On antimicrobial approaches to arrest and control chronic periodontitis

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UNIVERSITY OF GOTHENBURG

2009

Abstract
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The main objectives were to study the effect of frequently repeated supragingival plaque removal on the subgingival microbiota and periodontal pocket reduction and to analyze the adjunctive effect of different antimicrobial agents on the treatment of periodontal disease. Subjects with gingivitis or chronic periodontitis were included.

The patients in Study I underwent professional supragingival removal of plaque and calculus 2-3 times/week for 30 weeks. At sites with suprabony and infrabony pockets and furcation sites, repeated supragingival plaque removal reduced the total number of microorganisms, as well as the percentage of sites with *P. gingivalis*.

In Study II, six months of unsupervised use of a dentifrice containing 0.3% magnolia extract produced significantly less gingival inflammation than a corresponding control dentifrice. Furthermore, at sites with similar amounts of plaque, fewer signs of gingival inflammation were observed in the magnolia group than the control group.

In Study III, the effect of topically applied PVP iodine used as an adjunct both during basic SRP and at re-treatment during long term-maintenance was studied. PVP iodine, applied topically during subgingival instrumentation, may improve the outcome of SRP therapy.

In Study IV, the short- and long-term effects of the systemic administration of tetracycline in conjunction with SRP were studied. One year after active therapy, the probing attachment level in the test group was almost 3 times higher than in the control group. Re-examinations after 3, 5 and 13 years of SPT disclosed that this short-term benefit was not maintained in the longer perspective.

In Study V, the effects of topically applied minocycline in combination with surgery on PPD reduction were analyzed. Local minocycline as an adjunct to surgery produced a statistically significantly larger reduction in probing depth (0.3mm) compared with surgery alone.

Key words: Plaque control, microorganisms, magnolia, dentifrice, gingivitis, PVP iodine, maintenance, tetracycline, minocycline

ISBN 978-91-628-7737-8

Contents

Preface

The present thesis is based on the following studies, which will be referred to in the text by their Roman numerals

I. Hellström, M-K., Ramberg, P., Krok, L. & Lindhe, J. (1996). The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **23,** 934-940.

II. Hellström, M-K., Xu, T.,Volpe, A. & Ramberg, P. (2009). The effect of a dentifrice containing Magnolia extract on established plaque and gingivitis in man: A six month clinical study. *Manuscript*.

III. Rosling, B., Hellström, M-K., Ramberg, P., Socransky S. S. & Lindhe, J. (2001). The use of PVP-iodine as an adjunct to non-surgical treatment of chronic periodontitis. *Journal of Clinical Periodontology* **28,** 1023-1031.

IV. Ramberg, P., Rosling, B., Serino, G., Hellström, M-K., Socransky, S. S. & Lindhe, J. (2001). The long term effect of systemic tetracycline used as an adjunct to non-surgical treatment of advanced periodontitis. *Journal of Clinical Periodontology* **28,** 446-452.

V. Hellström, M-K., McClain, P. K., Schallhorn, R. G., Bellis, L., Hanlon, A. L. & Ramberg, P. (2008). Local minocycline as an adjunct to surgical therapy in moderate to severe, chronic periodontitis. *Journal of Clinical Periodontology* **35,** 525-531.

Abbreviations

BL = baseline

BoP = bleeding on probing

CAL = clinical attachment level

CHX = chlorhexidine

GI = gingival index

NS = non significant

OHI = oral hygiene instructions

PAL = probing attachment level

PI = plaque index

PPD = probing pocket depth

QHI = Quigley and Hein Index (dental plaque)

SD = standard deviation

SPC = supportive periodontal care

SPT = supportive periodontal therapy

SRP = scaling and root planing

Introduction

Gingivitis and periodontitis

Most periodontal diseases are *plaque-associated* inflammatory disorders. Gingivitis and chronic periodontitis are the most commonly occurring forms of periodontal disease (Caton et al. 1999, Lindhe et al. 1999). It is well documented that chronic periodontitis starts as an inflammatory lesion in the gingiva which, if left untreated over time, may progress to involve and cause damage and the breakdown of smaller or larger parts of the periodontal attachment apparatus (e.g. Saxe et al. 1967, Lindhe et al. 1973, 1975, Page & Schroeder 1976). Findings from epidemiological studies indicate that the prevalence of subjects with gingivitis is already high among children and young adults, that the number of subjects with chronic periodontitis (i) increases with age and that (ii) > 50% of the population above the age of 50 years exhibit signs of periodontal tissue breakdown in one or more parts of the dentition (Scherp 1964, Hugoson & Jordan 1982, Okamoto et al. 1988).

Gingivitis unequivocally develops in subjects with healthy gingiva who, during a 2-3 week period, refrain from tooth-cleaning (Löe et al. 1965) and thereby allow microorganisms to colonize the supragingival part of the tooth (Theilade et al. 1966). It is also well known that products released from this plaque or biofilm initiate a host defense (inflammation – gingivitis) that (i) effectively prevents bacteria from invading the host but also (ii) induces a series of local tissue alterations including the establishment of inflammatory cell infiltrates in the marginal gingiva ("initial" and "early lesions"; Page & Schroeder 1976). Concomitantly, parts of the junctional epithelium are lost and replaced with a "non-attached" pocket epithelium. A niche – a pocket – is hereby established between the tooth and the marginal soft tissue. The local environment is changed and a biofilm with a different bacterial composition may become established in this newly formed subgingival pocket compartment (Socransky et al. 1998, Haffajee & Socransky 2005, Socransky & Haffajee 2005). In this shift,

the microbial population in the biofilm changes from “an endogen poly-microbial opportunistic flora” (Dahlén & Frandsen 2002) in the supragingival location to become dominated in the subgingival setting by “gram-negative proteolytic and predominantly anaerobic” microorganisms (Dahlén 2009). The host response to this altered microbial challenge changes and an “established lesion” forms in the gingiva (Page & Schroeder 1976). The mechanisms involved in the transition of a gingival lesion into a more profound “advanced lesion” (Page & Schroeder 1976) that also involves the cementum, the periodontal ligament and the alveolar bone are currently not fully understood, but they are most probably related to the ability of the host to respond to the multitude of products that emanate from the biofilm.

Studies in man and experiments in animals have shown that gingivitis lesions, as well as lesions of chronic periodontitis, may resolve, following treatment regimens that include the removal of supra- and subgingival plaque and (its mineralized component) calculus (e.g. Heitz-Mayfield et al. 2003). It has also been demonstrated that, following this basic therapy, recurrent disease can be prevented in most cases and sites in subjects who are enrolled in careful professional and self-performed supportive treatment programs (Supportive Periodontal Therapy; SPT), including the regular removal of supragingival biofilm (e.g. Axelsson & Lindhe, 1974, 1978, 1981a, b, Becker et al. 1984, Axelsson et al. 2004).

The critical role of plaque in the etiology of gingivitis and chronic periodontitis was further evaluated in programs studying the effect of “preventive” measures delivered to schoolchildren and adults (Axelsson & Lindhe1974, Axelsson et al. 1991, 2004). In the studies referred to here, panelists were recalled on a regular basis (1/2 weeks to 1/3-6 months) to the dental clinic for professional tooth cleaning and information, as well as training in self-performed tooth-cleaning measures. In subjects participating in these programs, most gingivitis was

resolved and the progressive tissue destruction in chronic periodontitis was prevented or arrested.

The outcome of *self-performed* and/or *professional* supragingival plaque control

The effect of supragingival plaque control on the subgingival biofilm and associated clinical symptoms of periodontal disease has been examined in both clinical and animal studies. In some of the studies, it was observed that the plaque control program had an obvious effect on the subgingival microbiota (e.g. Tagge et al. 1975, Siegrist & Kornmann 1982, Smulow et al. 1983, Katsanoulas et al. 1992, McNabb et al. 1992, Dahlén et al. 1992, Al-Yahfoufi et al. 1995, Haffajee et al. 2001), while no such influence could be found in other studies (e.g. Listgarten et al. 1978, Kho et al. 1985, Beltrami et al. 1987, Loos et al. 1988).

Del Peloso Ribeiro et al. (2005) showed that there was a reduction in gingival inflammation after one session of *professional* supragingival plaque control.

Clinical, histological and biochemical observations

Tagge et al. (1975) reported on the clinical outcome of (i) subgingival root debridement and (ii) *self-performed supragingival plaque control* in patients with chronic periodontitis. In each of 22 subjects, 3 sites that exhibited a similar degree of inflammation, assessed by crevicular fluid measurements, were identified. Following a baseline examination, a gingival biopsy was obtained from one of these sites, while a second site was exposed to meticulous scaling and root planing. The third site was left without treatment. All the volunteers received instruction in the appropriate self-performed plaque control measures. After 2 months of *self-performed supragingival plaque control* the 2 experimental sites were subjected to a clinical re-examination and biopsy. In the

biopsy material, the size and extent of the inflammatory lesions were determined. The results revealed that both treatments reduced inflammation and pocket depth, but also that the combined treatment (subgingival debridement plus supragingival cleaning) was more effective than supragingival plaque control alone.

Del Peloso Ribeiro et al. (2005) investigated 25 patients with chronic periodontitis with at least 4 pockets of ≥ 5 mm. The patients underwent the extraction of hopeless teeth, the removal of plaque retention factors, instruction in good oral hygiene and a single episode of supragingival calculus and soft deposit removal, followed by *self-performed* oral hygiene for the following 21 days. The results after 21 days showed an increase in the number of sites with a probing depth of ≤ 3 mm and a decrease in the number of sites with a probing depth of ≥ 6 mm. A change in the subgingival environment examined by measuring the level of trypsin-like enzymes (BAPNA test) produced by microorganisms like *T. forsythensis, Treponema denticola and P. gingivalis* showed a significant reduction compared with baseline values.

Microbiological observations

In an animal study in monkeys, Siegrist & Kornman (1982) investigated the effect of supragingival plaque control on the subgingival microbiota of experimentally (ligature) induced periodontitis lesions. Following a baseline examination, some sites were exposed to *professional* supragingival cleaning (3 times a week for 6 weeks), while contralateral sites were left without cleaning. The re-examination revealed that the regularly repeated supragingival cleaning reduced (i) the total number of microorganisms and (ii) the percentage of black-pigmented bacteroides species in the subgingival biofilm.

Smulow et al. (1983) evaluated the effect of supragingival plaque control on the subgingival microbiota of deep (≥ 5 mm) pockets in 14 subjects with chronic periodontitis. In each subject, 4 sites were identified and they received different

therapies, i.e. (i) subgingival debridement alone (SRP), (ii) subgingival debridement plus *professional cleaning* 5 days/week for 3 weeks (SRP +SPT), (iii) SPT alone or (iv) no treatment. Bacterial samples were harvested prior to therapy and after 3 weeks. An analysis of the samples revealed that SPT alone was as effective as SRP + SPT in reducing the total number of colony forming units, as well as some anaerobic marker species.

Similar findings were reported by McNabb et al. (1992) who treated and monitored subjects with initially poor oral hygiene and gingivitis. During the first 12-week period, only 2 of 4 quadrants of the dentition were treated by scaling and *professional plaque control* (3 times/week), while, during the second 12-week period, the remaining 2 quadrants were also included in the treatment regimen. It was observed that supragingival cleaning (i) markedly reduced the number of bacteria in the dento-gingival region and (ii) caused a relative increase in gram-positive microorganisms, while (iii) the numbers of *P. gingivalis* and spirochetes were decreased.

Dahlén et al. (1992) studied the effect on the subgingival microbiota of a plaque control program that included supragingival scaling, repeated oral hygiene instruction and *professional tooth cleaning* during a 3-month period in subjects with moderately advanced chronic periodontitis. Sixty-two subjects and in these patients 134 sites were objected to both bacterial samples and clinical measurements obtained prior to therapy and after 2 years. The authors reported that the treatment resulted in (i) an increase in the number of sites with shallow pockets (≤ 3 mm), (ii) but no decrease in the number of sites with deep pockets (≥ 6 mm). Both in sites with shallow and remaining deep pockets, the supragingival therapy resulted in a biofilm with a markedly reduced bacterial density. Further, the number of subjects with samples positive for species such as *A. actinomycetemcomitans, P. gingivalis* and *F. nucleatum* was also markedly reduced between the baseline and the 2-year examinations.

Al-Yahfoufi et al. (1995) investigated the effect of the supragingival removal of calculus and soft deposits followed by *self-performed plaque* control in 10 subjects with shallow pockets and without previous experience of periodontal treatment. All the patients displayed presence of *P. gingivalis* and *P. intermedia*. Further, *A. actinomycetemcomitans* were found in 5 of the subjects at the start of the investigation. After 1 month, the authors concluded not only that the clinical situation was improved but also that the number of subgingival bacteria was markedly reduced.

Haffajee et al. (2001) reported that, as a consequence of *self-performed* plaque control measures, the composition of the subgingival microbiota associated with periodontitis was changed and the percentage of periodontopathic bacteria was reduced.

Kho et al. (1985) studied the effect of *self-performed* supragingival plaque control on the subgingival microbiota in deep periodontal pockets in 8 subjects with chronic periodontitis. The clinical assessments were made on the Ramfjord Index teeth and 8 bacterial samples, one from each subject with a PPD of ≥ 7mm (mean PPD 8.2mm), were obtained at the 8- and 16-week re-examinations. The volunteers received surpragingival scaling and oral hygiene instructions (OHI). During 2 weeks of intense OHI, PlI and BoP decreased, but increased again during the period of *self-performed* plaque control. Thus re-examinations performed after 16 weeks revealed that there was (i) no improvement in gingivitis (BoP), (ii) no reduction in PPD and (iii) no change in the composition of the subgingval microbiota at sites with deep pockets.

