CHAPTER 1

GENERAL INTRODUCTION
General introduction

Of the many thousand known bacterial species only a limited number has been associated with human diseases. A great deal of the known human bacterial pathogens occurs in healthy subjects, whereas others are not indigenous to the human microflora. These exogenous pathogens are often strict pathogens i.e. colonisation by these organisms results in pathological processes in most individuals. Bacterial diseases can occur at body sites that are normally sterile or at sites that are colonised by a complex microbial flora. The skin, the vagina, the digestive tract including the oral cavity and the upper respiratory tract are examples of mucosal surfaces that harbour large numbers of micro-organisms including viruses, bacteria, fungi and protozoa. Some bacterial diseases such as tuberculosis are caused by a specific organism while many other infections are associated with a number of different pathogens. Mucosal infections are often associated with multiple pathogens and may be considered mixed infections. Mixed infections can be caused by the resident microflora of the host or by invasion of exogenous pathogens. In medicine systemic antimicrobial agents are used to support the human defense system in order to eliminate bacteria from invaded sterile body sites, or to assist in restoring the normal microflora by either suppressing endogenous pathogens or by eliminating exogenous bacteria.

The oral cavity

One unique feature of the oral cavity is the presence of teeth that represent hard, non-shading surfaces that allow for the colonizing of specific bacterial species which develop into a unique microbial flora: the dental plaque. Periodontal infections are bacterial diseases that affect the soft and hard tissues surrounding the tooth. In gingivitis the infection is limited to the gingiva while in periodontitis the attachment apparatus of the teeth is also involved. In both diseases the bacterial plaque is responsible for the infection. All humans will develop gingivitis when a critical bacterial mass is formed that can no longer be controlled by the host defense system. A substantial proportion of the human population is inclined to develop periodontitis. Periodontitis is a disease that represents a major risk factor for tooth loss and it has been suggested that this chronic infection is linked to cardiovascular diseases and pre-term low birthweight (Beck & Slade 1996; Offenbacher 1996; Offenbacher et al. 1996). Periodontal lesions may be a focus to cause non-oral infections in both immuno-competent and immuno-compromised individuals (Van Winkelhoff & Slots 1999). Periodontal infection control is not only essential to maintain a functioning dentition; it may also contribute to general health.
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Genetic and environmental factors

Each individual acquires his or her own unique periodontal-, skin- and intestinal microflora (Holdeman et al. 1976; Moore & Moore 1994). It is not clear whether the inter-individual differences in the composition of the microflora are controlled primarily by genetic or by environmental factors. A genetic component was indicated by the fact that the periodontal microflora in identical twins were more similar than in fraternal twins (Moore et al. 1993). Genetic factors may also explain the observation that the mere presence of certain periodontal pathogens does not inevitably lead to development of periodontitis. This is in agreement with the supposition that the majority of periodontal bacteria are commensal oral organisms, although conflicting data exists on this matter. For example, in the study of Haffajee et al. (1998) the number of *A. actinomycetemcomitans*-positive sites was similar in healthy subjects and periodontitis patients. In addition, *A. actinomycetemcomitans* can be isolated from periodontally stable individuals (Müller et al. 1997). In contrast, Ashimoto et al. (1996) found >2 times higher prevalence of *A. actinomycetemcomitans* in periodontitis than in adult gingivitis. A longitudinal study in an Indonesian population revealed that the presence of *A. actinomycetemcomitans* was a risk marker for periodontal breakdown (Timmerman et al. 2000).

Differences in pathogenicity (AvNA) of individual bacterial strains (Laine & Van Winkelhoff 1998; Mombelli et al. 1998; Van Steenbergen et al. 1987; Van Winkelhoff et al. 1993) or prevalence of certain periodontal pathogens may also be a determining factor for the disease to develop. Epidemiological studies have shown that populations with a high prevalence of *A. actinomycetemcomitans* have a high prevalence of localized juvenile periodontitis (Papapanou 1996; Slots & Rams 1992). For instance, black people from Panama exhibit a relatively high occurrence of localized juvenile periodontitis (7.7%) as well as a high prevalence of oral *A. actinomycetemcomitans* (60%)(Eisenmann et al. 1983). Although the bacterial etiology for periodontitis is evident, it does not explain all the variability shown in several epidemiological studies. Periodontitis is a multi-factorial disease in which the periodontal microflora plays an essential role but seems not enough to explain the onset and progression of the disease. The host’s susceptibility is also a determining factor.

