean plaque index (PI) at baseline was 1.1 for the placebo group and 0.9 for the test group. At 3 months, the PI had dropped to 0.3 in both groups, and was maintained during the rest of the study. The mean changes in bleeding index and gingival index in the course of the study were similar in both groups. A mean reduction of probing pocket depth of 1.0 mm in the placebo group and 0.9 mm in the test group was observed during the first 3 months. No further reduction in PPD was noticed during the study period in either group. There was no statistically significant difference in the PPD reduction between the 2 groups. The mean change in clinical attachment level (CAL) from baseline to 3 months were similar in both groups (0.5 mm). Between 3 and 12 months, the CAL changed in neither group. In both groups, treatment resulted in a decrease in the number of spirochetes and motile rods positive patients, but no significant differences between both groups were noted in any of the dark field microscopy observations. Samples were tested for the presence of *A. actinomycetemcomitans*, *P. gingivalis*, *B. forsythus*, *P. intermedia*, *P. micros* and *F. nucleatum*. Analysis of the microbial data revealed no differences between the 2 groups. In conclusion, the findings demonstrated that, in comparison to placebo, systemic amoxicillin plus clavulanic acid provided no additional clinical and microbiological effects in the treatment of adult periodontitis patients.
Amoxicillin plus metronidazole in the treatment of adult periodontitis patients

Since the subgingival microflora in adult periodontitis consist of various putative pathogens that may differ in antimicrobial susceptibility, several investigators have advocated a combination of 2 antibiotics. Metronidazole and amoxicillin have been used successfully in the treatment of advanced periodontitis. Chapter 5 reports on a double-blind, placebo-controlled study in which the adjunctive effects of systemic amoxicillin and metronidazole were investigated in a group of adult periodontitis patients. Forty-nine patients with a diagnosis of generalised severe periodontitis participated in the study. Random assignment resulted in 26 patients in the placebo (P) group and 23 patients in the test (T) group. Clinical measurements and microbiological assessments were performed at baseline and 3 months after completion of the initial periodontal therapy with additional placebo or antibiotic treatment. Patients received either 375 mg amoxicillin in combination with 250 mg metronidazole or identical placebo tablets, every 8 hours for 7 days. Except for the plaque index, there was a significantly larger change of the bleeding index, probing pocket depth (PPD) and clinical attachment level (CAL) in the T-group as compared to the P-group after therapy. The greatest reduction in PPD was found in sites with initial PPD of ≥ 7 mm, 2.5 mm in the P-group and 3.2 mm in the T-group. Also the improvement in CAL was most pronounced in the PPD category ≥ 7 mm and amounted to 1.5 mm and 2.0 mm in the P- and T-groups, respectively. After therapy there was a significant difference between the P- and the T-group in the remaining number of patients positive for \( P. \) gingivalis, \( B. \) forsythus and \( P. \) micros. Four subgroups were created on the basis of the initial microbiological status with regard to \( P. \) gingivalis, i.e. \( P. \) gingivalis positive (Pg-pos) and negative patients (Pg-neg) in the P- and the T-groups.

The difference in reduction of PPD between Pg-pos and Pg-neg patients was particularly evident with respect to the changes in % of sites with a probing pocket depth ≥ 5 mm. This % reduced from 45% at baseline to 23% after treatment in the Pg-pos placebo subgroup and decreased from 46% to 11% in to the Pg-pos test subgroup. In contrast, the changes in % of sites with a probing pocket depth ≥ 5 mm in the Pg-neg placebo and Pg-neg test subgroup were similar. In conclusion, in this investigation in which adult periodontitis was used as a study model, additional systemic metronidazole and amoxicillin resulted in a better clinical and microbiological outcome than initial periodontal treatment only. No difference in reduction of PPD was observed between the P- and T-group in patients that where culture negative for \( P. \) gingivalis at baseline. Moreover, the outcome of the study suggests that patients diagnosed with \( P. \) gingivalis at baseline benefited most from this antibiotic treatment.
Additional clinical and microbiological effects of amoxicillin and metronidazole after initial periodontal therapy in patients positive for *A. actinomyctemcomitans*

In literature the combination of metronidazole and amoxicillin is especially advocated in patients positive for *A. actinomyctemcomitans*. In the previous study, only 9 out of 49 patients were culture positive for *A. actinomyctemcomitans* at baseline. Therefore, the aim of the study in Chapter 6 was to evaluate the clinical and microbiological effects of initial periodontal therapy (IT) and additional use of systemic amoxicillin and metronidazole (AM), in patients culture positive for *A. actinomyctemcomitans*. A total of 22 patients were enrolled in the study. The deepest, bleeding pocket in each quadrant was selected and at these 4 experimental sites clinical measurements and microbiological testing was carried out at baseline, after IT, *i.e.*, 21 weeks after baseline, and after AM, *i.e.*, 35 weeks after baseline. AM was given directly after IT. The mean plaque index (PI) amounted to 0.5 at baseline, 0.1 after IT and 0.3 after systemic AM. The mean bleeding index decreased from 1.6 to 1.2 after IT and to 0.7 after AM. The mean change of probing pocket depth (PPD) after IT amounted to 1.4 mm and was further reduced with an additional mean change of 1.1 mm after medication. Clinical attachment gain was 1.1 mm after IT and an additional 0.9 mm was observed after AM. One patient became negative for *A. actinomyctemcomitans* and 4 for *P. gingivalis* after IT. After AM, in comparison to baseline, suppression below detection level for *A. actinomyctemcomitans* was achieved in 19 out of 22, for *P. gingivalis* in 9 out of 17. On the basis of the microbiological results the study group was separated into 2 subgroups: group A consisted of subjects who had no detectable levels of *A. actinomyctemcomitans*, *P. gingivalis*, *B. forsythus* and <5% of *P. intermedia* after AM. Group B consisted of those who still showed presence of one of these 3 species and/or >5% levels of *P. intermedia*. After AM, group B showed significantly higher PI, BI, deeper PPD and less attachment gain than group A.