Similar findings were reported by Beltrami et al. (1987). Patients with deep periodontal pockets and vertical bone loss verified with X-rays in 2 pairs of contralateral sites were selected. They were given *professional supragingival plaque control* at some sites with deep pockets (mean PPD 6.8 mm) but no treatment at sites with a similar probing pocket depth. The professional cleaning of the test sites was repeated 3 times/week for 3 weeks. The patients maintained

their usual OH procedures including tooth brushing 1-2 times/day but without using interdental cleaning aids. Examinations, performed at baseline and after 3 weeks, revealed that the treatment failed to reduce gingivitis scores and probing depths, as well as the numbers of different bacterial morphotypes in the subgingival biofilm.

Loos et al. (1988) studied the clinical and microbiological effects of a treatment program that included various forms of *self-performed plaque control* measures. Fifteen patients with untreated chronic periodontitis were recruited and subjected to clinical and microbiological examinations. During the study period, the patients were given OHI including the use of dental floss or tape, synthetic yarn and interdental brushes. The patients plaque control was checked weekly. After 6 weeks, in an attempt to improve OH, the patients were instructed to gently insert the tip of the Perio-Aid® (Marquis Dental MFG. Co., Aurora, CO, USA) below the gingival margin and to move the device intrasulcularly as deep as possible once a day. The patients were re-examined after 12 weeks. As a result of the large variation in OH, the initial group of 15 individuals was divided into 2 subgroups based on the level of plaque. Six subjects obtained Pl scores of ≥ 25%, while 9 subjects obtained plaque scores of < 25% at both examinations. The authors concluded that the effect of the various self-performed plaque control regimens had no significantly different effect on clinical and microbiological parameters and failed to change the subgingival microbiota.

Darkfield microscopy observations

Listgarten et al. (1978) monitored 6 subjects with advanced chronic periodontitis. All the subjects received detailed instruction in proper *self-performed plaque control*. In addition, meticulous scaling and root planing was performed in 2 quadrants of the dentition. Clinical and microbiological examinations were performed prior to therapy, as well as after 8 and 25 weeks

of treatment. The authors reported that, while the non-surgical therapy markedly affected the subgingival microbiota, self-performed plaque control measures failed to change the numbers of coocoid cells, motile rods and spirochetes.
Katsanoulas et al. (1992) used a split-mouth design to study the effect of *professional supragingival plaque control* on the composition of the subgingival microflora. Thirteen subjects with untreated periodontal pockets of 4-6 mm received *professional supragingival plaque control* 3 times/week during a period of 21days in 2 test quadrants. The 2 remaining quadrants (control) were left without any *professional supragingival plaque control*. The subjects did not receive any oral hygiene instructions but were asked to brush their teeth once a day and not to use any interdental cleaning aids. Re-examination after 21 days showed a decrease in PII scores at the test sites but no other clinical changes. The darkfield microscopy demonstrated significantly fewer spirochetes and motile rods compared with the control sites.

In the majority of the studies demonstrating an effect by supragingival plaque control on the subgingival biofilm, it was observed that the plaque control program caused shrinkage of the gingiva and a reduced probing depth. As a result, it can be argued that the change in the subgingival microflora occurred subsequent to the occurrence of the reduced probing depth and that the supragingival environment per se did not have a direct influence on the subgingival flora. While supragingival plaque control may cause advanced soft tissue shrinkage at suprabony pockets, no significant change in the probing depth may occur at sites with infrabony pockets or pockets associated with furcation defects. The question of whether supragingival plaque control affects the subgingival biofilm should therefore be evaluated at sites with different bone morphology.

Mechanical debridement combined with periodontal surgery

Several clinical studies have shown that the clinical outcome will improve, particularly in the case of deep periodontal pockets, if a surgical procedure is also performed. Heitz-Mayfield et al. (2002) reported the effect of surgical therapy, in a meta-analysis comprising 6 randomized controlled trials (Isidor et al. 1984, Lindhe et al. 1982, Lindhe & Nyman 1975, Philström et al. 1983, Ramfjord et al. 1987, Kahldahl et al. 1996). These 6 studies all had a split-mouth design comparing non-surgical with surgical treatment in each patient. It was concluded that both treatment modalities are effective in the treatment of chronic periodontal disease. In shallow pockets, 1-3 mm, treated with open flap surgery, there was significantly more CAL loss compared with treatment with scaling and root planing. In medium-deep pockets, 4-6 mm, scaling and root planing showed significantly more CAL gain than treatment with open flap surgery. In pockets exceeding 6 mm, surgical therapy resulted in a significantly larger reduction in pocket depths and more clinical attachment gain than in corresponding pockets treated with scaling and root planing.

From a randomized, single-blind, parallel-arm study comprising 64 patients, Serino et al. (2001) reported the short- and long-term results of non-surgical and surgical periodontal treatment during a period of 13 years. They reported that SRP during basic therapy (first year) reduced 75% of the category of deep pockets (≥7mm) (28% to shallow pockets) compared with 92% in the surgery group (56% to shallow pockets). Furthermore, the numbers of shallow, medium and deep pockets were unchanged during the 12 years of maintenance. Four subjects (12.5%) in the surgery group and 8 subjects (25 %) in the SRP group displayed disease progression during the first 3 years of maintenance. The authors reported that, in subjects with advanced periodontal disease, surgical therapy produces better short- and long-term periodontal pocket reduction and added that this may lead to fewer subjects requiring additional adjunctive therapy.

Chemical plaque control

Antiseptics

The effectiveness of self-performed plaque control after a single oral hygiene instruction was reported not to be sufficient to resolve gingivitis in adults using a manual toothbrush (van der Weijden et al. 2005). The effectiveness of manual tooth brushing (van der Weijden 1998) and powered tooth brushing (Williams et al. 2004) on plaque removal, where the subjects performed their normal tooth brushing for 1 or 3 minutes, showed that approximately 61-69% of the initial amount of plaque still remained. These results are comparable with findings presented by Del la Rosa et al. (1979), who reported that subjects who had received oral hygiene prophylaxis and exercised *self-performed* tooth brushing daily for 28 days retained about 60% of the initial amount of dental plaque. Attempts have therefore been made to develop adjunctive means to improve the outcome of self-performed plaque control measures, by incorporating antiseptics in mouth rinses and toothpastes, for example (Lang & Brecx 1986, Axelsson & Lindhe, 1987, Svatun et al. 1989, 1990, 1993, DePaola et al. 1989, Deasy et al. 1991, Lindhe et al. 1993, Addy 1995, Rosling et al. 1997a, b, Charles et al. 2001, 2004, Davies et al. 2004, Stoeken et al. 2007).

Antiseptic agents such as chlorhexidine, phenols, essential oils and cethyl pyridinium chloride as well as povidone iodine and metal salts like zinc chloride have been incorporated in mouth rinses and toothpastes (Addy & Moran 1997, 2008).

Effects of chlorhexidine, phenolic compounds and triclosan on plaque and gingivitis

Since the early 1970s, chlorhexidine has been used as an adjunct in the prevention and treatment of periodontal disease. In a review, Fine (1995) reported that chlorhexidine rinsing resulted in a 48-61% plaque reduction

compared with 19-35% for essential oils and 0-30% for triclosan (a broad-spectrum phenolic). The reduction in gingivitis was between 27-67% and 20-75% for chlorhexidine and triclosan respectively, while essential oils reduced gingivitis by 15-37%. These results are in agreement with a number of clinical studies (e.g. Lindhe et al. 1993, Addy 1995, Rosling et al. 1997a, Charles et al. 2001, 2004, Davies et al. 2004).

Experimental in vitro studies have indicated that triclosan has anti-inflammatory properties that may in part explain its effect on gingivitis, despite the fairly mediocre effect on plaque (Creeth et al. 1993, Waaler et al. 1993, Barkvoll & Rölla 1994, 1995, Gaffar et al. 1990,1995, Kjaerheim et al. 1995, Mustafa et al. 2005). It has been reported from in vitro studies that triclosan inhibits the release of prostaglandin E_2 from IL-Iβ-stimulated gingival fibroblasts (Gaffar et al. 1995, Mustafa et al. 2005). The level of IL-Iβ in the crevicular fluid from adults with periodontal breakdown has been found to be correlated to the degree of gingival inflammation (Wilton et al. 1992).

To enhance the plaque-reducing effect, different antibacterial agents like co-polymer (polyvinylmethyl ether and maleic acid; PVM/MA) or zinc citrate have been incorporated in toothpastes containing triclosan. Synergistic effects of the co-polymer, as well as an increase in the substantivity of triclosan, have been reported by Gaffar et al. (1990, 1995), Lindhe et al. (1993), Ramberg et al. (1995) and Sreenivasan & Gaffar (2008) and, in the case of zinc citrate, by Svatun et al. (1989a, b, 1990, 1993).

Lindhe and co-workers (1993) reported that, in subjects with gingivitis, the regular use of a triclosan-containing dentifrice significantly reduced gingivitis beyond what could be explained by the concomitant reduction of plaque per se. The authors reported that, at surfaces with similar amounts of plaque, fewer clinical signs of inflammation were observed in the triclosan group than in the control group.

An extract from the magnolia tree has been used for centuries in oriental medicine, mainly in China, Japan and Korea. The extract originates primarily from the magnolia tree (*Magnolia officinalis*) and the main source is the dried stem, root or branch bark (Chang & But, 1986). In addition to being used in traditional medicine to treat depression, anxiety, gastrointestinal disturbances, fever, muscular pain and headaches, the extract has been incorporated in dietary supplements and topically applied cosmetic products (Hattori et al. 1986, Tsai et al. 1992, Ogata et al. 1997, Sarker 1997, Hsieh et al. 1998, Maruyma et al. 1998).

Chemical investigations of the cortex of the magnolia tree have led to the isolation of several phenolic compounds, such as magnolol, isomagnolol and honokiol, among others (Fujita et al. 1972). The two main active components are magnolol and honokiol and the antibacterial and anti-inflammatory effects originate from these two major phenolic compounds. Magnolol has been shown to be the most active component and positive results in terms of inhibiting tumor metastases have been reported in both in vitro and in vivo studies (Nagase et al. 2002, Ikeda et al. 2003). From experimental studies, it has been indicated that mangnolol, probably via its anti-oxidative effects, is able to prevent athero-sclerotic vascular diseases (Ou et al. 2007). The anti-inflammatory and anti-oxidant properties of the magnolia extract have been investigated by Wang et al. (1992, 1993), Lo et al. (1994) and Chen et al. (2006). Chen et al. (2006) showed that magnolol suppressed the IL-6-induced activity of intracellular cell-adhesion molecules (ICAM-1). Antibacterial effects by the magnolia extract have been reported by Ito et al. (1982), Lo et al. (1994), Park et al. (2004) and Chang et al. (1998), Ho et al. (2001).

Experimental in vitro results indicate that magnolol and honokiol have significant antimicrobial activity against *P. gingivalis, P. intermedia and A. actinomycetemcomitans,* for example, even if the antimicrobial effect is lower than that produced by chlorhexidine (Chang et al. 1998). The authors suggested

"that magnolol and honokiol may have potential therapeutic use as a safe oral antiseptic for the prevention and treatment of periodontal disease". There are also reports stating that magnolia extract has an effect on fungi (Ito et al. 1982, Bang et al. 2000).

There are reasons to suggest that the adjunctive effect of antibacterial and anti-inflammatory agents may be helpful in resolving inflammatory lesions of the gingiva in subjects highly susceptible to periodontal pathogens, or in subjects unable to perform optimal plaque control.

Adjunctive effect of antiseptics and antibiotics in conjunction with non-surgical and surgical periodontal therapy

Scaling and root planing may be performed either as a "closed" procedure – *non-surgical periodontal therapy* – or after surgical exposure of the root surface – *surgical periodontal therapy*. In a number of clinical trials, it was shown that both procedures, properly executed, might result in periodontal health (Ramfjord et al. 1973, 1987, Badersten et al. 1981, 1984, 1987a, b, Westfelt et al. 1985). Mechanical root debridement is a therapy that is technically demanding and, when performed at sites with deep periodontal pockets, varying amounts of plaque (and calculus) may be left behind (Waerhaug 1978, Caffesse et al. 1986, Matia et al. 1986, Buchanan & Robertson 1987). As a result, associated periodontal lesions may not heal properly following basic therapy (Badersten et al. 1981, 1984, Caffesse et al. 1986, Rosling et al. 2001) or recurrence of disease – due to de novo bacterial proliferation – may occur during the phase of supportive periodontal therapy (Magnusson et al. 1984). A further analysis of some of these studies reveals PAL loss of ≥ 1.5 mm in 5-10% of sites after 24-42 months. To enhance the outcome of mainly *non-surgical periodontal therapy*, antiseptics (e.g. chlorhexidine, PVP iodine, phenolic compounds) and antibiotics (e.g. tetracyclines, amoxicillin, metronidazole) have been used, either

applied topically or delivered via the systemic route, as adjuncts to scaling and root planning (Goodson et al. 1979, Lindhe et al. 1979, 1983, Rosling et al. 1986, Wennström et al. 1987, Rams & Slots 1996, Soskolne et al. 1997, Furuichi et al. 1997, Berglundh et al. 1998, van Winkelhoff et al. 1996). The results of these studies are, however, inconclusive. Some studies have demonstrated benefits from the adjunctive therapy on treatment outcome, while some investigators failed to demonstrate any improvement (for reviews see Mombelli & van Winkelhoff 1997, Tonetti 1997, Wennström 1997, Quirynen et al. 2002, Slots & Ting 2002, Herrera et al. 2008).

