Host response modulation in periodontics

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Host response modulation (or host modulation) is a term that has been introduced to the dental profession relatively recently. In the periodontal context, and in very simple terms, it means modifying or modulating destructive or damaging aspects of the inflammatory host response that develops in the periodontal tissues as a result of the chronic challenge presented by the subgingival bacterial plaque. Host response modulation is routinely practised by our medical colleagues, who use host modulation strategies in the treatment of disorders such as rheumatoid arthritis and osteoporosis. And while the term host modulation has only recently started to be widely used in general dentistry, the concept was first introduced to the research community in the late 1980s and early 1990s (34, 107). Indeed, in 1990 Williams (107) stated that 'there are compelling data from studies in animals and human trials indicating that pharmacologic agents that modulate the host responses believed to be involved in the pathogenesis of periodontal destruction may be efficacious in slowing the progression of disease'. Over the last two decades, a variety of pharmacological agents have been studied for a possible role as host modulators in the management of periodontal disease. These include nonsteroidal anti-inflammatory drugs, bis-phosphonates and the tetracycline family of compounds (and their chemically modified analogues). Newer agents that have the potential to be of benefit in periodontal treatment include anti-cytokine drugs (which have successfully been used in the treatment of rheumatoid arthritis), soluble cytokine blockers and lipoxins. To date, only one systemic medication has been licensed specifically as a host response modulator for the treatment of periodontal disease, and that is subantimicrobial dose doxycycline (a focus in this review). The inclusion of host response modulation into periodontal management strategies is an exciting development with significant potential for improving treatment outcomes. It is likely that the future will see a range of host modulation therapies developed that target different aspects of the inflammatory pathogenic processes which occur in the diseased periodontium.

Periodontal pathogenesis

To a large extent, the emergence of host response modulation as a treatment concept has resulted from our improved understanding of the pathogenesis of periodontal disease. A common observation in periodontal practice is that while gingivitis and mild periodontitis are relatively common in the population, severe periodontitis is less prevalent, despite plaque being a common finding in a majority of people. Thus, advanced periodontal disease is now considered to affect approximately 8–15% of adults (3, 9, 48), which is considerably less than that reported in epidemiological studies conducted in earlier decades (63). Certain individuals appear to be more susceptible to periodontal disease, and this increased susceptibility is largely determined by the immune-inflammatory response that develops in the periodontal tissues following chronic exposure to bacterial plaque.

Periodontal pathogenesis has been extensively reviewed by a number of authors (52, 54, 73) and it is not the purpose of this paper to cover this ground again. Suffice to say, the microbial challenge presented by subgingival plaque results in an upregulated host immune-inflammatory response in the periodontal tissues that is characterized by the excessive production of inflammatory cytokines (e.g. interleukins, tumor necrosis factor-α), prostanoids (e.g. prostaglandin E_2) and enzymes [including the matrix metalloproteinases (MMPs)]. These pro-inflammatory mediators are responsible for the majority of periodontal breakdown that occurs, leading to the clinical signs and symptoms of disease.

Perhaps more important than the levels of any single inflammatory mediator in the periodontal tissues is the relative balance between pro-inflammatory and anti-inflammatory cytokines and enzymes. Thus, pro-inflammatory mediators, such as prostaglandins and many cytokines, are balanced by anti-inflammatory cytokines and lipoxins (102). The destructive activities of MMPs are balanced by their inhibitors, the tissue inhibitors of metalloproteinases. Imbalances between pro-inflammatory and anti-inflammatory activities in the periodontal tissues are a major determinant of periodontal destruction.

The immune-inflammatory response against bacterial plaque can thus be viewed as a 'two-edged sword'. That is, the response is protective by intent, and provides antibodies and polymorphonuclear neutrophils that are responsible for the control of the bacterial infection. However, the inflammatory response, in certain individuals, results in the local production of excessive quantities of destructive enzymes and inflammatory mediators that result in the tissue destruction which is observed clinically. It is paradoxical (although not unique in human diseases) that the inflammatory response to the bacterial challenge is primarily responsible for the breakdown of the periodontal hard and soft tissues. Periodontal disease is characterized by high concentrations of MMPs, cytokines and prostanoids in the periodontal tissues, whereas periodontal health is characterized by the opposite (75).

Plaque bacteria therefore initiate the disease process, and bacterial antigens that cross the junctional epithelium into the underlying connective tissues drive the inflammatory response. Bacteria are therefore a necessary prerequisite for disease to develop but are insufficient to cause periodontal disease alone. For periodontal disease to develop, a susceptible host is also required, in other words a host in which excessive production of destructive enzymes (such as MMPs) and inflammatory mediators (e.g. interleukins and prostaglandins) are released during the cascade of destructive inflammatory events that occur as part of the inflammatory response (76). The purpose of host modulatory therapy is to restore balance between, on the one hand, pro-inflammatory mediators and destructive enzymes, and, on the other hand, anti-inflammatory mediators and enzyme inhibitors.

Conventional treatment strategies

The mechanical removal of plaque and calculus is considered as the standard treatment for periodontitis. This treatment approach has changed little over the years, notwithstanding considerable debate about issues such as the merits of manual vs. ultrasonic instrumentation, or the degree of root surface smoothness / hardness to be achieved. Traditionally, this process has been referred to as 'root planing', although the term 'root surface instrumentation' is now preferred as it is felt that 'planing' places too much of an emphasis on the removal of cementum and dentine from the root to create a smooth, hard surface, which has now been demonstrated as unnecessary for periodontal healing (15). The aim of root surface instrumentation is to disrupt physically the subgingival biofilm and reduce the bacterial bioburden, while removing plaque and calculus to as large an extent as is achievable. The objective of this treatment is to reduce the chronic challenge presented by the subgingival plaque bacteria, such that inflammatory responses in the periodontal tissues are reduced, leading to resolution of inflammation and shrinkage of the gingival tissues. Resulting shallower pockets (with further gains in attachment possibly arising from the formation of a long junctional epithelium) are easier to maintain for both the patient and the clinician, and favour a less pathogenic microflora.

The outcomes that can be expected following nonsurgical periodontal therapy are remarkably consistent. For example, for those pockets initially 4–6 mm deep, mean probing depth reductions of approximately 1.0–1.5 mm and mean attachment gains of 0.5–1.0 mm can be expected (15). For deeper pockets (7 mm or greater), mean probing depth reductions of 2.0–2.5 mm and mean attachment gains of 1.0–1.5 mm can be expected (15). In many patients, nonsurgical management alone (comprising oral hygiene instruction, root surface instrumentation and periodontal maintenance care) may be sufficient to result in clinical improvements and control of periodontal disease. However, there are many patients in whom treatment responses following conventional treatment are more limited and both patient and clinician may ask if anything further can be done. Our improved understanding of the pathogenesis of periodontal disease has led to the development of host modulation as a treatment strategy that can be used in addition to conventional treatment approaches. Thus, a combination of therapeutic approaches may offer the best chance for clinical improvements (82), and this could include:

- reduction in the bacterial burden (by root surface instrumentation and hygiene therapy).

- risk factor modification (by smoking cessation and improved diabetes control).
- host response modulation.