Periodontal therapy

Treatment of periodontitis aims at controlling the periodontal infection, arresting further attachment loss, survival of the maximum number of functioning teeth and at ensuring an acceptable aesthetic result. Supra- and subgingival debridement
to remove plaque and calculus, instruction in effective oral hygiene measures and periodontal surgery are essential therapeutic approaches in periodontal treatment. The treatment of periodontitis can arrest further periodontal attachment loss in most individuals. However, not all patients respond equally well to these treatment protocols and approximately 15% of the patients may suffer from refractory periodontitis (Haffajee et al. 1988). In addition, maintenance patients may experience recurrent disease activity despite regular recall. In these cases, repeated professional tooth cleaning and reinforcement of the oral home-care regime are instituted. Since bacteria are considered a primary cause of periodontal infections, antibiotic therapy may assist in controlling the subgingival microflora in patients with periodontitis. Potent antimicrobial agents such as metronidazole and tetracycline have been tested for their ability to enforce the conventional periodontal treatment (Helldén et al. 1979; Lindhe et al. 1983; Listgarten et al. 1978). Although additional clinical and microbiological effects were established, the differences between root planing and plaque control alone and mechanical treatment in conjunction with these antibiotics were considered not clinically significant (Lindhe et al. 1983). In most early studies on the effects of systemic periodontal antibiotic therapy, it was assumed that adult patients with periodontitis had a similar subgingival plaque composition. More recent investigations have shown that this view is not correct and significant differences in prevalence and levels of putative periodontal pathogens have been documented (Rodenburg et al. 1990; Slots & Listgarten 1988; Zambon 1996). Of the many different bacterial species that can occur in the deepened pockets, only a limited number have been associated with progressive periodontitis (American Academy of Periodontology 1996). These periodontal pathogens seem to occur in clusters rather than as single pathogens (Socransky et al. 1988).

Microbiological and clinical monitoring of treated patients with periodontitis have revealed that persistence of certain microbial species is associated with minimal treatment response or recurrence of progressive disease. Slots and Rosling (1983) observed an association between renewed periodontal attachment loss and detectable *A. actinomycetemcomitans* in patients with localised juvenile periodontitis. Later, Christersson et al. (1985) and Kornman and Robertson (1985) showed that optimal treatment outcome in localised juvenile periodontitis was related to elimination of *A. actinomycetemcomitans*. Similar observations were later reported in adult periodontitis in which a limited number of bacterial species were considered key pathogens (Bragd et al. 1987; Renvert et al. 1990; Takamatsu et al. 1999). These observations have led to the conclusion that one treatment goal in periodontics is suppression of certain bacterial species.
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Treatment implications

Most cases of periodontitis do not require systemic antimicrobial therapy to arrest disease progression (Axelsson & Lindhe 1978; Axelsson & Lindhe 1981; Badersten et al. 1981; Badersten et al. 1987; Claffey et al. 1988) with the possible exception of immuno-compromised patients (Murray 1994; Yalda et al. 1994). It has been documented that periodontal infections associated with A. actinomycetemcomitans, Porphyromonas gingivalis and possibly Bacteroides forsythus require significant suppression, and if possible, complete subgingival elimination in order to arrest periodontal breakdown in many patients (Socransky et al. 1999; Takamatsu et al. 1999; Van Winkelhoff et al. 1996; Wennström et al. 1987). The concept of specific microbial components in periodontitis is the basis for the selective use of systemic antibiotic therapies in the treatment of severe periodontitis (Van Winkelhoff & Winkel 1997) and it is the rationale for the studies described in this thesis.

Systemic antibiotic therapies

Antibiotics that have been tested in patients with periodontitis include tetracyclines, metronidazole, ornidazole, amoxicillin, erythromycin, spiramycin, clindamycin, cefixim and ciprofloxacin (Van Winkelhoff et al. 1996). In a number of controlled clinical trials, with a.o. tetracyclines and metronidazole, only small mean differences were noted in the change of probing pocket depth and clinical attachment levels between test and control patients. However, relatively large differences in therapy response were noted between individual patients (Helldén et al. 1979; Lindhe et al. 1983; Listgarten et al. 1978). These observations suggested no significant role of these antibiotics in the treatment of periodontitis in adult patients. However, patients in these studies were not selected on the basis of susceptibility profiles of the subgingival microflora. Moreover, patients were not selected on the basis of documented disease activity i.e. the disease may have been in quiescence in a number of patients. An alternative approach to test the effects of systemic antibiotics is to pre-select patients on the basis of microbiological composition of the subgingival microflora and/or known disease activity as was done in some of the studies of this thesis.