The adjunctive effect of PVP iodine

The natural element, iodine, was discovered in 1811 by the chemist Bernhard Courtois, but it was not until iodine was detoxified by chemical binding to macromolecules that this effective microbicide could be used without adverse reactions in human tissue. Free molecules of iodine may have an allergic effect, but, by combining molecular iodine and a surface tension lowering agent, i.e. polyvinyl- pyrrolidone (PVP iodine), the antimicrobial effect remains without causing any adverse events. Furthermore, the reduced surface tension of PVP iodine increases the ability of iodine to reach irregularities in the contaminated root surfaces (Gordon et al. 1993, Fleisher et al. 1997).

Elemental iodine and its iodophor (PVP iodine, i.e. iodine + polyvinyl-pyrrolidone) are broad-spectrum antiseptics that, upon direct contact, effectively kill both bacteria and yeast (Caufield et al. 1987). Furthermore, it was demonstrated that the water-soluble iodophor is cidal to both gram-positive and gram-negative bacteria, fungi, mycobacteria and viruses (Schreier et al. 1997, Kawana et al. 1997) and that PVP iodine kills periodontitis-associated microorganisms (Caufield et al. 1987, Higashiutsumi et al. 1993). It has also been reported that resistance to antiseptics and antibiotics does not influence the sensitivity of bacteria to PVP iodine (Kunisada et al. 1997, Michel & Zach

1997). On the other hand, bacterial resistance has not been reported following either short- or long-term exposure to PVP iodine (Gordon 1993, Lanker-Klossner et al. 1997).

Allergic and toxic reactions to PVP iodine are rarely reported (Neidner 1997). However, it should not to be used in subjects allergic to iodine or in pregnant or nursing women. Furthermore, PVP-iodine rinses can have an adverse effect on thyroid function (Ferguson et al. 1978, Nobukunu et al. 1997, Greenstein 1999).

PVP-iodine mouth rinse

Clark et al. (1989) tested 5% PVP iodine and 1.5% hydrogen peroxide in patients with chronic gingivitis as an adjunct to their standard oral hygiene. The subjects rinsed 3 times a day for 24 weeks and were irrigated subgingivally with 5% PVP iodine and 1.5% hydrogen peroxide every 3 weeks during this time period. The patients showed significantly reduced gingival inflammation compared with the control rinse (water).

Maruniak et al. (1992) reported that rinsing with 5% PVP iodine and 1.5% hydrogen peroxide (Perimed®) was effective in reducing plaque and gingivitis in subjects who, during a 2-week period, abstained from mechanical tooth cleaning. Seventy-one subjects were asked to rinse with Listerine®, Perimed®, Peridex® (0.2% chlorhexidine) or plain water. After 14 days, the papillary bleeding index (PBS) was significantly lower in the subjects who rinsed with either Perimed® or Peridex® compared with Listerine® and water.

PVP iodine applied topically in the dentogingival region may also reduce bacteremia following surgical procedures in the oral cavity (Rahn et al. 1993, 1995, Dajani et al. 1997, Fleischer & Reimer 1997, Cherry et al. 2007). Rahn et al. (1995) irrigated the gingival sulcus before surgical tooth extraction with 10% PVP iodine and compared this effect with 0.2% chlorhexidine or only water in 40 patients. The frequency of observed bacteremia after surgery was 27.5% for PVP-I, 45% for 0.2% chlorhexidine irrigation and 52.5% for water.

PVP iodine delivered with an ultrasonic scaler

In a split-mouth design, Rosling et al. (1986) studied the effect of 0.5% PVP iodine administered through the cooling system of an ultrasonic scaler as an adjunct to periodontal therapy. They observed that there was more gain in clinical attachment at sites with deep pockets that were irrigated with PVP iodine than at sites that were irrigated with saline.

This finding was confirmed by Christersson et al. (1988) who, in a similar trial, demonstrated that irrigating periodontal pockets with PVP iodine as an adjunct to non-surgical periodontal therapy resulted in more sites with attachment gain of ≥ 2 mm (80% of all sites) than a corresponding regimen using saline as the irrigating substance (55% of sites).

Forabosco et al. (1996) used 0.5% PVP iodine with an ultrasonic scaler as an adjunct to surgical and non-surgical treatment in patients with chronic periodontitis using a split-mouth design. Twelve months after therapy, they reported that, at sites with initially deep pockets, PVP-iodine application resulted in better healing outcome than at sites not exposed to antimicrobial irrigation.

Clinical and/or microbiological effect of PVP iodine

In a split-mouth design including 16 adult subjects with at least 1 pocket of ≥ 6 mm, Hoang et al. (2003) investigated the effect of (i) subgingival flushing with 10% PVP iodine during 5 minutes in conjunction to SRP, (ii) SRP alone, (iii) flushing with 10% PVP iodine alone for 5 minutes or (iv) flushing with sterile saline for 5 minutes. All the patients had received appropriate OH instructions prior to the experimental phase.

Clinical and microbiological examinations were performed at baseline and 5 weeks post treatment. The microbiological results showed that subgingival irrigation with 10% PVP iodine for 5 minutes in conjunction with SRP resulted in a 95% reduction in the number of pathogens in 43.8% of the study sites,

whereas the three other groups showed similar reductions in only 6.3% to 12.5% of the study sites.

In a split-mouth designed study, Leonardt at al. (2006) evaluated the adjunctive effect of 0.5% PVP-iodine solution in conjunction with ultrasonic scaling. Twenty patients received one of four randomly distributed treatments: (i) ultrasonic scaling combined with either 0.5% PVP-iodine solution or saline in two quadrants and (ii) irrigation alone with saline or PVP iodine in the other two quadrants. The clinical evaluation was performed in one single-rooted tooth/quadrant with an initial PPD of ≥ 6mm. The 6-month evaluation revealed no differences in the reduction of PPD in the teeth subjected to ultrasonic scaling and irrigation with either PVP iodine or saline. The quadrants subjected to ultrasonic scaling with or without 0.5% PVP-iodine solution showed a significant reduction in both PPD and BoP, but the two treatment modalities were not significantly different. The results of the microbiological examination (Leonhardt et al. 2007) showed a reduction in periodontal pathogens by ultrasonic scaling but with no adjunctive effect from PVP iodine.

During a period of 6 months, Grossi et al. (1997) investigated 115 subjects with periodontal disease and non-insulin-dependent diabetes mellitus. The subjects were divided into 5 treatment groups. All the participants received ultrasonic scaling and the patients in treatment groups 1, 2 and 3 also received systemic doxycycline, 100 mg/day for 2 weeks. Moreover, in the patients in group 2, the water used as a cooling agent during the ultrasonic scaling was replaced with 0.12% chlorhexidine. For the patients in group 3, the cooling agent was replaced with 0.5% PVP iodine. The patients in group 4 received additional subgingival irrigation with 0.12% chlorhexidine, while the subjects in group 5 received only ultrasonic scaling with water. The results indicated that the subjects that received ultrasonic scaling in combination with systemic doxycycline experienced a greater PPD reduction and a greater reduction in subgingival microbiota than the corresponding subjects that had not been treated with

doxycycline (groups 4 and 5). It was concluded that the outcome of periodontal therapy including ultrasonic scaling combined with systemic doxycycline did not produce any additional effect when combined with the local application of CHX or PVP iodine in the cooling water.

In most studies, PVP iodine appears to be a potent antimicrobial agent that can be used as an adjunct to mechanical instrumentation in the treatment of periodontitis.

The effect of tetracyclines as an adjunct to non-surgical treatment in patients with periodontal disease

Tetracycline and the analogs doxycycline and minocycline are broad-spectrum antibiotics which are effective against both aerobic and anaerobic bacteria, gram-positive as well as gram-negative. Tetracyclines are bacteriostatic at clinically achieved concentrations (Schnappinger & Hillen 1996). Antimicrobial agents used systemically are transported by the bloodstream and reach the site of periodontal infection and the target, the microbes, in the subgingival biofilm via the crevicular fluid, but they simultaneously also enter the saliva, other tissues and different body components (van Winkelhoff et al. 1996). Tetracycline and analogs to tetracyclines (i) inhibit bacterial protein synthesis (Maxwell 1968) and (ii) cause damage to the cytoplasmic membrane of the bacterial cell. The long-term use of tetracyclines may favor the development of resistant strains of periodontal bacteria (Kornmann & Karl 1982, Lacroix & Walker 1995).

Tetracyclines, administered via the systemic route, have been used in clinical studies in patients with both aggressive periodontitis and chronic periodontitis as an adjunct to mechanical debridement. To have a synergistic effect in combination with the mechanical therapy, the antibiotics have to reach the microbiota in the biofilm at concentrations high enough to have a bacteriostatic

effect. It has been suggested that the microbiota living in biofilms could be 1000-1500 times more resistant to antibiotics than planctonic living cells (Marsh 2005). Other theories based on findings from scanning electron microscopy and laser confocal microscopy of in vitro cultured biofilms suggest that the channels found in the biofilm for the transportation of nutrients and waste products are thought to be sufficiently wide for the relatively small molecular size of most antibiotics (< 500 MW) to diffuse through these channels. Other theories state that antibiotics may adhere to extracellular products in the biofilm matrix, i.e. the glycolax, which will reduce or even inactivate the action of the antibiotic (Costerton et al. 1981, 1995). The view that the microbiota in a biofilm is more resistant to antibiotics is the subject of debate. Marsh (2005) concluded that new research about dental biofilms reveals properties that are typical of biofilms and microbial communities in general and a clinical consequence would therefore be reduced susceptibility to antimicrobial agents. Observations that tetracycline may reduce the collagenase activity in the inflammatory connective tissue and inhibit osteoclast activity have been reported by Vernillo and coworkers (1994). Tetracyclines and other antibiotics administered in doses that are too low to kill the microorganisms or the overuse of antibiotics can develop microbial resistance (van Winkelhoff et al. 2000). The risk of antibiotics inducing the development of resistant strains means that antibiotics should not be used routinely without a correct periodontal diagnosis and only in individuals with defined periodontal conditions (Herrera et al. 2008, Lindhe & Palmer 2002). The consensus report of the sixth European workshop on periodontology indicated that, if systemic antibiotics are part of the periodontal therapy, they should be adjunctive to mechanical debridement (Herrera et al. 2008). It was suggested and concluded that optimal conditions, i.e. mechanical supra- and subgingival debridement, of higher quality, produced a better result from the adjunctive systemic antibiotic treatment and that the time between the SRP and

the antibiotic intake was important and should be reduced to a minimum, in order to avoid biofilms rebuilding and reorganizing (Sanz & Teugel 2008).

Studies have documented that a regimen of 250 mg of tetracycline HCL given 4 times a day improved the resolution of gingivitis and promoted a gain in clinical attachment in patients with *aggressive periodontitis,* as adjuncts to mechanical debridement (Genco et al. 1981, Lindhe & Liljenberg 1984, Novak et al. 1988, 1991). Analogs of tetracycline, such as minocycline and doxycycline, used as adjuncts in the treatment of chronic periodontitis, resulted in the same degree of healing as tetracycline, but the effect of the drugs on treatment outcome have varied (e.g. Williams et al. 1979, Ciancio et al. 1982, Müller et al. 1993a, b, Freeman et al. 1992, Loesche et al. 1996, Akincibay et al. 2008).

Systemically administered tetracyclines as an adjunct to mechanical therapy in the treatment of chronic periodontitis

Listgarten et al. (1978) and Helldén et al. (1979) reported that systemic tetracycline used as an adjunct to scaling and root planing had no significant effect on treatment outcome.

Lindhe et al. (1983) combined an initial standard dose of tetracycline HCL with a subsequent dose regimen of 250 mg/day for 1 year in the treatment of 14 patients with chronic periodontitis. The authors reported that this type of tetracycline administration used in combination with mechanical therapy improved clinical parameters and reduced motile bacteria more than SRP alone.

In most studies, systemically administered tetracyclines used in conjunction with mechanical debridement appear to have a short-term beneficial effect in the treatment of periodontitis.

Topically administered tetracyclines (doxycycline, minocycline) as adjuncts to mechanical therapy in the treatment of chronic periodontitis

Even if the therapy modalities that are currently available are effective, a great deal of interest has been focused on using antibiotic formulations administered locally in periodontal pockets, either as a single therapy, or in combination with non-surgical periodontal treatment (MacAlpine et al. 1985, Heijl et al. 1991, Garret et al. 1999, Wennström et al. 2001, Williams et al. 2001, Aimetti et al. 2004, McColl et al. 2006). The effect of topically applied antibiotics in conjunction with surgery has been reported by a few investigators, i.e. Palmer et al. (1996) and Needlemann et al. (2000).

Topically applied antibiotics in patients with periodontal disease used as an adjunct to conventional treatment are thought to reach the site of action in the subgingival biofilm. Goodson (1989) suggested that it is possible to maintain an adequate concentration of a topically applied antibiotic to promote antimicrobial effects.