As healthcare professionals, we should strive to continually improve patient choice and enhance the patient's experience as they progress through a course of treatment under our care. Central to this is the concept of patient education and involvement in decision-making about treatment strategies. A paternalistic stance, in which the clinician makes all decisions for the patient based on their superior knowledge, is no longer appropriate. Thus, patients must become partners with the clinician and assume a degree of responsibility for their care. The importance of this in periodontal treatment is quite clear, given that patient compliance (with oral hygiene, periodontal maintenance and smoking cessation) has a profound effect on treatment outcomes. This is not to say that responsibility for a good treatment outcome can be devolved entirely to patients. Rather, it is our responsibility to make sure that patients are aware of the importance of their own self-management, and we as clinicians facilitate this through education, motivation, empowerment and, of course, the provision of excellent clinical care. These concepts fit well with management protocols in which adjunctive host modulation is combined with conventional treatment strategies. Anecdotally, host modulation therapy is typically welcomed by the patient once they have been informed of the rationale for such an approach.

Host modulation agents: historical perspective

Host modulatory therapy is a treatment concept that aims to reduce tissue destruction and stabilize the periodontium by downregulating or modifying destructive aspects and / or upregulating protective or regenerative components of the host response. Host modulatory therapies could include systemically or locally delivered pharmaceuticals that are prescribed as adjuncts to other forms of periodontal treatment. Host modulatory therapies offer the opportunity to move periodontal treatment strategies to a new level. Historically, periodontal treatment has focused on reducing the bacterial challenge by root surface instrumentation. However, the outcomes after conventional treatment of this chronic disease are not always optimal, predictable or stable. Periodontal disease can be viewed as a balance between (i) a persisting bacterial burden and pro-inflamma-

tory destructive events in the tissues, and (ii) resolution of inflammation and downregulation of destructive processes. Reducing the bacterial bioburden by root surface instrumentation targets one aspect of the pathogenic process by reducing the antigenic challenge that drives the inflammatory response in the tissues. However, complete elimination of all subgingival bacteria is not achievable (or even desirable), and recolonisation by putative pathogens occurs. Host response modulation therefore offers the potential for downregulating destructive aspects of the host response so that, in combination with conventional treatments to reduce the bacterial burden, the balance between health (characterized by resolution of inflammation and wound healing) and disease progression (characterized by continued pro-inflammatory, destructive events) is tipped in the direction of a healing response.

In periodontitis, the host is responsible for most of the tissue breakdown that occurs, leading to the clinical signs of disease. Host response modulators offer the potential for modulating or reducing this destruction by ameliorating excessive or pathologically elevated inflammatory processes to enhance opportunities for would healing and periodontal stability. A variety of drug classes have been evaluated as host response modulators, including the nonsteroidal anti-inflammatory drugs, bisphosphonates, and tetracyclines.

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs inhibit the formation of prostaglandins, including prostaglandin E_2, which is produced by a variety of resident and infiltrating cell types in the periodontium (including neutrophils, macrophages, fibroblasts and epithelial cells) in response to lipopolysaccharide. Prostaglandin E_2 is a key inflammatory mediator in periodontal disease as it upregulates osteoclastic bone resorption (45, 74), and prostaglandin E_2 levels are significantly increased in the tissue and gingival crevicular fluid of patients with periodontal disease compared to healthy controls (37, 74). Nonsteroidal anti-inflammatory drugs inhibit the formation of prostaglandins by blocking the cyclo-oxygenase pathway of arachidonic acid metabolism. They are used to reduce tissue inflammation and pain, and are indicated in a variety of chronic inflammatory diseases. The ability of nonsteroidal anti-inflammatory drugs to block prostaglandin E_2 production, thereby reducing inflammation and inhibiting osteoclast activity, has been investigated in patients with periodontal disease.

Thus, studies have shown that systemic flurbiprofen (109), indomethacin (108), naproxen (44) and others (43), administered daily for periods of up to 3 years, significantly slowed the rate of alveolar bone loss compared to patients treated with placebo. It is noteworthy that in one of these early papers published in 1993, the authors commented that 'research into nonsteroidal anti-inflammatory drugs in periodontal therapy may ultimately prove to have only opened the door to research into host modulation as an additional, but exciting, approach to periodontal disease prevention and treatment' (45).

However, the nonsteroidal anti-inflammatory drugs suffer from some serious disadvantages that essentially preclude their use as an adjunctive treatment for periodontal disease. Daily administration for extended periods of time (years rather than months) is necessary for periodontal benefits to become apparent, and the nonsteroidal anti-inflammatory drugs are associated with significant unwanted effects, including gastrointestinal problems, hemorrhage (as a result of decreased platelet aggregation), and renal and hepatic impairment. Also, once patients cease taking nonsteroidal anti-inflammatory drugs, a return to, or even acceleration of, the rate of bone loss seen prior to drug therapy occurs, sometimes referred to as a 'rebound effect'.

The selective cyclo-oxygenase-2 inhibitors were investigated in the anticipation that they could offer potential in the treatment of periodontitis. The enzyme cyclo-oxygenase, which metabolizes arachidonic acid, exists in two functionally distinct isoforms: cyclo-oxygenase-1 (which is constitutively expressed and has antithrombogenic and cytoprotective functions); and cyclo-oxygenase-2 (which is induced after stimulation with various cytokines, growth factors and lipopolysaccharide). Inhibition of cyclo-oxygenase-1 by nonselective nonsteroidal anti-inflammatory drugs results in the majority of the unwanted effects associated with nonsteroidal anti-inflammatory drug use, such as gastrointestinal ulceration and impaired hemostasis. Induction of cyclo-oxygenase-2 results in the production of elevated quantities of prostaglandins, and therefore inhibition of cyclo-oxygenase-2 by selective inhibitors results in a reduction of inflammation without the unwanted effects commonly seen after long-term nonsteroidal anti-inflammatory drug use. Preliminary studies in animal models showed that selective cyclo-oxygenase-2 inhibitors slowed alveolar bone loss (5, 42), and human studies confirmed that prostaglandin production in the periodontal tissues was modified (103). However, the selective cyclo-oxygenase-2 inhibitors were later identified to be associated with significant and life-threatening adverse events, resulting in several of these drugs being withdrawn from the market (21).

In summary, nonsteroidal anti-inflammatory drugs have been extensively reviewed as potential host response modulators for the treatment of periodontal disease (90), but unwanted effects (including the serious adverse effects of the selective cyclo-oxygenase-2 inhibitors) preclude their use as adjuncts to periodontal treatment.

Bisphosphonates

The bisphosphonates disrupt osteoclastic activity and thereby inhibit bone resorption. Bisphosphonates represent a class of chemical compounds structurally related to pyrophosphate, which regulates mineralization by binding to hydroxyapatite crystals, but is not stable *in vivo*, undergoing hydrolysis of its P-O-P bond as a result of pyrophosphatase activity (90). The replacement of the oxygen atom with a carbon atom (creating a P-C-P bond) results in the formation of a bisphosphonate molecule that is chemically stable and resists hydrolysis via pyrophosphatase and alkaline phosphatase. Thus, bisphosphonates bind to hydroxyapatite crystals and prevent their dissolution. They also increase osteoblast differentiation and inhibit osteoclast activation, and are used extensively in the management of osteoporosis and other bone-resorptive conditions.