It was concluded that after IT there was a reduction of the plaque index, bleeding index and probing pocket depth and a gain of clinical attachment level. After the amoxicillin and metronidazole treatment, an additional clinical improvement was shown for all clinical parameters, except for the plaque index. Initial periodontal therapy did not result in a decrease of prevalence of *A. actinomyctemcomitans*, *P. gingivalis* and *B. forsythus*. However, additional use of amoxicillin and metronidazole resulted in a significant reduction of the prevalence of *A. actinomyctemcomitans*, *P. gingivalis* and *B. forsythus*. It should be noted that not each patient benefited from additional systemic antimicrobial therapy. Patients who benefited most were subjects without detectable *A. actinomyctemcomi-
tans, P. gingivalis, B. forsythus and <5% of P. intermedia after AM. They fulfilled an important aim of treatment in periodontics, i.e. low plaque and bleeding indices and shallow pockets.

Conclusions

The studies described in this thesis were designed to answer some critical questions with respect to systemic antibiotic use in the treatment of severe periodontitis in adult patients and should therefore not be interpreted as treatment protocols that are directly applicable for clinical practice. For example, patients were not treated surgically although the clinical indication for this treatment was indicated in a number of study patients. In two studies (Chapters 4 and 5), patients were treated with systemic antibiotics without clinical nor microbiological selection, a procedure that is not recommended in clinical practice. Also, no additional local antimicrobial treatment, e.g. chlorhexidine, during systemic antibiotic therapy was used. In the study described in Chapter 6 no renewed debridement prior to antibiotic therapy was applied.

One important finding was the observation that amoxicillin and clavulanic acid displays a large activity in vitro against the majority of periodontal bacteria but showed no additional clinical and microbiological effects in vivo (Chapters 3 and 4). Also, it has been shown that not all patients equally benefit from an adjunct systemic antimicrobial therapy (Chapters 5 and 6). One determining factor for an antibiotic to be effective seems to be the composition of the subgingival microflora (Chapters 2, 5 and 6). For instance, the number and levels of susceptible pathogens at baseline was related to the extent of improvement of metronidazole therapy in refractory adult periodontitis (Chapter 2). Also, patients with detectable subgingival P. gingivalis appeared to benefit more than patients without this microorganism from systemic metronidazole and amoxicillin (Chapter 5). One possibly important clinical finding in this study was the observation that patients with subgingival P. gingivalis at baseline that were treated with antibiotics showed 50% less ≥ 5 mm pockets after therapy in comparison to placebo-treated P. gingivalis patients. This implies that, in patients culture positive for P. gingivalis, the antibiotic therapy had significantly reduced the number of teeth in need for surgical treatment (Chapter 5). In Chapter 6, it is confirmed that scaling and root planing alone is not able to significantly decrease the number of patients with detectable A. actinomycetemcomitans, B. forsythus and P. gingivalis. The data from the present thesis show that the adjunctive use of antibiotics in the treatment of adult patients with periodontitis enhances the results that can be obtained by supra- and subgingival debridement. It also shows that the composition of the subgingival microflora is a factor that is associated with the
clinical outcome of treatment. These results suggest that microbiological testing of periodontitis patients prior to prescribing antibiotics is warranted. It may also indicate a more rational use of systemic antibiotics in periodontics, which is important to minimize the emerge of resistant micro-organisms.

**Future research objectives**

In the studies presented in this thesis a limited number of periodontal pathogens were detected and monitored. The selection of these bacterial species was largely based on the consensus report of the World Workshop in Periodontics 1996. However, a significant part of the subgingival microflora in periodontitis is unknown. Therefore, future research could focus on other periodontal pathogens more difficult to culture. Knowledge of these pathogens may further improve the selective use of certain antibiotics. Another interesting area for future research is the sequencing of systemic antimicrobial therapies in periodontics. Antibiotics are used on different time points in periodontal therapy and the most optimal protocol has still to be determined. The same holds true for regimes and dosing of antibiotics in periodontics. Also the selection criteria on the basis of which patients would be treated with either a single antibiotic therapy or a combined antibiotic therapy are largely unknown. New information on these future research objectives may probably contribute to a more predictable treatment outcome.