Different strategies in the local administration of tetracyclines have been developed for several decades. During the 1980s and early 1990s, results were published from studies in which tetracycline was combined with irrigation (MacAlpine et al. 1985, Silverstein et al. 1988, Nylund & Egelberg 1990, Christersson et al. 1993), followed by publications in which ethylene vinyl acetate fibers loaded with tetracycline were used (Goodson et al. 1991a, b, Newman et al. 1994, Drisko et al. 1995, Aimetti et al. 2004). The difficulties and time-consuming procedure involved in the placement of the tetracycline fibers in the pockets resulted in the development and use of minocycline and doxycycline in ointments and gels, as well as resorbable polymers which could easily be administered subgingivally (Garrett et al. 1999, van Steenberghe et al. 1999, Walker et al. 2000, Wennström et al. 2001, Williams et al. 2001, Meinberg et al. 2002, McColl et al. 2006). Walker et al. (2000) examined the microbiota in 23 patients after treatment with topical applications of a gel

containing 8.5% doxycycline. Questions had been raised about whether high concentrations of locally applied antibiotics eliminate or suppress the normal "beneficial" flora and create an opportunity for colonization by microorganisms that are pathologically resistant either to doxycycline or to other related antibiotics. The patients in the test group received topically applied gel containing 8.5% doxycycline at baseline and the control group received instruction in good oral hygiene, but no SRP was performed in either of the groups. Subgingival plaque sampling was performed at baseline and after 1, 3 and 26 weeks. The authors concluded that doxycycline treatment significantly reduced the anaerobic flora in subgingival plaque but did not result in a change in either the number of resistant bacteria or the overgrowth of fungi, for example.

In a 6-month multicenter trial, Wennström et al. (2001) examined the effect of 2 different non-surgical treatment approaches to chronic periodontitis, both involving the use of a locally delivered doxycycline. One hundred and five adult patients with at least 8 periodontal sites in 2 quadrants with PPD of ≥ 5 mm were randomly assigned to one of two treatment groups: scaling/root planing (SRP) with local analgesia or supra- and subgingival ultrasonic instrumentation without analgesia ("debridement"). The "SRP" group underwent a single episode of full-mouth supra-/subgingival scaling and root planing under local analgesia and, at the 3-month recall visit, full-mouth supra-/subgingival debridement with ultrasonic instrumentation was administered. In addition, 8.5% doxycycline gel was administered at sites with a remaining PPD of ≥ 5 mm. The subjects in the "debridement" group were treated for 45 minutes with ultrasonic instrumentation and the application of doxycycline gel at sites with PPD of ≥ 5 mm. At the 6-month examination, no statistically significant differences in PPD or CAL were found between the two treatment groups. The mean total treatment time for the "SRP" group was 3.11 hours, compared with 2.00 hours for the patients in the "debridement" group ($p<0.001$). The authors

concluded that “simplified subgingival instrumentation combined with local application of doxycycline in deep periodontal sites can be considered as a justified approach for non-surgical treatment of chronic periodontitis”.

Williams et al. (2001) examined the adjunctive effect of minocycline microspheres and SRP from an 18-center study. Three parallel groups, a total of 748 patients at baseline, received treatment with (i) SRP (Control I), (ii) SRP plus placebo vehicle (Control II) or (iii) SRP and 2% minocycline in a microsphere vehicle (Test). At the 9-month re-examination, the subjects in the test group showed a mean reduction of 1.32 mm in pockets of ≥ 5 mm at baseline, with 1.08 mm in corresponding pockets in the control group. The mean difference between the test and control group was 0.24 mm ($p<0.001$). The authors concluded that SRP plus minocycline microspheres was more effective than SRP in reducing probing depths in periodontitis patients

Van Steenberghe et al. (1999) presented clinical and microbiological observations from a multicenter study comprising 93 patients with moderate to severe periodontitis in which all the subjects received supra- and subgingival debridement at baseline, 6 and 12 months.

In addition, the patients in the test group (46) received adjunctive treatment with 2% minocycline ointment. At 2 weeks and 3, 6 and 12 months, all the subjects were instructed in normal oral hygiene measures and were asked to practice *self-performed* oral hygiene. After 15 months, pockets with an initial depth of ≥ 5mm in the test group showed a significant improvement after subgingival instrumentation (PPD reduction 1.9 versus 1.2 mm and PAL gain 0.9 versus 0.5 mm). The microbiological results showed that *P. gingivalis, P. intermedia and C. rectus* were significantly reduced in the test group. For *A. actinomycetemcomitans,* no significant differences could be seen between the test and control group.

Topically administered minocycline during supportive periodontal treatment in patients with chronic periodontitis

Meinberg et al. (2002) monitored 48 patients during a 12-month period of SPT who had been treated with conventional non-surgical debridement for moderate to advanced chronic periodontal disease. The patients were randomly assigned to 2 treatment groups in which SPT treatment in the control group was performed with subgingival debridement in all pockets with BoP and a pocket depth of ≥ 5 mm. In 24 subjects assigned to the test group, the subgingival application of 1 mg minocycline microspheres (Arestin®, Oral Pharma Inc., Warminster, PA, USA) was repeated at 1, 3 and 6 months in all pockets with BoP and a pocket depth of ≥ 5 mm, but without concomitant subgingival instrumentation. At the 1-months re-examination of the patients in the test group, an additional PPD reduction of 0.5mm was demonstrated compared with the control group.

McColl et al. (2006) treated residual pockets in 40 patients with BoP and PPD of ≥ 5mm for one year. The test group (20 subjects) received 2% minocycline gel subgingivally every 3 months and, at the same intervals, the subjects in the control group were treated with subgingival mechanical debridement. The plaque control was checked and, if necessary, reinforced. Clinical and microbiological examinations performed every 3 months in both groups showed a reduction in the numbers of sites with PPD of ≥ 5mm at the 3-month re-examination, a reduction that was maintained during the study period. There were no statistically significant differences between the groups at any examination interval. The TVC increased during the 12-month period in both groups, but the increase was more pronounced in the SRP group for *P. gingivalis, T. forsythus* and *T. denticola* from 6 months on.

In most studies, there appeared to be a short-term beneficial effect from locally administered antibiotics (tetracyclines) used in conjunction with mechanical debridement in the treatment of periodontitis.

Summary of studies included in the present thesis.

Objectives

The aims of the present series of studies performed in patients with gingivitis and chronic periodontitis were:

To assess the effect of frequently repeated supragingival plaque removal on the subgingival microbiota at periodontal sites with suprabony, infrabony and furcation lesions in patients with chronic periodontitis (**Study I)**

To assess the effect of a dentifrice containing 0.3% magnolia extract in gingivitis patients on dental plaque and gingivitis (**Study II)**

To assess the effect of topically applied PVP iodine in patients with chronic periodontitis, used as an adjunct during both basic non-surgical therapy and long-term supportive periodontal therapy (**Study III)**

To study the short- and long-term effect of the systemic administration of tetracycline in conjunction with non-surgical treatment in patients with advanced chronic periodontitis (**Study IV)**

To assess the effect of topically applied minocycline microspheres as an adjunct to surgical periodontal treatment in patients with moderate to advanced chronic periodontitis (**Study V**)

Study duration

Study I: 30 weeks

Studies II & V: 6 months

Studies III & IV: 13 years

Clinical examinations

The following clinical parameters were assessed in all teeth except the third molars **(Studies I, II & IV)** and in all available non-molar teeth **(Studies III & V).**

Dental plaque

The amount of dental plaque was assessed after staining with a disclosing solution, erythrosin (Diaplac®, Wallco AB, Enköping, Sweden), on 4 surfaces (distal, buccal, mesial, buccal) per tooth (**Studies I, III, IV & V**) as present/absent (Ainamo & Bay 1976). Furthermore, in **Study I** on **selected sites** the dental plaque was assessed on 4 surfaces according to the criteria of the PlI (Silness & Löe 1964). In **Study II** plaque was assessed on 6 surfaces (disto,-buccal, buccal,mesio buccal, disto-lingual, lingual, mesio-lingual) according to the criteria of the Turesky modification of the QHI Index (Quigley & Hein Index System; Quigley and Hein, 1962, Turesky et al. 1971).

Criteria according to the Plaque Index (PlI) (Silness & Löe 1964)

Score 0: no plaque

Score 1: a film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after the application of disclosing solution or by using the probe on the tooth surface.

Score 2: moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye.

Score 3: abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Criteria according to the Turesky modification of the Quigley and Hein Index (QHI) (Quigley and Hein, 1962, Turesky et al. 1971)

Score 0: no plaque

Score 1: separate flecks of plaque at the cervical margin of the tooth

Score 2: a thin continuous band of plaque (up to one mm) at the cervical margin of the tooth

Score 3: a band of plaque wider than one mm but covering less than one-third of the crown of the tooth

Score 4: plaque covering at least one-third of the crown of the tooth but less than two-thirds of the crown of the tooth

Score 5: plaque covering two-thirds or more of the crown of the tooth

Gingival inflammation

The degree of inflammation was assessed at 4 sites per tooth according to the criteria specified in the Gingival Index System (GI) (Löe 1967) **(Study I, selected sites, and Study II).**

Criteria according to the Gingival Index (GI*)* (Löe 1967)

Score 0: absence of inflammation

Score 1: mild inflammation – slight change in color and little change in texture

Score 2: moderate inflammation – moderate glazing, redness, edema and hypertrophy; bleeding on probing

Score 3: severe inflammation – marked redness and hypertrophy; tendency towards spontaneous bleeding

Pocket depth (PD) was assessed at 4 sites/tooth (distal, buccal, mesial, lingual) from the free gingival margin to the base of the pocket using a periodontal probe (Hu-Friedy, GF-W, USA) to the nearest 1 mm (**Study I)** or at 6 sites/tooth (disto-buccal, buccal, mesio-buccal, disto-lingual, lingual, mesio-lingual) using a periodontal probe (PCPUNC 15® Hu Friedy, USA) (**Studies II, III, IV & V).**

Recession was assessed from the cemento-enamel junction (CEJ) to the gingival margin or from a restorative margin if the CEJ was not present **(Studies I & V).**

Clinical attachment level (CAL) was calculated as PD plus recession (**Studies I & V).**

Probing attachment level (PAL) was assessed by measuring the distance between fixed landmarks on individually fabricated stents and the bottom of the pocket (**Studies III & IV).** The reading of PAL was performed in connection with the PD measurement and without changing the location of the probe tip.

Bleeding on probing (BoP) was assessed at 4 sites per tooth (mesial, buccal, distal, ligual) as present or absent, in conjunction with the PPD measurements (Ainamo & Bay 1975) (**Studies I, III, IV & V).**

Microbiological sampling. A bacterial sample was obtained from each of the selected sites using 2 sterile, medium-sized paper points **(Study I)**. Supragingival plaque and calculus were removed and the tooth surface was dried with sterile cotton pellets. The paper points were inserted into the pocket until definite resistance was met and were then kept in place for 30 seconds. The paper point samples were placed in an anaerobically prepared transport medium and processed the same day.

Radiographic bone level (Rxbl). A set of full-mouth intraoral radiographs was obtained at the baseline examination **(Studies I, III, IV &V)** and at the final

examination (**Study V)** using a standardized parallel technique (Eggen 1969). The location of the marginal alveolar bone in relation to the cemento-enamel junction (CEJ) was measured at the mesial and distal aspects of all non-molar teeth present. The radiographs were analyzed using a digitizing system connected to a specially developed computer program (Status XR, AEC, Göteborg, Sweden) that automatically calculated the linear distances that were identified (**Studies III, IV & V).**

Registrations

One examiner performed all the measurements in **Studies I, II** & **V** (Center A)**.** There were 2 calibrated examiners in **Studies IV** & **V** (Center B) and 3 calibrated examiners in **Study III.**

Patient selection

In **Study I,** the subjects were selected from patients referred to the Department of Periodontology, University of Gothenburg, for the treatment of moderate to severe periodontitis.

In **Study II,** the subjects were volunteers with no signs of destructive periodontal disease, recruited at the Specialist Center, Department of Periodontology, Uddevalla, Sweden.

In **Studies III** and **IV,** the patients were recruited from a larger group of patients who, in 1982-1984, were referred to the Department of Periodontology, Helsingborg, Sweden, for the treatment of advanced periodontal disease.

In **Study V,** the subjects were selected from patients referred to the Specialist Center, Department of Periodontology in Uddevalla, Sweden (Center A) and to a private dental practice in Aurora, Colorado, USA (Center B)**.**

All the studies were performed in collaboration with the Department of Periodontology, Institute of Odontology, University of Gothenburg, Sweden.

Data analysis

Student's t-test was used to analyze difference over time between mean values (on subject level) for clinical and microbiological parameters in study I. Differences between the test and control groups as differences over time and analysis of variance were performed in study III, IV and V. In study V differences between the groups at patient entry were examined using Fisher's exact tests. Nonparametric Wilcoxon statistics were used to validate findings of the parametric t-test and Levine's test assessing homogeneity of variance. The Gail and Simon test was performed to determine whether there was any evidence of treatment by center interaction. In study II one factor ANOVA using the treatment where the factor was used to detect if significant differences between the products existed.

For each of the variables mean values and standard deviation were calculated for the treatment group and subject level.

Material and methods

Study I

Twelve subjects (5 males) aged 44 to 69 years with moderate to severe periodontal disease participated in the study. The subjects were otherwise healthy and had not received any periodontal therapy during the last 12 months or received antibiotics during a 3-month period preceding the study.

Based on a full-mouth screening examination, including the assessment of plaque, (+/-) gingivitis (+/-) and probing depth on 4 surfaces per tooth (Nyman & Lindhe 1983), 6 to 8 sites per subject were selected which had a probing depth of ≥ 5 mm. Of these sites, at least one had a pocket with a suprabony location and an infrabony location and at least one site was located at a furcation defect of Degree II (Hamp et al. 1975).

The total sample comprised 25 suprabony sites, 20 infrabony sites and 22 furcation sites.

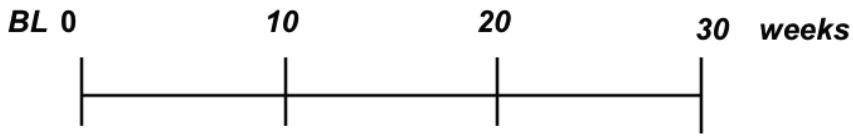

Figure 1. Baseline examination. Microbiological samples
Professional plaque control 2-3 times /week
Reexamination and microbiological samples at 30 weeks

A full-mouth clinical examination was performed at baseline and after 30 weeks, including examination of the selected sites where assessments of GI, PlI, PPD and PAL and bacterial samples were made for microbiological examinations.