Given these properties, it is not surprising that bisphosphonates have been investigated as adjuncts in the treatment of periodontal disease, and in a study in dogs of naturally occurring periodontitis, the use of alendronate was associated with a significant increase in bone density compared with placebo (85). In experimentally induced periodontitis in animal studies, bisphosphonates reduced alveolar bone resorption (92, 106), and in human studies, bisphosphonates increased alveolar bone density (22). In randomized, placebo-controlled clinical trials in humans with periodontal disease, bisphosphonate use resulted in statistically significant reductions in the proportion of teeth demonstrating alveolar bone loss after 9 months (84) and statistically significant improvements in alveolar bone height (87). However, a recent systematic review concluded that the small number of studies that have been published, each containing few subjects and investigating different outcome measures, prevented meaningful conclusions from being drawn about the benefits of bis-

phosphonates when used as an adjunctive treatment in periodontal disease (84). Following this, a more recently published study of a randomized clinical trial of 335 patients with periodontal disease who received either alendronate or placebo, reported that after 2 years of therapy there were no differences in alveolar bone level or bone density between the two treatment groups (47). However, in a subgroup of patients with low mandibular bone mineral density, alendronate significantly reduced bone loss compared with placebo.

A recent development has been the publication of several case reports of avascular necrosis of the jaws, particularly the mandible, following bisphosphonate therapy, with an increased risk of bone necrosis following dental extractions. This has been termed bisphosphonate-associated osteonecrosis and is a significant and clinically serious complication of bisphosphonate therapy (55). Current guidance is to avoid extractions in patients who have received long-term bisphosphonate therapy and seek expert opinion (55). At the present time, there are no bisphosphonate drugs that are approved and indicated for use as adjuncts in the treatment of periodontal disease.

Subantimicrobial dose doxycycline

Subantimicrobial dose doxycycline remains, at present, the only systemic host response modulator specifically indicated as an adjunctive treatment for periodontitis and will therefore be a focus in this review. Subantimicrobial dose doxycycline is approved by the US Food and Drug Administration, the UK Medicines and Healthcare products Regulatory Agency, and by similar agencies in other countries throughout the world, and was introduced under the trade name Periostat® (CollaGenex Pharmaceuticals Inc., Newtown, PA). It is a 20-mg dose of doxycycline hyclate that is taken twice daily for periods of 3–9 months as an adjunct to root surface instrumentation in the treatment of periodontitis.

Doxycycline, similarly to other members of the tetracycline family, has the ability to down-regulate MMPs, a family of zinc-dependent enzymes that are capable of degrading a variety of extracellular matrix molecules, including collagens (6, 88). MMPs play a key role in the tissue destruction observed in periodontal disease and are secreted by the majority of cell types in the periodontium, including macrophages, neutrophils, fibroblasts, keratinocytes, endothelial cells and osteoclasts. In healthy tissues, MMPs are produced primarily by fibroblasts (MMP-1

or collagenase-1) and are concerned with the maintenance of the periodontal connective tissues. Transcription of genes coding for MMPs is upregulated by inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α (61). Regulation of MMP activity involves specific, endogenous tissue inhibitors of metalloproteinases and α-macroglobulins, which form complexes with active MMPs and their latent precursors (86, 88). Tissue inhibitors of metalloproteinases are produced by fibroblasts, macrophages, keratinocytes and endothelial cells (7).

In healthy tissues, collagen homeostasis is a controlled process that is mediated extracellularly by MMP-1 (fibroblast collagenase) and intracellularly by a variety of lysosomal acid-dependent enzymes. Excessive quantities of MMPs are secreted in inflamed periodontal tissues, and the balance between MMPs and their inhibitors is disrupted, resulting in breakdown of the connective tissue matrix (6, 97). Neutrophils are key infiltrating cells in periodontitis that accumulate in large numbers in inflamed periodontal tissues. Neutrophils have evolved to respond rapidly and aggressively to external stimuli and they release large quantities of destructive enzymes very rapidly (6). The predominant MMPs in periodontitis – MMP-8 and MMP-9 – are secreted by neutrophils (33) and are very effective in degrading type 1 collagen, the most abundant type of collagen in the periodontal ligament (62). MMP-8 and MMP-9 levels increase with increasing severity of periodontal disease and decrease after treatment (28, 33). The excessive release of large quantities of MMPs in the periodontium leads to significant breakdown of structural components of the connective tissues, contributing to the clinical signs of disease.

The rationale for using doxycycline at subantimicrobial doses as a host response modulator is that it inhibits the activity of MMPs by a variety of synergistic mechanisms independent of any antibiotic properties (summarized in Table 1). Early studies on the use of tetracyclines to inhibit MMPs identified, interestingly, that those metalloproteinases which were produced in excessive quantities in inflamed periodontal tissues were more sensitive to inhibition by tetracyclines than those MMPs that were constitutively expressed. Thus, MMP-13 is more sensitive to tetracycline inhibition, with an inhibitory concentration$_{50}$ (IC_{50}) of <1 μM (i.e. the concentration of tetracycline required to reduce the MMP concentration by 50%), than MMP-8 ($IC_{50} \approx 30$ μM), and MMP-1 (fibroblast collagenase) is the least sensitive ($IC_{50} > 200$ μM) to inhibition by tetracyclines *in vitro* (31). Furthermore, doxycycline was shown to be more

Table 1. Downregulation of destructive events in the periodontium by doxycycline results from modulation of a variety of different pro-inflammatory pathways [adapted from Golub et al. (31)]

Effect of doxycycline
• Direct inhibition of active MMPs by cation chelation (dependent on Ca^{2+}- and Zn^{2+}-binding properties)
• Inhibits oxidative activation of latent MMPs (independent of cation-binding properties)
• Downregulates expression of key inflammatory cytokines (interleukin-1, interleukin-6 and tumor necrosis factor-α) and prostaglandin E_2
• Scavenges and inhibits production of reactive oxygen species produced by neutrophils
• Inhibits MMPs and reactive oxygen species thereby protecting α_1-proteinase inhibitor, and thus indirectly reducing tissue proteinase activity
• Stimulates fibroblast collagen production
• Reduces osteoclast activity and bone resorption
• Inhibits osteoclast MMPs

MMP, matrix metalloproteinase.

effective than other tetracyclines in reducing collagenase activity in the gingival crevicular fluid of chronic periodontitis patients (35). Doxycycline was also confirmed as being a more effective inhibitor of MMPs than either minocycline or tetracycline; doxycycline has a much lower inhibitory concentration ($IC_{50} = 15$ μM) than minocycline ($IC_{50} = 190$ μM) or tetracycline ($IC_{50} = 350$ μM), indicating that a much lower dose of doxycycline is necessary to reduce a given collagenase level by 50% compared with minocycline or tetracycline (10, 29). Furthermore, doxycycline has also been found to be more effective in blocking neutrophil collagenase activity (MMP-8) than fibroblast collagenase activity (MMP-1) (33, 94), suggesting that doxycycline can provide a safe method of reducing pathologically elevated collagenase levels without interfering with normal connective tissue turnover.