Based on previous studies (Goené et al. 1990; Pavičić et al. 1994; Van Winkelhoff et al. 1989; Van Winkelhoff et al. 1992), in this thesis it has been tried to further substantiate the clinical and microbiological effects of metronidazole and amoxicillin as single therapeutic regimes as well as the combination of both drugs.
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Metronidazole

Metronidazole is a member of the nitro-imidazole group of antibiotics. It is an antimicrobial agent with a bactericidal activity against a wide range of anaerobic bacterial species, a feature that makes this drug particularly suitable for the treatment of acute and chronic periodontal infections. Moreover, development of resistance to metronidazole is relatively rare (Dubreuil et al. 1989; Tally et al. 1984; Van Winkelhoff et al. 1999).

Metronidazole has been tested in conjunction with scaling and root planing in different adult patient populations including mentally retarded patients (Clark et al. 1983), maintenance patients with recurrent periodontitis (Gusberti et al. 1988) and as an adjunct to periodontal surgery (Mahmood & Dolby 1987). In a series of studies, Loesche et al. (1984, 1991, 1992) have described the clinical and microbiological effects of adjunctive metronidazole in adult periodontitis patients. One salient finding was a significant reduction in the number of teeth in need of periodontal surgery in the metronidazole group in comparison to placebo treated patients. Elter et al. (1997) performed a meta-analysis of the effect of systemic metronidazole as an adjunct to scaling and root planing in the treatment of adult periodontitis. They concluded that adjunctive metronidazole may offer a benefit over scaling and root planing alone, in pockets with a probing depth of 4 mm or greater.

In the study of Gusberti et al. (1988) only 5 maintenance patients with recurrent disease activity were involved but the data suggested that metronidazole clinically improved the periodontal condition for at least 9 months. Mombelli and co-workers (1989) evaluated another member of the nitro-imidazole group, Omidazole®. It was used as an adjunct to root planing in the therapy of 10 patients suffering from recurrent periodontal disease. Eleven months after therapy, a significant decrease of pocket probing depth, no further clinical attachment loss and a decrease of the bleeding index was shown. The above-mentioned studies include only patients with either adult periodontitis or adult patients with recurrent disease activity. As far as we know, no study has been performed on the effects of metronidazole in patients refractory to initial periodontal therapy.

Amoxicillin

Amoxicillin, a β-lactam penicillin, has a broad antimicrobial spectrum that includes both strict anaerobes and facultative anaerobic bacterial species. In vitro, whole subgingival plaque samples from periodontitis patients are extremely sensitive to amoxicillin (Walker et al. 1983). Amoxicillin is susceptible to bacterial enzymes capable of degrading β-lactam antibiotics and β-lactamase producing bacteria...
occur in deepened periodontal pockets in adult periodontitis patients (Walker et al. 1987). Clavulanic acid, a compound with a similar chemical structure as amoxicillin, is capable of inhibiting the degrading activity of the bacterial enzymes, thereby protecting the amoxicillin against inactivation. Several authors have suggested that this combination of drugs is effective in periodontal infection control, especially in refractory adult periodontitis (Haffajee et al. 1996; Magnusson et al. 1994). However, this antimicrobial drug has not been tested in untreated adult periodontitis in a double-blind placebo-controlled study.

**Amoxicillin and metronidazole**

Since the subgingival microflora in periodontitis consists of multiple periodontal pathogens with different antimicrobial susceptibility profiles, the use of more than one antibiotic may be a more predictable approach in periodontal therapy. Combination antibiotic therapy can broaden the antimicrobial range of the antibiotic therapy and thus prevent the emergence of bacterial resistance by using antibiotics with overlapping antimicrobial spectra. Moreover, one can lower the dose of individual antibiotics by exploiting synergy between selected drugs against specific target organisms (Pavičić et al. 1991; Pavičić et al. 1994). Metronidazole plus amoxicillin has been used successfully in the treatment of advanced periodontitis, especially in *A. actinomycetemcomitans*-associated periodontal infections (Goené et al. 1990; Pavičić et al. 1994; Van Winkelhoff et al. 1989; Van Winkelhoff et al. 1992).

**Aim of this thesis**

The purpose of this thesis was to further investigate, in adult periodontitis patients, the microbiological and clinical effects of 1) metronidazole in patients refractory to initial periodontal therapy culture positive for *Bacteroides forsythus*, 2) initial periodontal therapy followed by amoxicillin and clavulanic acid, 3) initial periodontal therapy followed by amoxicillin and metronidazole, 4) initial periodontal therapy and the additional effects of amoxicillin plus metronidazole in patients culture positive for *Actinobacillus actinomycetemcomitans*. In addition, the occurrence of β-lactamase producing periodontal bacteria was determined.
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