The total viable count (TVC) and the percentage of *P. gingivalis* were calculated.

At the baseline examination, the patients were instructed and trained in good OH, followed by thorough supragingival scaling. During the subsequent 30 weeks, the patients were recalled 2-3 times a week for PTC by a dental hygienist (Axelsson & Lindhe 1974) and the patients' oral hygiene was checked and, if necessary, corrected.

Study II

One hundred and two subjects, aged 18-67 years, participated in the study. Subjects with no destructive periodontal disease but with gingival inflammation (GI) ≥ 1.0 and dental plaque ≥ 1.5 (QHI) were recruited.

The subjects (i) should have a minimum of 24 teeth, (ii) should not have used antibiotics during the last 3 months prior to the study, (iii) should not have been on regular anti-inflammatory medication and (iv) should be non-smokers.

The subjects were randomly assigned to a test group (magnolia) and a control group. The magnolia group comprised 51 subjects who were assigned to a

dentifrice containing 0.3% magnolia extract and 0.243% sodium fluoride (1100 ppm F). All the participants in the control group (51) used a corresponding fluoride dentifrice. The subjects were asked to continue to exercise their regular *non-supervised, self-performed plaque control* twice a day for the next 6 months. Plaque (QHI) and gingivitis (GI) were registered at baseline and after 3 and 6 months.

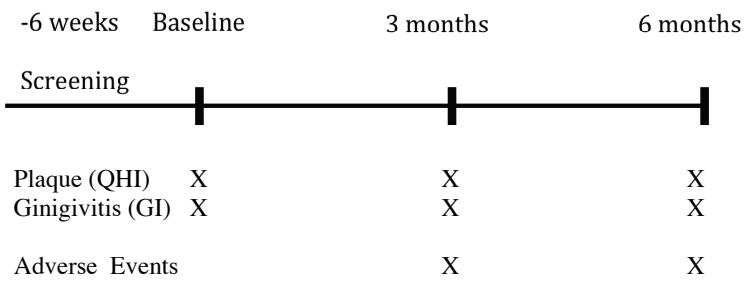

Figure 2. Study II outline

Study III

Two hundred and twenty-three patients with advanced destructive periodontitis were recruited; they had (i) a minimum of 8 non-molar teeth, (ii) a probing pocket depth of ≥ 6 mm at ≥ 2 teeth in each quadrant and (iii) radiographic bone loss exceeding 40% in the same teeth.

The study included one phase of basic therapy and 12 years of supportive periodontal therapy (SPT).

Following the first baseline examination (***Baseline I*****),** the patients were stratified into 2 treatment groups (test and control). The groups were balanced in terms of age, gender, PPD and RxBL. The basic therapy included instruction in good self-performed OH measures and SRP. The OH instruction was repeated on an "individual need basis", both during the basic treatment period and at recall visits during SPT. The SRP was carried out under local anesthesia and using an ultrasonic device (Odontoson M®, Copenhagen, Denmark). The supra- and

subgingival instrumentation was performed with the concomitant administration of 0.1% povidone-iodine (test group) or water (control group) as the cooling solution for the ultrasonic device.
The non-surgical treatment required an average of 4-6 sessions, each lasting 50-60 minutes, using 950 ml PVP iodine (test group) or water (control group).

Following ***Baseline I,*** all subjects were examined after 3, 6 and 12 months. The 12-month re-examination marked the end point of the outcome of *basic therapy* and became the starting point for the evaluation of the effect of maintenance therapy ***(Baseline II)***. Comprehensive re-examinations, including PI, BoP, PPD and PAL, were performed 3, 5 and 12 years after *Baseline II,* i. e. the patients were monitored for a period of 13 years.
All the subjects were included in a *maintenance program* that included SPT every 3–4 months during the 12-year period. In conjunction with the recall visits, sites that exhibited BoP and PPD of ≥ 5 mm were subjected to subgingival therapy identical to the treatment provided during the basic therapy.
Subjects who demonstrated an *annual* further loss of PAL of ≥ 2 mm at ≥ 4 sites between year 1 and 3 after *Baseline II* were removed from the study and referred for re-treatment.

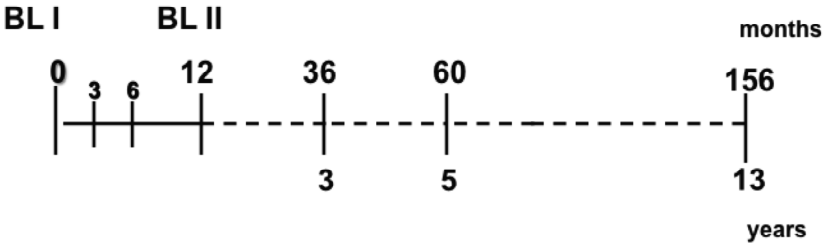

Figure 3. Study design. **Baseline I** (BLI): Initial examination. 0:Start of Basic therapy: Nonsurgical debridement: SRP + iodine (test). SRP alone (ctrl).
Baseline II (BLII): Evaluation of basic therapy. Start of SPT 3-4 times/year.
SRP + iodine (test). SRP alone (ctrl)

Drop outs

During Baseline I – 13 years 8 subjects in the test group and 25 subjects in the control group withdrew from the trial for reasons unrelated to the study. These subjects were not included in the final data analysis.

Study IV

Thirty-five subjects (16 males) aged 24 to 60 years were included in the test group. Eighty gender-matched subjects were recruited for the control group (38 males).

The subjects should be > 20 years of age and have ≥ 16 teeth of which ≥ 2 teeth must be molars. The subjects should not have any systemic conditions which required antibiotic coverage for SRP, should not be pregnant, or allergic to tetracycline or have taken antibiotics during the previous 6 months.

Clinical examinations including the assessment of plaque, BoP, PPD and PAL were performed at baseline and at re-examination after 1, 3, 5 and 13 years. Following the baseline examination, all patients were given instruction in effective self-performed plaque control measures.

During the 3-week interval, all the participants received 4-6 sessions of SRP under local anesthesia. Each SRP session required about 60-90 minutes of treatment. The subjects in the test group started the antibiotic regimen, including 250 mg of tetracycline hydrochloride (Tetracycline®, NM Pharma, Enköping, Sweden) 4 times a day for a period of 3 weeks. No drug therapy was given to the subjects in the control group. During the entire basic therapy phase, all the patients rinsed with a 0.2% CHX solution twice a day.

Following the termination of the basic therapy phase, all the patients were enrolled in a maintenance care program, including SPT 3-4 times per year. At each recall appointment, the quality of the self-performed plaque control measures was evaluated and, if necessary, corrected. Sites that bled on gentle probing and with a PPD of ≥ 5 mm were anesthetized and underwent renewed mechanical instrumentation.

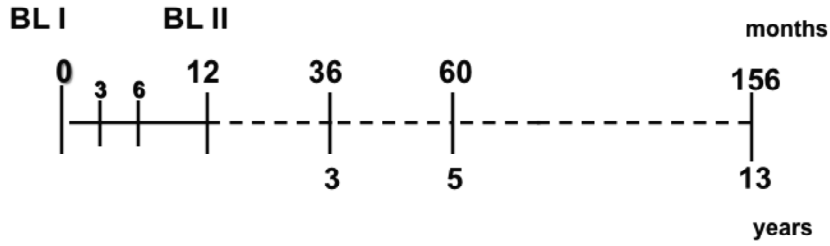

Figure 4. Baseline I (BLI): Initial examination.0: Start of Basic therapy: Nonsurgical debridement: (i) SRP +systemic Tetracycline (test). (ii) SRP alone (ctrl).
Baseline II (BLII): Evaluation of basic therapy. Start of SPT, test & control, 3-4 times/year.

Drop outs

During the course of the 13 years of monitoring, a total of 26 subjects were lost. Seven subjects in the test group and 19 subjects in the control group withdrew from the trial for reasons unrelated to the study. Only subjects who remained in the study for the entire 13-year period were included in the analysis.

Study V

This study was a 6-month double-blind, parallel, prospective, 2-center study. Sixty patients aged 25 to 80 years who met the following inclusion criteria were included: a minimum of 12 non-molar teeth in 4 quadrants, ≥ 3 non-molar teeth per quadrant with 2 or more periodontal sites with bleeding on probing (BoP) and ≥ 1 site with a PPD of ≥ 6 mm.

The baseline examination including PlI, BoP, PPD and PAL were assessed in all non-molar teeth.

The patients were randomized in a test and a control group. The patients in the test group received modified Widman flap surgery and minocycline microspheres (SMM) and the control group of 28 subjects (12 male) only received modified Widman flap surgery (SO).

Following the baseline examination, all the patients were given instruction in effective self-performed plaque control and, if necessary, the plaque control was reinforced on each visit. Two quadrants in the test group was given local minocycline microspheres at baseline, immediately following each of 2 surgical therapies performed in weeks 2 and 3 and week 5. Each treatment site received a single dose of Arestin® (1mg of minocycline). The other surgery quadrants followed the same treatment regimen but without any antibiotic therapy. The 2 remaining quadrants were treated with scaling and root planing, with or without Arestin®.

STUDY OUTLINE

	Baseline	Week 2	Week 3	Week 5	Week 13	Week 26
PD, CAL BoP & PI	X				X	X
Quadrant A	SRP+M	M M		MM		
Quadrant B	SRP+M	M M		MM		
Quadrant C	M M	MVF+MM		MM		
Quadrant D	M M		MVF+MM M	M		

PD: Pocket depth; CAL: Clinical Attachment level; BoP: Bleeding on Probing;
SRP: Scaling and Rootplaning; MVF: Modified Widman Flap; MM: Minocycline Microspheres
Surg: Surgery

Drop outs

Thirty-one subjects were randomized to the SO group and 29 subjects to the SMM group and 1 subject in each group discontinued the study; 1 patient in the

SO group was removed because of a protocol violation and 1 patient in the SMM group due to non-serious reported joint pain.

Results

Study 1

Clinical observations

At the baseline examination, 82-100% of all sites harbored gross amounts of plaque (PlI ≥2) and 100% bled on probing. At the 30-week examination, the percentage of sites with gross amounts of plaque was reduced to 14-19% and the percentage of sites with bleeding on probing was reduced to 20-32%. The reduction was statistically significant for all selected sites ($p>0.001$).

Suprabony sites

There were statistically significant reductions in mean probing depth ($p<0.001$) and the mean probing attachment level ($p<0.05$) between the examinations, as well as an increase in gingival recession ($p<0.001$). At baseline, 10 of the 25 selected suprabony sites had a probing depth of 5 mm, 12 were 6 mm and 3 were > 6 mm deep. At the 30-week examination, all but 2 of the sites showed a reduction in probing depth. As a result, 7 sites were ≤ 3 mm, 16 were 4 mm and 2 were 6 mm deep. In other words, the professionally supervised plaque control regimen improved the clinical conditions at all suprabony sites, irrespective of their baseline depth.

Infrabony sites

There were no significant alterations in the mean probing depth (PPD) and the mean probing attachment level (PAL) during the 30-week interval.

At 30 weeks, only 5 sites exhibited improved probing depth values; 1 site was 4 mm, 6 sites were 5 mm, 7 sites were 6 mm and 6 sites were still ≥ 6 mm deep. No site displayed a PPD reduction of > 1 mm and at 3 sites a PPD increase of ≥ 1 mm occurred. All infrabony sites showed the presence of plaque and bleeding

gingiva at baseline, while, at the 30-week re-examination, only 18% of the selected sites harbored visible amounts of plaque and only 20% were BoP positive.

The probing depth change between baseline and 30 weeks was less conspicuous at sites with infrabony sites than at suprabony sites. In fact, there were no significant alterations in the mean probing depth (PPD) and the mean probing attachment level (PAL) during the 30-week interval. At baseline, 3 of 20 infrabony sites were 5 mm deep, 13 were 6 mm and 4 sites had a PPD of > 6 mm. At 30 weeks, only 5 sites exhibited improved probing depth values; 1 site was 4 mm, 6 sites were 5 mm, 7 sites were 6 mm and 6 sites were still 6 mm deep. No site displayed a PPD reduction of > 1 mm and at 3 sites a PPD increase of ≥ 1 mm occurred.

Furcation sites

Of 22 furcation sites involved in the study, 82% showed the presence of visible plaque and 100% bled on gentle probing at baseline. At the end of the 30-week interval of professional supragingival plaque control, the plaque and gingivitis scores had decreased to 19% and 32% respectively.

There was a statistically significant reduction in the mean PPD ($p<0.01$) but not in the mean PAL between baseline and the 30-week examination. The amount of gingival recession was, however, significantly higher. At most furcation sites (21/22), the PPD at baseline varied between 5-6 mm. During the course of the therapy, 5 of the 9 sites in the 5 mm category and 5 of the 12 sites in the 6 mm category exhibited a PPD reduction of ≥ 1 mm. One site had a deeper probing depth value at 30 weeks than at baseline.

Microbiological observations

Suprabony sites

There was a statistically significant reduction in the mean TVC (x 10^5) counts between baseline and the 30-week examinations and the TVC counts were notably reduced for all suprabony pocket sites. Whereas at baseline the TVC counts were higher in deeper pockets than in more shallow pockets, no such trend could be seen at the end of the trial. The percentage of *P. gingivalis* in the subgingival samples was reduced in all PPD categories, but this reduction was not statistically significant.