The ability of doxycycline to downregulate MMP activity was recognized as representing a novel treatment strategy for the management of periodontitis. However, a major concern with the long-term administration of doxycycline was the possibility of development of antibiotic resistance. Indeed, when high (antibiotic) doses of tetracycline (250 mg daily for 2–7 years) had previously been given as treatment for refractory periodontitis, up to 77% of the patients' culturable subgingival microflora exhibited tetracycline resistance (53). Therefore, a low dose of 20 mg twice daily was introduced, which was shown, after 2 weeks, to inhibit collagenase activity by 60–80% in the gingival tissues and crevicular fluid of patients

with chronic periodontitis (28). Subsequent studies of relatively short duration (1–3 months) indicated that this dosage regimen could prevent periodontitis progression without the emergence of doxycycline-resistant organisms or other typical antibiotic side-effects (36). These initial studies paved the way for progressively larger and longer clinical trials of the efficacy of subantimicrobial dose doxycycline in the management of periodontal disease.

Clinical studies of subantimicrobial dose doxycycline

One of the early clinical trials involved just 14 patients with chronic periodontitis who, after removal of subgingival plaque and calculus, were randomized to either 20 mg of doxycycline twice daily or placebo for 2 months, then no study medications for 2 months, then 20 mg of doxycycline twice daily or placebo again for 2 months (16). The doxycycline regimen resulted in significant improvements in probing depths and attachment levels, but did not affect plaque or gingival inflammation. Furthermore, crevicular fluid collagenase levels were significantly reduced in the doxycycline group, as was α_1-proteinase inhibitor degradation. In a clinical study of 75 patients assigned to one of five treatment groups (including various dosage regimens of low-dose doxycycline and placebo) for 12 weeks after initial scaling and prophylaxis, followed by 12 weeks of no drug then a second episode of scaling and prophylaxis and 12 more weeks of drug therapy, those patients who received subantimicrobial dose doxycycline (20 mg twice daily) demonstrated significant reductions in crevicular fluid collagenase levels compared with the placebo group (32). In another small study by the same research group, subantimicrobial dose doxycycline was given to 12 patients with chronic periodontitis for 2 months following a course of subgingival instrumentation and six patients were prescribed placebo (30). After 2 months of subantimicrobial dose doxycycline, there were significant decreases in the crevicular fluid concentrations of MMP-8 and MMP-9 and carboxyterminal peptide (a pyridinoline-containing fragment of type 1 collagen) compared with placebo. This was the first study to show that subantimicrobial dose doxycycline resulted in simultaneous reductions in MMP activity together with a concomitant reduction in the levels of collagen degradation products. Of interest, root surface instrumentation alone has no effect on crevicular fluid carboxyterminal peptide levels (1).

Following on from these small-scale clinical studies, a variety of larger clinical trials have been conducted to investigate the efficacy of subantimicrobial dose doxycycline when used as an adjunct to nonsurgical therapy, and the clinical data from these studies are summarized in Table 2. The data in this table demonstrate that clinical outcomes are improved when adjunctive subantimicrobial dose doxycycline is prescribed and these benefits are particularly clear in the larger studies of longer duration. Thus, in the largest clinical trials to date (12, 83), mean attachment gains and probing depth reductions were statistically significantly greater in patients treated with adjunctive subantimicrobial dose doxycycline compared with placebo. Mean changes in probing depth or attachment level (whether stratified by baseline probing depth or calculated for the full mouth) are not particularly useful for revealing, in a meaningful way, the benefits, or not, of a particular treatment, however (46), and as clinicians we never calculate mean probing depths (for example) when we are evaluating the outcomes of the treatment we have provided. Rather, we focus on individual sites; thus, threshold change data, in which we calculate the proportion of sites demonstrating probing reductions of ≥2 or ≥3 mm are particularly useful.

These data are presented for some of the studies in Table 2, and reveal very clearly the clinical benefit of subantimicrobial dose doxycycline. Thus, in the studies by Caton et al. (12) and Preshaw et al. (83), approximately one-third more sites demonstrated probing depth reductions of ≥2 mm in the subantimicrobial dose doxycycline groups compared with the placebo groups, and nearly twice as many sites demonstrated probing depth reductions of ≥3 mm in the doxycycline groups compared with the placebo groups. These changes represent tangible and clinically significant benefits for the patients treated with subantimicrobial dose doxycycline compared with those treated by root surface instrumentation alone. While it is not possible to predict with certainty the likely clinical benefits of using adjunctive subantimicrobial dose doxycycline in an individual patient or at a particular periodontal site, these data do support that, on balance, within a patient population, significantly more periodontal sites will experience clinically significant improvements in probing depths and attachment levels (of ≥2 or ≥3 mm) compared with patients treated by root surface instrumentation alone.

In a study of 30 subjects, ≤45 years of age, with severe, generalized chronic periodontitis, the subjects received intensive periodontal therapy comprising full-mouth subgingival debridement and oral hygiene instruction each week for 4 weeks plus 6 months of adjunctive subantimicrobial dose doxycycline or placebo, with follow-up for approximately 9 months (70). Ten subjects completed the study in each group, and the mean reduction in probing depths at deep sites (baseline probing depths of ≥7 mm) in the doxycycline group was 3.02 mm compared with 1.42 mm in the placebo group. In the doxycycline group, 38% of pockets of ≥7 mm were reduced by ≥4 mm (representing a huge clinical improvement) compared with <10% of pockets in the placebo group. Furthermore, in the doxycycline group, more than double the number of pockets initially ≥7 mm improved by ≥3 mm when compared with the placebo group (55% vs. 24%, respectively). These data demonstrate a very clear, clinically significant benefit of subantimicrobial dose doxycycline when combined with appropriate and effective conventional therapy.

In a 9-month, randomized, placebo-controlled study of 24 institutionalized geriatric patients (≥65 years of age), patients treated with root surface instrumentation and subantimicrobial dose doxycycline for 9 months demonstrated significantly greater probing depth reductions and attachment gains compared to patients treated with root surface instrumentation and placebo (66). Indeed, in deep sites (baseline probing depths ≥7 mm), the mean probing depth reduction in the subantimicrobial dose doxycycline group was more than three times greater than that observed in the placebo group (Table 2). Furthermore, at month 9, no sites in the subantimicrobial dose doxycycline group demonstrated attachment loss or probing depth increases of ≥2 or ≥3 mm from baseline. However, in the placebo group at month 9, 47 sites (17.4%) demonstrated attachment loss of ≥2 mm, 30 sites (11.1%) demonstrated attachment loss of ≥3 mm, 13 sites (4.8%) demonstrated probing depth increases of ≥2 mm and five sites (1.9%) demonstrated probing depth increases of ≥3 mm. The authors concluded that subantimicrobial dose doxycycline prevented disease progression, which may be particularly important in elderly patients. The clinical benefits observed in the doxycycline group were particularly impressive in this study, which may reflect (i) that this was an institutionalized population over whom complete control was exercised regarding compliance with plaque control and with taking the study medication, (ii) study medication was prescribed for a full 9 months, and (iii) this was a population of non-smokers.