Infrabony sites

The TVC (x 10^5) counts at sites with a PPD ≥ 5 mm at baseline were significantly reduced between the 2 examination intervals ($p<0.01$). This reduction occurred at initially deep as well as shallower sites and also at sites which maintained their probing depth. Moreover, the percentage of *P. gingivalis* was markedly reduced at all sites between baseline and the 30-week examination.

Furcation sites

The mean TVC was markedly reduced during the course of the trial. The percentage of *P. gingivalis* was significantly higher ($p<0.05$) at baseline than at the 30-week examination. Independent of the initial probing pocket depth, both the TVC count and the percentage of *P. gingivalis* were reduced between the 2 examinations.

Study II

Ninety-four subjects completed the study. Eight subjects (5 from the test group) withdrew from the study for reasons not related to the ongoing trial.

Plaque

There were no statistically significant differences between the groups at the baseline registration. The mean QHI score was 2.90 (SD±0.39) and 2.82 (0.32) in the magnolia group and the control group respectively.

At the 3-month examination the mean QHI score was 2.50 (0.30) in the test group and 2.52 (0.28) in the control group, but the reduction was significantly higher ($p<0.01$) in the magnolia group.

At the 6-month examination the mean QHI plaque score was 2.65 (0.40) and 2.72 (0.33) in the magnolia group and the control group respectively. The mean QHI reduction was statistically significant in both the magnolia group and the control group ($p<0.001$ and $p<0.05$ respectively), but it was significantly larger in the magnolia group than in the control group (0.25±0.40 vs. 0.09±0.28; $p<0.01$).

Percentage distribution of various QHI score categories

At the baseline examination, an average of 3.6% (5.6) of the tooth surfaces in the magnolia group had a QHI score of 0/1, while the corresponding figure in the control group was 4.4% (4.8). The percentage of tooth surfaces with gross amounts of plaque (QHI 3/4/5) was 70.0% (19.7) and 65.4% (17.1) in the magnolia group and the control group respectively.

At the 3-month examination the percentage of tooth surfaces with a QHI score of 0/1 had increased to 12.6% (12.6) in the magnolia group and 11.2 % (10.9) in the control group. The percentage of surfaces with large amounts of plaque (QHI 3/4/5) had decreased to 52.1% (22.0) in the magnolia group and 56.2% (19.3) in the control group. The change was statistically significant within the two treatment groups ($p<0.001$), but the participants receiving the magnolia regimen obtained a significantly higher reduction in QHI scores 3/4/5 than the control group (17.8% vs. 9.2%) ($p<0.01$).

At the 6-month examination the mean percentage plaque score of 0/1 was 7.2% (12.6) in the magnolia group and 5.3% (7.2%) in the control group. The corresponding percentage of surfaces with scores of 3/4/5 was 59.8% (19.3) and 62.1% (18.1) respectively.

The change in the various QHI score categories between baseline and 6 months was statistically significant in the magnolia group ($p<0.001$) but not in the control group. The participants receiving the magnolia regimen obtained a significantly higher reduction in QHI scores of 3/4/5 than the control group (10.2% vs. 3.2 %) ($p<0.05$).

Gingivitis

At baseline the mean GI score was 1.34 (0.16) and 1.30 (0.12) in the magnolia group and the control group respectively.

At the 3-month examination the mean GI score was 1.07 (0.18) in the magnolia group versus 1.16 (0.14) in the control group. The reduction was statistically significant in both treatment groups ($p<0.001$), but it was significantly higher in the magnolia group than in the control group; 0.27 (0.13) vs. 0.15 (0.14); $p<0.001$.

At the 6-month examination the mean GI score was 1.08 (0.14) in the magnolia group and 1.19 (0.12) in the control group. The mean GI reduction was statistically significant in both the magnolia group and the control group ($p<0.001$), but it was significantly higher in the magnolia group (0.26 ± 0.11) than in the control group 0.11 (0.12) ($p<0.001$).

Percentage distribution of various GI score categories

At the baseline examination the mean percentage of healthy gingival sites was 2.9% (3.6%) in the magnolia group and 2.8% (3.2%) in the control group. The percentage of sites with a GI score of 2/3 was 36.5% (14.0%) and 32.8% (11.3%) in the magnolia group and the control group respectively.

At the 3-month examination the mean percentage of healthy gingival sites (GI 0) had increased to 10.7% (9.3%) in the magnolia group and 6.9% (6.8%) in the control group. The percentage of sites with a GI score of 2/3 had decreased to 17.4% (11.1%) in the magnolia group and 22.3% (10.7%) in the control group.

The changes between baseline and the 3-month examination were statistically significant in both treatment groups ($p<0.001$).

At the 6-month examination the percentage of sites (GI 0) was 7.2% (6.6%) in the magnolia group and 3.7% (3.8%) in the control group. The percentage of sites with a GI score of 2/3 was 14.8% (9.1%) in the magnolia group and 22.9% (9.6%) in the control group. The change in the various GI score categories was statistically significant in the two treatment groups ($p<0.001$), apart from the GI score 0 category in the control group. The subjects in the magnolia group displayed a significantly larger increase in the percentage of healthy gingival sites (3.5% vs. 0.9%; $p<0.01$) and a significantly higher reduction in inflamed gingival sites compared with the control group (21.7% vs. 9.8 %; $p<0.001$).

Number of sites with various GI scores

The number of sites with GI scores of 0, 1 and 2 in the magnolia group and the control group at baseline and 6 months were calculated. The number of healthy sites (GI = 0) increased from 207 sites to 517 sites (150%) in the magnolia group. The corresponding increase in the control group was 213 sites to 278 sites (31%).

The number of gingival units showing overt signs of gingival inflammation in the magnolia group (GI score 2) was reduced from 2582 sites to 1045 sites (60%), while the corresponding reduction in the control group was from 2483 sites to 1734 sites (30%).

At the 3-month and 6-month examinations, significantly less clinical signs of gingivitis were observed in the magnolia group than in the control group at sites adjacent to tooth surfaces harboring the same amount of plaque.

Study III

Drop outs and excluded subjects

During the basic therapy phase 9 subjects, 1 in the test group and 8 in the control group, withdrew from the trial for reasons unrelated to the study. Seven subjects in the test group and 17 subjects in the control group withdrew from the study during the maintenance phase. In addition, during the first 2 years after active treatment, 31 subjects from the control group and 9 from the test group were excluded due to further disease progression (≥ 2 mm PAL loss at ≥ 4 teeth per year). The percentage of *" loser subjects"* was higher in the control group (25.2%) than in the test group (13.4%). No subjects with additional CAL loss ≥ 2 mm were identified between years 3 and 13.

As a result, the data analyses were based on 58 subjects in the test group and 92 subjects in the control group, who remained in the study during the entire 13-year period of monitoring.

Baseline I

The mean age of the subjects was 44 years in both groups and 51% and 57% were males in the test and control groups respectively. The mean number of non-molar teeth was 18.7 in the test group and 18.3 in the control group. Some 30% of all tooth surfaces exhibited the presence of plaque and 66-69% of all gingival units were BoP positive.

The mean PPD was 3.9 mm (0.9 mm) in the test group and 3.7 mm (0.9 mm) in the control group. In both groups, about 50-54% of all sites had shallow pockets (≥3 mm), 28-29% medium-deep pockets (4-5 mm) and 18-21% had deep pockets (≥6 mm). The mean RxBL was 6.5 mm (1.2 mm) in the test group and 6.7 mm (1.3 mm) in the control group. No statistically significant differences in the clinical or radiographic variables were found between the two groups at *Baseline I*.

The effect of basic therapy (*Baseline I – 12 months*)

During the basic therapy phase, 0.5 (2.0) teeth in the test group and 0.3 (2.0) in the control group were extracted. In both groups, a significant improvement was observed in oral hygiene conditions. The plaque score was reduced from 30% at *Baseline I* to about 4-8% at 3 months and the percentage of BoP-positive sites was reduced from about 70% to 20-25% and remained at these reduced levels in both study groups at the 6- and 12-month re-examinations.

The mean PPD value decreased in the test group from 3.9 mm at *Baseline I* to 2.8 mm (mean reduction 1.1 mm) at 3 months. The corresponding decrease in the control group was 0.8 mm (from 3.7 mm to 2.9 mm). The difference in PPD reduction between the test group and the control group was statistically significant ($p<0.05$). The reduced mean PPD value in both groups was maintained at the 6- and 12-month re-examinations.

Both treatment groups showed a gain in PAL at 3 months; test group 0.5 mm (0.5 mm), control group 0.3 mm (0.5 mm) ($p<0.001$). In the test group, the PAL gain was maintained at the 6- and 12-month examinations, while the control group displayed a tendency to lose the initial attachment gain. As a result, at 12 months, the subjects in the test group displayed an average PAL gain from baseline of 0.4 mm, while the corresponding gain in the control group was 0.1 mm ($p< 0.001$).

The data from the 3-month re-examination revealed that the PAL gain in the test group in initially medium-deep pockets (4–5 mm) was 0.7 mm, while it was (≥6 mm) 1.5 mm in deep pockets. The corresponding PAL gains in the control group were 0.4 mm and 1.1 mm respectively. The gain in both site categories was significantly higher in the test group than in the control group ($p<0.01$).

***Baseline II* findings**

At the 12-month examination, there was an average of 18 non-molar teeth in the 2 groups, between 6-8% of the tooth surfaces harbored plaque and in 22-25% of all sites the gingival units were BoP positive. The overall mean PPD was significantly ($p<0.01$) lower in the test group than in the control group (2.7 mm vs. 2.9 mm). The percentages of shallow (≤ 3mm), medium-deep (4-5 mm) and deep (≥6 mm) pockets in the test group were 79%, 19% and 2% respectively. The corresponding percentages in the control group were 74%, 21% and 5%.

Re-examinations after 3, 5 and 13 years

During the 12 years of SPT, an average of 1.3 teeth were lost in the test group and 2.4 teeth in the control group ($p < 0.05$). During the maintenance period, the mean plaque scores varied between 3% and 17% in the 2 study groups. The percentage of BoP-positive sites varied during the same period between 17-33%. The mean PPD increased in both groups between *Baseline II* and the 13-year examination. The mean annual PPD increase was 0.02 mm on average in the test group and 0.03 mm on average in the control group. The mean PPD at the final re-examination was 2.9 mm in the test group and 3.3 mm in the control group ($p < 0.01$).

The frequency of shallow pockets (≤ 3 mm) remained high in both groups in the interval between *Baseline II* and 13 years (67-76%), while the frequency of deep pockets (≥ 6 mm) in the control group tended to increase over time; from 5% at *Baseline II* to 8% at the final examination.

In both groups, there was some loss of PAL during the 12 years of SPT. The annual PAL loss amounted to 0.06 mm in the test group and 0.08 mm in the control group. During the entire 13-year period of observation (*Baseline I* – 13 years), the total mean PAL loss in the test group was 0.28 mm. The corresponding loss in the control group was 0.87 mm ($p<0.001$).

Study IV

Baseline examination

The patients in the 2 study groups were 41.2 and 42.1 years of age on average. In the test group, 60% were smokers, while the corresponding figure in the control group was 56%.

The patients in both groups had a mean of 25 teeth (3rd molars excluded), of which 18 were non-molars.

In the test group, 33% of all tooth surfaces harbored plaque and the corresponding figure in the control group was 28%, while 71% (test) and 67% (control) of the gingival units bled on gentle probing (BoP). The individual mean PPD values were 4.2 mm for the test group and 3.9 mm for the control group ($p<0.05$), while the mean amount of bone loss assessed on radiographs (RxBL) was 6.5 mm in the test group and 6.6 mm in the control group. The frequency of shallow pockets (≤ 3 mm) was 43% in the test group and 49% in the control group, while deep pockets (≥ 7 mm) occurred at a frequency of 15% and 12% in the 2 groups. None of the parameters assessed at baseline differed significantly between the 2 groups, apart from the mean PPD ($p<0.05$).

Re-examinations

1 year

During the first year of non-surgical basic therapy, 0.5 teeth were extracted in both the test group and the control group. The re-examination at the 1-year follow-up revealed that, in both treatment groups, there was a statistically significant reduction in (i) the percentage of plaque-harboring surfaces (-26% and -23%) and BoP+ sites (-49% and -38%), (ii) mean PPD values (-1.0 mm and -0.7 mm) and (iii) the percentage of deep pockets (-11% and -8%). In addition, in both groups, there was a significant clinical attachment level gain in both the test group (0.47 mm) and the control group (0.16 mm). The PAL gain in the test

group and the control group was significantly larger than in the control group ($p<0.001$).

In both groups, there was some PAL loss at sites with initially shallow pockets, while there was a gain at sites with an initial PPD of ≥ 4 mm. Furthermore, the PAL gain was more pronounced at sites which had a PPD of ≥ 7 mm at baseline than at sites in the 4-6 mm PPD category. The PAL gain in the test group was significantly ($p> 0.05$) greater than in the controls. In addition, for both medium-deep and deep pockets, the PAL loss at shallow pocket sites was significantly smaller in the test group than in the control group (-0.10 mm vs.-0.35 mm) ($p< 0.05$).

3, 5 and 13 years

The findings from the re-examinations after 3, 5 and 13 years showed that, during the course of SPT, a mean of 1.7 (2.1) teeth were lost in the test group and 2.7 (3.7) teeth in the controls ($p< 0.01$). The percentage of BoP-positive sites remained at a low level throughout the 12-year period. The mean individual PPD value, which was reduced between baseline and year 1, was still reduced at the 5-year re-examination. Between 5 and 13 years, however, there was a significant PPD increase in both groups ($p< 0.05$). The percentage of sites with deep pockets therefore increased in both groups from an average of 3-4% to 8% (test group) and 7% (control group).

Moreover, the initial (baseline to year 1) gain in the mean probing attachment level gradually diminished. In the control group, the attachment gain was lost at the 3-year re-examination. The 0.47 mm PAL gain that was initially obtained in the test group was lost after 5 years of SPT.

The annual PAL loss that occurred in both groups after the first year was (i) small and varied between 0.07 and 0.11 mm and (ii) was of similar magnitude in the 2 subject groups.

Study V

Baseline examination

In the SO group, 25.5% of the pockets were ≥ 5 mm, while the corresponding figure in the SMM group was 26.3%. The mean PPD of all 5 mm pockets was 5.8 mm and 5.7 mm in the SO and the SMM group respectively. The CAL was 6.6 mm in the SO group and 6.7 mm in the SMM group. None of the parameters assessed at baseline differed significantly between the 2 groups.

Re-examinations

During the 6 months, 12 patients lost 14 teeth, but only 3 of these teeth were non-molar teeth in the surgery quadrants.

The re-examination at the 3- and 6-month follow-up revealed that, in both treatment groups, there was a statistically significant reduction in

(i) BoP+ sites, 62% at 3 months and 56% at 6 months in the SO group and 64% at 3 months and 64% at 6 months in the SMM group, with no statistically significant difference and

(ii) mean PPD reduction, 2.29 (0.09) mm at 3 months and 2.18 (0.10) mm at 6 months in the SO group and 2.48 (0.10) and 2.5 (0.10) mm respectively for the SMM group. The difference in the mean PPD between the two groups at the 6-month examination was statistically significant ($p < 0.05$).

(iii) In smokers, a reduction in BoP+ sites of 64% (5) at 3 months and 54% (4) at 6 months was observed in the SO group and 70% (5) and 66% (4) at 3 months and 6 months in the SMM group, with no statistically significant difference between the groups.

(iv) Smokers displayed a mean PPD reduction of 2.17 (0.11) mm and 2.05 (0.09) mm in the SO group at 3 and 6 months respectively. For the SMM group, the corresponding PPD reduction was 2.40 (0.11) and 2.30 (0.09) mm respectively. The difference between the two groups at the 6-month examination was statistically significant ($p < 0.05$).

(v) Calculations of the number and percentage of sites showing a PPD reduction of ≥ 1, ≥ 2 and ≥ 3 mm between baseline and 3 months by smoking status in the SO group and SMM group respectively revealed that non-smokers in the SMM group had significantly fewer periodontal sites of ≥ 2. This difference also remained at 6 months. For smokers in the SO and SMM groups, this difference was even more pronounced at sites of both ≥ 2 and ≥ 3 mm at both 3 and 6 months for patients in the SMM group.

(vi) No differences in CAL were observed in this study and this is in agreement with the study by Williams et al. (2001).

The differences between the smokers in this study showed a significant difference between the treatment groups in terms of PD change in favor of the patients in the SMM group. Despite this, the non-smokers experienced more pocket depth reduction in both treatment groups.

Discussion and Conclusions

Study I

The *suprabony sites* showed a decrease in the probing depth, which was explained partly by the recession of the gingival margin during the 30-week interval and partly by an improved probing attachment level. A probing attachment gain of ≥ 1 mm occurred at 13 sites, while a gain of > 1 mm occurred at only 4 sites. At the *infrabony sites,* the probing depth reduction was due to the recession of the gingival margin ($p<0.05$) and not to any obvious improvement in PAL. Between baseline and 30 weeks, a PAL gain of 1 mm was observed at 5 sites, while no sites with an attachment gain of > 1 mm could be found. Probing attachment loss (≥ 1 mm) was found at 4 sites.

At the *furcation sites,* the main reason for the reduced PPD was gingival recession, while a few sites exhibited attachment gain. Eleven sites disclosed a gingival recession of ≥ 1 mm and only 5 sites showed a PAL gain (≥ 1 mm). Attachment loss amounting to ≥ 1 mm occurred at 2 sites with an attachment level at baseline of 7 mm.

The probing depth measurements in the present study revealed that the overall number of sites with ≤ 3 and 4 mm deep pockets increased, while the number of 5 mm deep pockets decreased. This observation is in agreement with data presented by Dahlén et al. (1992) and Sato et al. (1993), who reported that subjects who were enrolled in a carefully monitored but *self-performed plaque control program* over periods of 2-3 years exhibited an increase in sites with a PPD of ≤ 3 mm and a decrease in the number of pockets in the 4-5 mm categories. In the study referred to, it was also stated that improved oral hygiene failed to alter the number of sites with ≥ 6 mm deep pockets. However, in the present study, in which *professional, supragingival tooth cleaning* (3 times /week) was practiced, sites with initially deep suprabony pockets also exhibited a pronounced PPD reduction during the course of the experiment; from an average of 5.9 mm at baseline to 3.8 mm after 30 weeks of therapy. This result confirms findings by McNabb et al. (1992) and emphasizes the fact that prolonged periods of professionally administered supragingival tooth cleaning may result in a significant recession in the gingival margin, even at sites with deep suprabony pockets. It should be observed, however, that, in the current study, the improvements in the probing measurements and the amount of soft tissue recession were less pronounced at infrabony and furcation sites.

Microbiological observations

One important observation made in the current trial was that the supragingival plaque control regimen had a marked effect on the subgingival microbiota. At

sites with suprabony and intrabony pockets, as well as at furcation sites, the treatment therefore caused a reduction in the total number of microorganisms that could have been harvested, as well as the percentage of *P. gingivalis*. This observation is in agreement with findings previously reported by Smulow et al. (1983), McNabb et al. (1992), Dahlén et al. (1992), among others, and confirms that the subgingival microbiota is at least partly dependent on the supragingival environment. On the other hand, it may be argued that the probing depth reduction, which occurs during periods of enhanced plaque control, due to continuous gingival recession, may change the subgingival environment and thereby explain the ensuing change in the microbiota. This would then imply that oral hygiene regimens per se only have an indirect impact on the subgingival flora. In the present subject sample, however, it was found that, even at sites where no pocket depth reduction occurred, the TVC and the percentage of *P. gingivalis* were substantially reduced. This observation (i) tends to validate the suggestion by McNabb et al. (1992) "that the niche occupied by *P. gingivalis* in moderately deep pockets may be rather tenuous" and (ii) indicates that this hypothesis is also valid for deep pockets with different locations and anatomy.

Haffajee et al. (2001) reported that, as a result of *self-performed plaque control* measures, the composition of the microbiota associated with periodontitis in the pocket was altered and the percentage of periodontopathic bacteria was lowered. These results are in agreement with results observed by Del Peloso Ribeiro et al. (2005), who found a significant reduction in the microbiota associated with periodontitis compared with baseline values in the subgingival environment detected by measuring the level of trypsin-like enzymes (BAPNA test) possessed by microorganisms such as *T. forsythensis, Treponema denticola* and *P. gingivalis* after 21 days of a *self-performed plaque control* program.

The finding that improved supragingival plaque control may affect the subgingival environment and its microflora appears to disagree with results previously reported by Listgarten et al. (1998), Kho et al. (1992), Beltrami et al. (1986) and Loos et al. (1998), for example. In this context, differences in the experimental design must be considered. While, in the current trial, the participants were exposed to *professionally administered tooth cleaning* and concomitant oral hygiene instruction several times a week over a 30-week period, the oral hygiene improvements in the studies referred to were mainly dependent on patient compliance *(self-performed plaque control)*. As a result, differences in the quality of the supragingival infection control and differences in bone topography may explain why different conclusions were reached with respect to the effect of supragingival plaque control on the subgingival biofilm.

It was also observed that, at both categories of sites – suprabony and infrabony, there was a marked reduction in the subgingival microbiota. Both the TVC and the number of the marker species (*P. gingivalis*) were markedly reduced at both types of site between the baseline examination and the assessments made at the end of the clinical trial.

There are therefore reasons to suggest that the subgingival microbiota is markedly influenced by the biofilm present in the supragingival environment and the associated inflammation in the overt gingiva. In this respect, it must be understood that, at sites with deep infrabony defects, supragingival plaque control may not change the subgingival biofilm sufficiently to prevent the progression of destructive periodontitis (Westfelt et al. 1998).

Conclusions

The findings indicate that professionally administered and frequently repeated supragingival tooth cleaning, combined with careful self-performed plaque control, had a marked effect on the subgingival microbiota of moderate to deep periodontal pockets. At sites with suprabony and infrabony pockets, as well as at

furcation sites, meticulous, prolonged supragingival plaque removal reduced the total number of microorganisms that could be harvested, as well as the percentage of sites with *P. gingivalis*.

Supragingival plaque control – managed by professionals and self-performed efforts maintained for several months – resulted in the resolution of gingivitis and a reduction in the probing depth (PPD) at tooth sites with suprabony lesions and at furcation lesions. No corresponding PPD reduction occured at sites with infrabony lesions at which the hard tissue wall of the defect obviously prevented a substantial soft tissue recession.

Study II

The finding that 6 months of *non-supervised* tooth brushing with a dentifrice containing 0.3% magnolia extract had a more pronounced effect on dental plaque and gingivitis than a corresponding control dentifrice is in agreement with observations reported from other studies in which antiseptic and anti-inflammatory dentifrices and mouth rinses were included as adjunctives to selfperformed oral hygiene (Lindhe et al. 1993, Rosling et al. 1997, Charles et al. 2001, 2004). Furthermore, in the patients who used the magnolia dentifrice, less gingival inflammation was observed at sites harboring similar amounts of plaque compared with subjects using an ordinary fluoride dentifrice. Schätzle and coworkers (2004) reported that teeth that were observed with healthy gingival conditions at every examination interval during a 26-year period had a 99.5% chance to be maintained compared with 65.4% for teeth that were always diagnosed with gingival bleeding. Methods to enhance plaque control and reduce the inflammation may therefore increase tooth survival. The herb extract magnolia has been shown to retard plaque and gingival inflammation in a test group of individuals compared with a control group, without producing any adverse events. However, in the present study, the subjects were not instructed in optimal plaque control and the degree to which an optimized

mechanical plaque regimen may have influenced the results without any antiseptic or anti-inflammatory agents was not investigated in the present study. Compared with other antiseptic and anti-inflammatory agents incorporated in dentifrices and mouth rinses, 0.3% magnolia extract exerts comparable effects on gingivitis to those obtained by e.g. by triclosan and Listerine®.

Conclusions

It was observed that six months of unsupervised tooth brushing with a dentifrice containing 0.3% magnolia extract resulted in a significantly larger reduction in plaque and gingivitis compared with a control dentifrice and less gingival inflammation at sites with similar amounts of plaque.

Study III

It was observed that PVP iodine, applied topically during non-surgical periodontal therapy, influenced treatment outcome. During the first year of observation, there was both a PPD reduction and a probing attachment level (PAL) gain in the test group that was more pronounced than in the control group However, the effect of PVP iodine during the maintenance phase (1-13 years) was less marked than during the basic therapy phase. It was also observed that the effect of PVP iodine was more pronounced in initially deep pockets than in shallow pockets. It is therefore suggested that the use of PVP iodine in non-surgical treatment, as well as during supportive periodontal therapy in patients with advanced periodontal disease, may lead to an enhanced, more long-lasting outcome, i.e. reduced signs of gingivitis, more shallow pockets and more PAL gain.

These findings are in agreement with data previously presented from clinical studies by Rosling et al. (1986), Christersson et al. (1988) and Forabosco et al. (1996), in which 0.5% PVP iodine was used in conjunction with non-surgical, ultrasonic scaling. Hoang et al. (2003) presented microbiological results showing that subgingival flushing with 10% PVP iodine for 5 minutes as an

adjunct to SRP resulted in a reduction in the total number of pathogens of at least 95% in 43.8% of the study sites, whereas, in three other treatment groups, a corresponding reduction from 6.3% to 12.5% of study sites was observed.

However, in the current investigation, a lower concentration of the agent was used. The decision to use a 0.1% solution of PVP iodine in the current study was based on findings reported by Caufield et al. (1987). They used the membrane transfer technique to demonstrate that both 0.1% and 0.5% solutions of iodine had a bactericidal effect on *Aggregatibacter actinomycetemcomitans, Porhyromonas gingivalis* and *Prevotella intermedia* within a 5-minute period of exposure.

However, the beneficial effect of PVP iodine in conjunction with ultrasonic scaling has not been verified in studies by Grossi et al. (1997) or Leonardt et al. (2006, 2007). Grossi et al. (1997) did not find any adjunctive effect from either CHX, or PVP iodine when used in the cooling system during ultrasonic debridement. In the study by Grossi et al. (1997) the patients also received systemic administration of doxycycline during the active treatment phase. The study did not include any treatment group subjected to ultrasonic scaling combined with PVP iodine alone. In the study by Leonhardt et al. (2006, 2007), the clinical outcome of ultrasonic scaling combined with 0.5% PVP iodine was examined in 20 patients. A split-mouth design, including ≥ 1 single-rooted tooth/quadrant with ≥ 1 site with an initial PPD of ≥ 6 mm, was used. All the defects had a suprabony location. The 6-month evaluation revealed no differences in the reduction of PPD, i.e. at the sites subjected to ultrasonic scaling with either PVP iodine or saline used in the cooling system. The quadrants subjected to ultrasonic scaling with or without 0.5% PVP iodine solution showed a significant reduction in both PPD and BoP, but the two treatment modalities were not significantly different. The results of the microbiological examination (Leonhardt et al. 2007) showed a reduction in periodontal pathogens by ultrasonic scaling but with no adjunctive effect from

PVP iodine. A distinction that could have had an impact on the result is the different study design in the two trials. A split-mouth design can minimize the difference between test and control sites, as the antiseptic is distributed into the oral cavity and affects the microbes not only in the saliva but also in niches in the tongue and mucosa (Quirynen et al. 2000). Furthermore, the untreated control sites may serve as reservoirs for translation and re-population of pathogentic microorganisms to the test sites which also may have an inpact on the results (Hoang et al. 2003).