Table 2. Clinical data reported in randomized clinical trials of subantimicrobial dose doxycycline used as adjunctive treatment for chronic periodontitis

Year	Author	Study duration (drug duration)	Study groups	n[1]	Mean CAL gain (mm)[2] 4–6 mm pockets	≥7 mm pockets	Mean PD reduction (mm)[2] 4–6 mm pockets	≥7 mm pockets	Percentage of sites with CAL gain[3] ≥2 mm	≥3 mm	Percentage of sites with PD reduction[3] ≥2 mm	≥3 mm
2000	Caton et al. (12)	Study: 9 months (drug: 9 months)	SRP + SDD	90	1.03*	1.55*	0.95**	1.68**	46	22	47*	22*
			SRP + placebo	93	0.86	1.17	0.69	1.20	38	16	35	13
2002	Novak et al. (70)	Study: 9 months (drug: 6 months)	SRP + SDD	10	1.00	1.78	1.20	3.02*	29	15	48	26**
			SRP + placebo	10	0.56	1.24	0.97	1.42	21	11	21	6
2004	Preshaw et al. (83)	Study: 9 months (drug: 9 months)	SRP + SDD	107	1.27**	2.09**	1.29**	2.31**	58*	33**	62**	37**
			SRP + placebo	102	0.94	1.60	0.96	1.77	44	20	45	21
2004	Emingil et al. (23)	Study: 12 months (drug: 3 months)	SRP + SDD	10	0.21 (all sites)	1.59* (all sites)						
			SRP + placebo	10	0.05 (all sites)	1.32 (all sites)						
2004	Lee et al. (57)	Study: 9 months (drug: 9 months)	SRP + SDD	24	1.56* (all sites)	1.63* (all sites)						
			SRP + placebo	17	0.80 (all sites)	1.19 (all sites)						
2004	Choi et al. (14)	Study: 120 days (drug: 120 days)	SRP + SDD	15	2.2* (test sites)	1.6* (test sites)						
			SRP + placebo	17	0.6 (test sites)	1.1 (test sites)						
2005	Gurkan et al. (40)	Study: 6 months (drug: 3 months)	SRP + SDD	13	1.12	2.15	1.80	3.38				
			SRP + placebo	13	0.78	1.76	1.46	2.57				
2005	Preshaw et al. (81)[4]	Study: 9 months (drug: 9 months)	SRP + SDD, nonsmokers	116	1.23*	1.89**	1.22**	2.16*	59**	33**	63***	37***
			SRP + SDD, smokers	81	1.03	1.71	1.01	1.80	44	21	45**	20
			SRP + placebo, nonsmokers	135	0.96	1.43	0.88	1.53	43	19	44	18
			SRP + placebo, smokers	60	0.85	1.58	0.80	1.62	37	15	31	13
2005	Mohammad et al. (66)	Study: 9 months (drug: 9 months)	SRP + SDD	12	2.14**	3.18*	1.57***	3.22***				
			SRP + placebo	12	0.02	0.25	0.63	0.98				
2006	Gorska & Nedzi-Gora (38)	Study: 3 months (drug: 3 months)	SRP + SDD	33	0.33* (all sites)	0.29* (all sites)						
			SRP + placebo	33	0.04 (all sites)	0.08 (all sites)						
2007	Needleman et al. (67)	Study: 6 months (drug: 3 months)	SRP + SDD (all smokers)	18	0.65 (all sites)	1.40 (all sites)						
			SRP + placebo (all smokers)	16	0.40 (all sites)	0.98 (all sites)						

CAL, clinical attachment level; PD, probing depth; SDD, subantimicrobial dose doxycycline; SRP, scaling and root planing – the term used to describe the treatment in the original publications.

*P < 0.05, **P < 0.01 and ***P < 0.001 compared with placebo.

[1] n (subject numbers) are based on subjects completing the study or the intent-to-treat population, according to the original publication.

[2] Mean clinical attachment level gains and probing depth reductions were calculated either within categories of baseline probing depth, 4–6 mm or ≥7 mm as indicated (12, 40, 66, 70, 81, 83), or for all sites (23, 38, 57, 67) or for test sites (defined as the deepest site in the treated sextant) (14).

[3] Threshold change data are based on sites with probing depth ≥6 mm at baseline. These data were not reported in the original publications by Caton et al. (12) and Novak et al. (70), but were calculated from the raw data. Threshold change data were determined using the Generalised Estimating Equations model with adjustment for within-subject correlations among sites.

[4] Smoking data for Preshaw et al. (81) are combined data from Caton et al. (12) and Preshaw et al. (83). P values for mean attachment gain and probing depth reduction data indicate significantly greater improvements in nonsmokers who received SDD compared with placebo within the smoking category. P values for threshold analyses indicate a significant difference from placebo within the smoking category.

Some of the studies described in Table 2 evaluated whether subantimicrobial dose doxycycline had any effect on biochemical markers of inflammation, in addition to studying clinical outcomes. Thus, Emingil et al. (23) identified that after root surface instrumentation followed by 3 months of treatment with either subantimicrobial dose doxycycline or placebo, with monitoring for a further 9 months, reductions in crevicular fluid MMP-8 levels were identified in both groups as a result of treatment, and these reductions were significantly greater in the doxycycline group than in the placebo group at 6 months. Similarly, laminin-5 γ2 chain fragment levels, which are associated with the migration of epithelial cells during periodontal pocket formation, were significantly reduced in the subantimicrobial dose doxycycline group compared with the placebo group (24). Lee and co-workers (57) also studied the effect of subantimicrobial dose doxycycline on the crevicular fluid levels of MMP-8 and MMP-9. The significantly greater improvements in attachment levels and probing depths that they identified at all time-points in the doxycycline group were accompanied by significantly greater reductions in MMP-8 and MMP-9 levels in the doxycycline group compared with the placebo group over the course of the study.

In the study by Choi et al. (14), crevicular fluid concentrations of MMP-8 also demonstrated statistically significantly greater reductions following subantimicrobial dose doxycycline therapy compared with placebo, although there were no differences between the groups with regard to MMP-9 concentrations. Interestingly, this study also showed that following treatment, crevicular fluid concentrations of tissue inhibitor of metalloproteinase-1 significantly increased in both groups, supporting the effect of treatment on reducing inflammation in the periodontium.

In the study by Gurkan and colleagues (40), patients with severe, generalized chronic periodontitis were randomized to full-mouth supragingival instrumentation at baseline followed by 3 months of subantimicrobial dose doxycycline or placebo. At 6 months, clinical improvements were evident in both groups, although there were no statistically significant differences in mean probing depth reductions or attachment gains between the groups. However, significantly more sites (73% of sites) in the subantimicrobial dose doxycycline group that had probing depths of ≥7 mm at baseline demonstrated probing reductions of ≥3 mm compared with the placebo group (50% of sites) (P = 0.011). Furthermore, at the end of the 3-month medication period,

crevicular fluid concentrations of transforming growth factor-β1 were significantly higher in the subantimicrobial dose doxycycline group than in the placebo group, indicating that subantimicrobial doxycycline may contribute to connective tissue healing and collagen matrix formation via an increase in transforming growth factor-β1.

The concentrations of MMP-8, MMP-9 and tissue inhibitor of metalloproteinase-1 were measured in saliva and peripheral blood before and after subantimicrobial dose doxycycline therapy for 3 months in the study by Gorska & Nedzi-Gora (38). Thirty-three patients were prescribed subantimicrobial dose doxycycline for 3 months after root surface instrumentation and 33 patients were prescribed placebo. Statistically significantly greater attachment gains and probing depth reductions were observed in the subantimicrobial dose doxycycline group, as shown in Table 2, but there was no apparent effect of doxycycline therapy on serum or saliva levels of the MMPs or tissue inhibitor of metalloproteinase-1 compared with the placebo group.

In a randomized, single-blind study to determine the relative effectiveness of various systemic adjunctive therapies, 92 patients with chronic periodontitis were assigned to receive root surface instrumentation alone ($n = 23$), or combined with 500 mg of azithromycin per day for 3 days ($n = 25$), 250 mg of metronidazole three times per day for 14 days ($n = 24$), or subantimicrobial dose doxycycline 20 mg twice per day for 3 months ($n = 20$) (41). The patients were then monitored for 12 months. The majority of patients in each treatment group demonstrated improvements in probing depths and attachment levels, and the patients receiving adjunctive treatments exhibited greater improvements overall in probing depths (although this did not achieve statistical significance). Sites with initial probing depths of >6 mm demonstrated significantly greater probing depth reductions and attachment level gains in the subjects receiving metronidazole or azithromycin compared with the other groups, and the authors concluded that the use of adjunctive systemic antibiotics or adjunctive subantimicrobial dose doxycycline should be limited to those subjects who have more potential to receive the maximum benefit from these agents (i.e. the subjects with the most advanced periodontal disease) (41). These authors also identified an increase in mean probing depths and attachment levels at the 6-month recall period in the subantimicrobial doxycycline group (i.e. 3 months after completion of 3 months of drug therapy), possibly indicating a relapse after

cessation of doxycycline. This is in contrast to another study in which no evidence for relapse was identified 3 months after patients finished a 9-month course of subantimicrobial dose doxycycline (13). This may indicate that a 9-month regimen of subantimicrobial dose doxycycline is more effective at maintaining the initial benefits observed after 3 months of medication, supporting prolonged dosing for 9 months to achieve maximum benefit with less chance of relapse upon stopping the medication. Also, methodological differences between the two studies may have contributed to these different outcomes: in the study by Caton et al. (12), data analyses included only those sites with baseline probing depths of > 3 mm; and when only sites with initially deep pockets were analysed in the study of Haffajee et al. (41), an increase in clinical parameters at the 6-month time-point was not seen.

Taken collectively, the clinical studies summarized in Table 2 reveal a clear clinical benefit of subantimicrobial dose doxycycline when used as an adjunct to root surface instrumentation, supporting the findings of a recent systematic review which concluded that subantimicrobial dose doxycycline used as an adjunct to nonsurgical therapy is beneficial in the management of chronic periodontitis over 12 months (90). The clinical benefits are particularly apparent with longer dosing periods (e.g. 9 months rather than 3 months) and especially in patients with more advanced disease. The clinical significance of the improved outcomes following subantimicrobial dose doxycycline is most notable when considering the proportions of sites demonstrating clinically significant probing depth reductions and attachment gains (e.g. ≥2 or ≥3 mm) rather than when considering mean probing depth and attachment changes. This make sense, and, as clinicians, we use outcomes such as changes in the proportions of deep pockets when we evaluate our patients as part of routine clinical care (rather than calculating mouth mean probing depth changes, which have limited use in assessing a patient's response to treatment). As was concluded by Haffajee and co-workers (41), not all patients assigned to receive a specific adjunctive treatment respond clinically in the same manner, and this reinforces the importance of determining the factors that influence treatment outcome so that the most appropriate therapy can be provided to individual patients.

Subantimicrobial dose doxycycline was also used as an adjunct to surgical management in a pilot study of 24 patients undergoing access flap surgery (27). Over 12 months, those patients treated with suban-

timicrobial dose doxycycline for the first 6 months demonstrated greater reductions in probing depths of surgically treated sites of ≥ 6 mm compared with the placebo group, and greater reductions in carboxyterminal peptide, with a rebound in carboxyterminal peptide levels when the study medication was stopped. Additionally, there were no significant differences in the subgingival microflora between the doxycycline and control groups at any time-point.

Subantimicrobial dose doxycycline in smokers

Most periodontists would consider that the patient group which is most difficult to manage is the smokers. Smokers tend to have more advanced periodontal disease than nonsmokers and more limited outcomes following treatment (50). However, research has shown that if smokers stop smoking, treatment outcomes following nonsurgical therapy are improved (80). Two studies to date have focussed specifically on the use of subantimicrobial dose doxycycline in smokers with periodontal disease. The first of these (81) was a meta-analysis of two previously published clinical trials (12, 83). This meta-analysis of 392 patients (intent-to-treat population) was stratified into four subgroups depending on smoking status (current smoker or nonsmoker) and whether subantimicrobial dose doxycycline was prescribed (doxycycline or placebo) (Table 2). Nonsmokers included both ex-smokers and never-smokers, and it was not possible to identify from the data how long ago the ex-smokers had stopped smoking.

A hierarchical treatment response was observed following 9 months of adjunctive subantimicrobial dose doxycycline, such that nonsmokers who received subantimicrobial dose doxycycline demonstrated the greatest attachment gains and probing depth reductions (81). Smokers who received placebo demonstrated the smallest clinical improvements following treatment. Smokers who received subantimicrobial dose doxycycline demonstrated an intermediate treatment response that was broadly equivalent to that seen in nonsmokers who received placebo. In sites with baseline probing depths of 4–6 mm, month-9 attachment gains were 19–45% better in nonsmokers who received subantimicrobial dose doxycycline compared with all other subgroups (P < 0.05) and were 21% greater in smokers who received subantimicrobial dose doxycycline compared with smokers who received placebo (P < 0.05). Furthermore, month-9 probing depth reductions were 21–53% greater in nonsmokers who received sub-

antimicrobial dose doxycycline compared with all other subgroups (P < 0.01) and were 26% greater in smokers who received subantimicrobial dose doxycycline compared with smokers who received placebo (P < 0.05). In sites with a baseline probing depth of ≥7 mm, month-9 attachment gains were significantly greater (range 20–32% greater) in nonsmokers who received subantimicrobial dose doxycycline than in either of the placebo groups (smokers or nonsmokers) (P < 0.01) (Table 2).

This meta-analysis also considered the number of periodontal sites that achieved clinically significant thresholds of attachment gain and probing depth reduction (≥2 or ≥3 mm) (81). As seen in Table 2, a hierarchical treatment response was again observed, such that nonsmokers who received subantimicrobial dose doxycycline demonstrated the greatest number of sites achieving these thresholds of change. The smallest number of sites achieving these thresholds was observed in the smokers who received placebo. Smokers who received adjunctive subantimicrobial dose doxycycline, and nonsmokers who received placebo, demonstrated a similar, and intermediate, number of sites achieving these thresholds of change. When considering sites that at baseline had probing depths of ≥6 mm, subantimicrobial dose doxycycline in smokers resulted in significantly more sites that demonstrated probing depth reductions of ≥2 mm (45% of sites) compared with smokers who received placebo (31% of sites) (P < 0.01).