The effect of PVP iodine during the maintenance phase (1-13 years) was less marked than during the basic therapy phase. However, the data indicated that the adjunctive use of PVP iodine for the supportive periodontal therapy at each 3- to 4-month recall visit in patients with advanced periodontal disease resulted in more shallow pockets and more probing attachment gain than was seen in the patients in the control group.

Conclusions

The findings from the present study demonstrated that the topical application of 0.1% povidone (PVP)-iodine, used as a cooling agent in conjunction with ultrasonic, subgingival root debridement, established conditions that improved the outcome of non-surgical therapy. At the first re-examinations after active therapy (the 3-, 6- and 12-month examinations), the subjects in the test group exhibited significantly lower mean probing pocket depth values and had significantly more gain in probing attachment than the subjects in the control group. It was further observed that the effect of PVP iodine was more pronounced in initially deep pockets than in shallow pockets. In addition, the number of “loser subjects” (**losers**), i.e. subjects exhibiting further disease progression during the first phase of SPT, was much smaller in the test group than in the control group. Whether measured by (i) the percentage of **losers**, (ii) the initial gain in attachment or (iii) long-term PAL monitoring, the test group

exhibited approximately half the rate of disease progression compared with the control group.

Study IV

The finding that adjunctive treatment with systemic tetracycline may improve non-surgical periodontal therapy is in agreement with findings reported by Listgarten et al. (1978), Slots et al. (1979), Lindhe et al. (1983), Lundström et al. (1984) and Palmer et al. (1996), for example. However, in the present trial, it was noted that, in shallow pockets, the PAL loss was significantly smaller in the test group than in the control group and the PAL gain at deeper sites was significantly greater in the test group than in the control group. These observations appear to indicate that tetracycline, in one way or another, may have protected from attachment loss in shallow pockets and promoted attachment gain at deeper sites. A hypothesis of this kind is supported by the findings of Vernillo et al. (1994), who demonstrated that tetracyclines not only have antibacterial properties but may also inhibit the extracellular activity of collagenase released from mammalian cells. This metalloproteinase is of importance in the breakdown of collagen in inflammatory lesions and is also inhibited in later phases of proper wound healing. In addition, tetracyclines are potent inhibitors of osteoclast function. There are therefore reasons to suggest that tissue damage caused by subgingival instrumentation during the basic treatment phase at both shallow and deep sites may have been diminished in the patients treated with tetracycline. Furthermore, it was observed that tetracycline, administered systemically during the active phase of therapy, had a positive impact on treatment outcome in terms of PAL gain.

Conclusion

It was observed that tetracycline, administered systemically during the active phase of therapy, had a positive impact on treatment outcome in terms of PAL gain. One year after active therapy, the probing attachment level in the test group was almost 3 times higher than in the control group. Re-examinations after 3, 5 and 13 years of SPT disclosed that this short-term benefit was not maintained in the longer perspective.

It was noted that the small amount of annual loss of attachment that occurred between years 1 and 13 did not differ between the 2 groups (0.07 mm and 0.11 mm).

Study V

It was observed that minocycline microspheres applied topically during surgical periodontal therapy improved the treatment outcome. During these 6 months of observation, there was a PPD reduction in the test group that was more pronounced than in the control group, where the treatment included surgical periodontal therapy without any adjunctive antibiotic. Subgingival applications of minocycline microspheres produced a significantly larger reduction (0.3 mm) in mean probing depth in combination with periodontal surgery in adults with moderate to severe, chronic periodontitis than surgery alone in both smokers and non-smokers. These results are not in agreement with Palmer et al. (1996) or Needlemann and coworkers (2000), who did not find any additional effect from adjunctive antibiotic therapy during periodontal surgery. Palmer et al. (1996) treated 38 patients with aggressive periodontitis (i) with SRP and 250 mg x 4 of systemic antibiotics for 14 days and concluded that this was a useful adjunct to non-surgical treatment. At re-examination after 3 months, (ii) periodontal surgical treatment with a modified Widman flap and a renewed prescription of 250 mg x 4 of systemic antibiotics for 14 days in teeth with a PPD of ≥ 5 and BoP were given. The authors concluded after 12 months that “no further

advantage was obtained by the antibiotic in the surgical phase, although this may also be a result of the smaller number of subjects and fewer sites treated in the surgical phase". Needlemann et al. (2000) studied the additional effect of 25% metronidazol gel as an adjunct to Modified Widman Flap (MWF) surgery. Forty-three patients with moderate to advanced periodontal disease were followed in a randomized, single-blind, parallel-armed study for 12 months. At the 12-month re-examination, no adjunctive effect from the local antibiotitic was observed, with a PD reduction of 3.7 and 3.6 mm in the test and control group respectively.

Smoking as a lifestyle factor in periodontitis-susceptible individuals has been shown significantly to increase attachment and tooth loss (Bergström et al. 1989, Axelsson et al. 1998, Paulander et al. 2004). Substances from tobacco produced during smoking have been shown to enhance the metalloproteinase action and have a negative impact on the collagen in periodontal connective tissue (Goodson 1989). Bergström et al. (1989) found 56% of smokers in a group of 155 patients suffering from severe periodontitis. From a random sample of subjects of equal numbers (control group) but with no knowledge of their periodontal status, the percentage of smokers was calculated. In this control group, 34% of the subjects were smokers. Furthermore, Paulander and coworkers (2004) investigated the age group of 50 years in a cross-sectional study. They found significantly more attachment and tooth loss in smokers than in non-smokers. Vernillo and coworkers (1994) reported that tetracycline may reduce collagenase activity in the inflammatory connective tissue and also inhibit osteoclast activity. These findings may in part explain why smokers who have increased attachment loss and collagenase and osteoclast activity compared with non smokers, may respond with less attachment loss and reduced pocket depths when treated with minocycline.

Conclusion

After 6 months, the subgingival application of minocycline microspheres produced a significantly greater reduction (0.3 mm) in mean probing depth in combination with periodontal surgery in adults with moderate to severe, chronic periodontitis than surgery alone, in both smokers and non-smokers.

Future considerations

Six months use of a dentifrice containing 0.3 % magnolia extract in patients with gingivitis resulted in less gingival inflammation (about 10%) than in patients brushing with a dentifrice without the magnolia extract.

In patients with severe chronic periodontitis no difference in the percentage of bleeding sites was observed after treatment with ultrasonic SRP with or without 0.1% Povidone iodine used as a cooling agent. However, both treatment regimens reduced inflammation in the periodontal tissues more than 40 %. Furthermore, SRP with or without adjunctive treatment with systemic tetracycline reduced the inflammation with an additional 10% compared to only SRP (49% vs 38%). Finally, in patients who were treated with surgery and topical application of minocycline around 60% less bleeding on probing was observed.

Thus, there was a clinical albeit moderate effect on the level of gingival inflammation in subjects who brushed with a dentifrice containing magnolia extract. Also, the additional effect of tetracyclines, either administered systemically or topically, or iodine in adjunction to SRP and/or surgery had a limited effect on gingival bleeding.

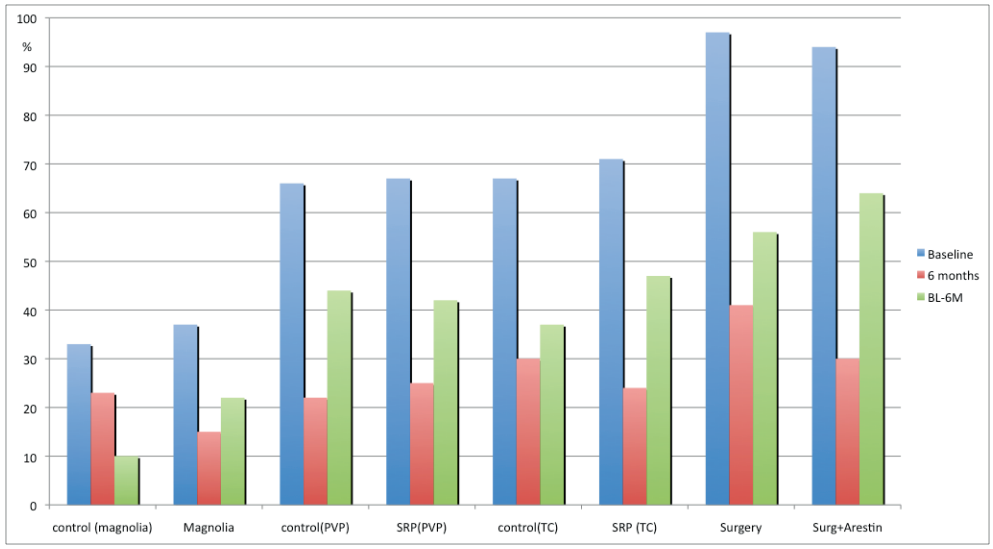

Figure 6. Percentage of sites with bleeding on probing (BoP)

The use of antiseptics/antibiotics as an adjunct to mechanical treatment during the active phase of periodontal therapy resulted in an additional mean pocket reduction of 0.3-0.4 mm. Furthermore, despite an increasing pocket depth which developed during the maintenance phase the differences observed between the test and control groups after active treatment was still maintained. Actually, during the PVP iodine regime the mean pocket depth reduction obtained after the active treatment was even larger at the 13-year examination than in the control group (0.4mm). This may be explained by the fact that all patients in the test group received SRP+PVP iodine treatment every third month.

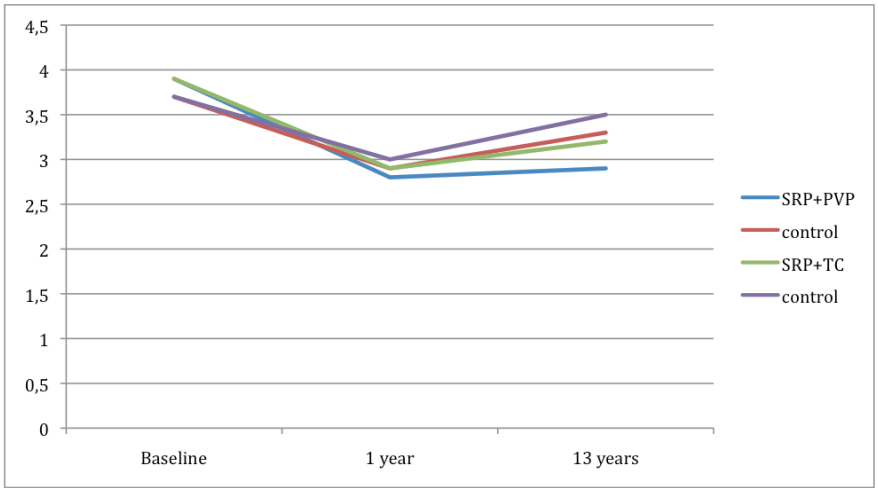

Figure 7. Mean Probing pocket depth

The effect of meticulous plaque control on pockets with a PPD of ≥5 mm but with various bone topography had an obvious clinical effect on pocket reduction only in those sites with a horizontal bone level whereas no such effects were observed at sites with infrabony defects. However, it was found that surgical treatment irrespective of the bone morphology of the periodontal pockets resulted in the similar pocket reduction that was observed for suprabony pockets after professional delivered supragingival plaque control. Thus, the results indicate that periodontal surgery in infrabony/vertical defects may be more justified than for sites with only suprabony defects. Furthermore, topical application of minocycline in conjunction to surgery resulted in a minor additional pocket reduction of 0.3 mm.

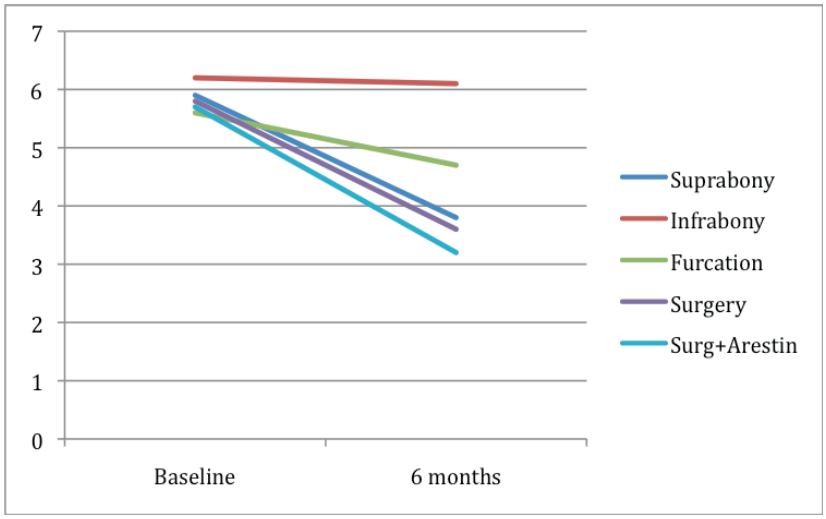

Figure 8. Mean pocket reduction (mm)

Meticulous supragingival plaque control combined with surgical/non-surgical therapy and additional antiseptics/antibiotics may be beneficial for patients with advanced periodontal disease although the mean additional effect is small. However, this minor adjunctive effect was observed in studies where the subjects had performed thorough plaque control and may not be considered to be decisive for the long term maintenance of the periodontal health of the patient.

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