A criticism that could be levelled at this meta-analysis (81) is that the comparisons between smokers and nonsmokers were not direct comparisons based on the original randomization scheme and therefore could be subject to bias, as was suggested by the authors of a later study that prospectively recruited smokers only to a randomized clinical trial of subantimicrobial dose doxycycline (67). In this investigation of 34 patients, 16 patients in the test group received nonsurgical therapy plus 3 months of subantimicrobial dose doxycycline, and 18 patients in the control group received nonsurgical therapy plus 3 months of placebo. The absolute changes in mean probing depth reductions and attachment gains at 6 months did not differ significantly between the two groups (but were greater in the subantimicrobial dose doxycycline group) (Table 2). However, the velocity of change was significantly greater for the subantimicrobial dose doxycycline group compared with the placebo group for clinical attachment gain (0.19 mm / 6 month greater, P < 0.05) and probing depth reduction (0.30 mm / 6 month greater, P < 0.001). These authors concluded that there was

no evidence of a benefit of using subantimicrobial dose doxycycline as an adjunctive treatment in smokers, which is in contrast to the findings of the meta-analysis discussed previously (81).

Clearly, further research is required to answer the question of whether subantimicrobial dose doxycycline confers any clinical benefit in smokers. The meta-analysis by Preshaw and co-workers (81) involved a large number of subjects (n = 392) enrolled into two separately conducted multicentre clinical trials, and involved 9 months of drug therapy. However, the recruitment of subjects was not undertaken specifically to involve smokers; the creation of the smoking and nonsmoking subgroups was performed retrospectively. The study by Needleman and colleagues (67) was a small, single-centre study, with just 30 patients completing the study. Subjects received study medication for only 3 months and were followed-up for 6 months. The authors recognized that this study was under-powered as a result of the treatment effects being smaller than originally anticipated when powering the project. However, the advantage of this study was that smokers were specifically recruited, thereby avoiding all bias, but whether this advantage in a small number of patients (n = 34) truly outweighs the disadvantages of possible bias in the retrospective analysis of a much larger subject population (n = 392), in the study of Preshaw et al. (81), seems doubtful. Clearly, further research is required, but additional support for the benefits of subantimicrobial dose doxycycline in smokers can be derived from, for example, the clinical study by Novak and co-workers (70), in which almost 50% of subjects were current smokers and 70% had a history of tobacco use, and yet hugely improved clinical outcomes were reported in the subantimicrobial dose doxycycline group compared with the placebo group.

The mechanism by which subantimicrobial dose doxycycline may be of clinical benefit in smokers with periodontal disease has not been clearly identified, but presumably relates to the ability of doxycycline to downregulate MMPs, together with other anti-inflammatory properties (as summarized in Table 1). Smoking has been associated with increased cytokine production, with peripheral blood mononuclear cells from smokers secreting significantly greater levels of interleukin-1β than cells from nonsmokers upon exposure to cigarette smoke (89). Smokers have also been reported to have significantly higher crevicular fluid levels of tumor necrosis factor-α than nonsmokers (8) and show suppressed levels of the crevicular fluid protease inhibitors α1-antitrypsin and α2-macroglobulin (77), suggesting that smoking

has a direct effect on the inflammatory response. Nicotine has also been shown to alter gingival fibroblast function, resulting in decreased collagen formation and increased collagenase activity (100), and smokers have increased crevicular fluid neutrophil elastase activity compared with nonsmokers (95). The exposure of coronary endothelial cells to cigarette smoke condensate results in the up-regulation of genes involved in matrix degradation (MMP-1, MMP-8 and MMP-9) and the increased production of cytokines, suggesting a complex pro-inflammatory response to cigarette smoke that probably involves the recruitment of leukocytes, cytokine signalling and MMP upregulation (68). Thus, based on what is known of the effect of smoking on inflammatory responses, subantimicrobial dose doxycycline would appear to offer the potential for improving clinical outcomes even in smokers, as confirmed in one of the two studies to date that have specifically investigated this issue (81), but not in another (67).

Microbiological and safety concerns

A concern expressed by many colleagues initially was that the prolonged use of doxycycline, even at a dose of 20 mg twice daily, may lead to the development of antibiotic resistance. However, no evidence for antibiotic resistance has been identified in any of the studies that have investigated this issue to date. This has been confirmed in several randomized studies in which subgingival plaque samples were collected from patients prior to and after 9–27 months of subantimicrobial dose doxycycline therapy or placebo (98, 99, 105). In these studies, susceptibility testing on the most prevalent organisms was performed using tetracycline, amoxicillin, doxycycline, minocycline, erythromycin and clindamycin, and 50% minimum inhibitory concentrations (MIC_{50}) and 90% minimum inhibitory concentrations (MIC_{90}) values were calculated for each antibiotic/organism combination. A consistent finding in these studies was that subantimicrobial dose doxycycline did not result in any overgrowth or replacement by opportunistic oral flora, there were no significant shifts in the MIC_{50} or MIC_{90} data, and there was no development of multidrug resistance to the antibiotics.

In the study by Walker and colleagues (105), plaque samples were collected at baseline and then every 3 months during 9 months of medication, and again at 3 months after cessation of drug therapy. Relative to baseline, statistically significant reductions in the proportions of spirochetes and motile rods, and an increase in the proportion of coccoid forms, were seen in both the subantimicrobial dose doxycycline and placebo groups, indicating a return to a flora more associated with periodontal health. No differences in other microbiological parameters were detected between groups, with the exception that the spirochetal proportions present in the subantimicrobial dose doxycycline group were significantly lower at certain time-points than in the placebo group, and these time-points were preceded by a significant decrease in the number of sites sampled that bled on probing. It was considered that the decrease in spirochetal groups in the subantimicrobial dose doxycycline group resulted from increasingly aerobic conditions in the pockets in this group as a result of the clinical improvements derived from the doxycycline therapy, as opposed to any effect of doxycycline on the spirochetes themselves. Because spirochetes are more sensitive to local oxygen tension than other pathogens and have relatively low redox requirements for growth (65), a reduction in the proportion of spirochetes is not unexpected in pockets that exhibit reduced probing depths. The authors of this paper concluded that the microbial differences observed were attributed to the anti-collagenase and anti-inflammatory properties of subantimicrobial dose doxycycline and not to an antimicrobial effect (105), and a consistent finding in these various studies has been that long-term subantimicrobial dose doxycycline does not alter or contribute to changes in antibiotic susceptibility of the subgingival microflora compared with placebo (98, 99, 105).

Similar findings were reported by Lee and co-workers (57) in a 9-month placebo-controlled study of the use of subantimicrobial dose doxycycline in which subgingival plaque samples were collected at baseline and at months 1, 3, 6 and 9. These authors identified a general tendency for the number of cocci, nonmotile rods and aerobes to increase over time and for the number of spirochetes, motile rods and anaerobes to decrease over time (both indicating a return to a flora more associated with health), but with no significant differences between the subantimicrobial dose doxycycline and placebo groups. In a later study, carried out to investigate whether subantimicrobial dose doxycycline exerts any antimicrobial effects in other body compartments (specifically the intestinal or vaginal microflora), 70 patients were randomized to receive either subantimicrobial dose doxycycline or placebo for 9 months, and fecal and vaginal samples were collected at baseline and at months 3 and 9 (104). Samples were examined for total anaerobic counts,

opportunistic pathogens and doxycycline resistance (≥ 4 µg/ml). All isolates that survived subculture were identified and their susceptibilities were determined to tetracycline, amoxicillin, doxycycline, minocycline, erythromycin and clindamycin. No differences between the treatment groups were detected at 3 or 9 months, either in the predominant bacterial taxa present or in their antibiotic susceptibilities, and these authors concluded that there was no evidence that subantimicrobial doxycycline exerted an effect on the composition or doxycycline resistance level of either the fecal or the vaginal microflora.

Regarding safety data, the plasma concentration of doxycycline following a subantimicrobial dose (20 mg) is low, with pharmacokinetic studies revealing peak concentrations of 0.7–0.8 µg/ml and steady-state concentrations of approximately 0.4 µg/ml (11), which are well below the concentrations of 3–4 µg/ml that can be expected following antibiotic doses of 100–200 mg of doxycycline (105). Thus, unwanted effects are less likely to occur as a result of treatment with subantimicrobial doses, compared with antibiotic doses, of doxycycline. Indeed, many dermatologists now prescribe subantimicrobial doses of doxycycline for prolonged periods for the treatment of acne in preference to the antibiotic doses they were previously using, as the same clinical improvements are achieved (presumably as a result of the anti-inflammatory properties of doxycycline as opposed to any antibacterial effect) but without the same frequency of adverse events, and without the development of antibiotic resistance or any effect on the microflora of the skin (93). In the periodontal literature, randomized clinical trials (Table 2) have regularly reported that subantimicrobial dose doxycycline is well tolerated with no significant differences in adverse event profiles compared with placebo, and no evidence at all of any unwanted effects that could be attributed to any antimicrobial activity (12, 83). Overall adverse event rates of approximately 0.15% have been reported (82), and, taken collectively, these studies support the safety of using subantimicrobial dose doxycycline.

Combinations of host response modulators

Host modulating drugs that target different aspects of the pathogenic processes in periodontitis have been combined to maximize therapeutic outcomes. Thus, in 19 patients with chronic periodontitis who were scheduled for periodontal flap surgery, the patients were randomly allocated to receive subantimicrobial dose doxycycline (20 mg twice daily), flurbiprofen (50 mg four times per day), or a combination of the two drugs, for 3 weeks (56). Gingival biopsies were obtained from the planned surgery sites before and after drug therapy and were analyzed for $\alpha 1$-proteinase inhibitor, its breakdown product, various MMPs and neutrophil elastase. Three weeks of subantimicrobial dose doxycycline alone produced a significant reduction in host-derived neutral proteinases, whereas flurbiprofen alone produced no reduction. However, the combination therapy produced a statistically significant synergistic reduction of collagenase, gelatinase and serpinolytic ($\alpha 1$-proteinase inhibitor-degrading) activities (of 69, 69 and 75%, respectively) and a lesser reduction of elastase activity (46%). These authors concluded that, consistent with other studies of chronic inflammatory diseases (e.g. rheumatoid arthritis), the combination of subantimicrobial dose doxycycline and the nonsteroidal anti-inflammatory drug synergistically suppressed MMPs and other neutral proteinases in the gingiva of patients with chronic periodontitis.

A similar effect has also been described when chemically modified tetracyclines are administered together with flurbiprofen in arthritic rats: the tetracycline levels were increased in the joints when flurbiprofen was administered even though serum levels of the tetracycline were the same as when the tetracycline was administered alone (58). The mechanism by which flurbiprofen promoted the uptake of the tetracycline in the local inflammatory lesion is not known, but could relate to an improved local blood flow in the lesion (thus improving the local delivery of the chemically modified tetracycline) as a result of the flurbiprofen therapy. Similarly, CMT-8 (a nonantimicrobial chemically modified doxycycline), has been combined with a bisphosphonate (clodronate) in rats with experimental periodontitis (60). After 1 week of treatment with either CMT-8 alone or the bisphosphonate alone, there were slight reductions in the levels of MMP-8 and MMP-9 in the gingival tissues. However, combination of these agents 'normalized' the pathologically elevated levels of these MMPs, indicating a synergistic inhibition in this animal model (60).

Subantimicrobial dose doxycycline has also been combined with the locally delivered doxycycline gel (10%; Atridox®, CollaGenex Pharmaceuticals Inc., Newtown, PA), placed into pockets of ≥ 5 mm, in combination with root surface instrumentation in a placebo-controlled study of 171 subjects (69). This combination therapy resulted in significantly greater

clinical improvements than instrumentation alone over 6 months, with mean probing depth reductions (in pockets that were ≥7 mm at baseline) of 2.4 mm in the combination group compared with 1.7 mm in the control group (P < 0.01).

The future of host response modulation

Host response modulation has emerged as a valid treatment concept for the management of periodontal disease and represents a significant step forward for clinicians and patients. To date, only subantimicrobial dose doxycycline has been approved specifically as a host response modulator for the treatment of periodontitis and the majority of clinical trials of this drug have clearly demonstrated a benefit. Most of these studies have been undertaken in secondary care settings with well-managed populations of patients and therefore probably represent the best that is achievable in terms of periodontal care and treatment outcomes. Further research is necessary to evaluate the efficacy of subantimicrobial dose doxycycline in primary care, and also to focus on very long-term outcomes, such as prevention of tooth loss. The health economics of therapy should also be investigated: thus, if subantimicrobial dose doxycycline confers significant clinical improvements, the cost of medication may be offset by the reduced need for additional treatment such as periodontal surgery.

Future developments in relation to subantimicrobial dose doxycycline will include modified-release formulations that achieve sustained plasma concentrations of doxycycline over 24 h, but only require once per day dosing, thereby improving patient compliance. Furthermore, the development of chemically modified tetracyclines is welcomed, as this will completely eliminate all concerns about any possible antimicrobial effects of these agents. Given the safety of subantimicrobial dose doxycycline, it is likely that host response modulators with similar safety profiles will be welcomed by practising clinicians if proven to have a clinical benefit and minimal unwanted effects. Such drugs that target relatively specific aspects of pathogenic processes (and therefore tend to have more predictable outcomes) might be preferred in comparison to agents that have profound, and possibly unpredictable, effects on human inflammatory responses, such as evidenced in the recent, disastrous clinical trial of TGN1412 (the activating 'superagonist' anti-CD28 monoclonal antibody) (96).

Given the huge and ever-expanding range of pathogenic pathways that play a role in periodontal tissue destruction [for example, the interleukin-1 cytokine family is now far more complex than previously realized (4)], it is inevitable that the future will see a range of different host response modulators developed. Furthermore, most biological responses involve a range of different mechanisms, and therefore blocking one single inflammatory pathway may not achieve the desired outcome because receptor-mediated responses could be activated by alternate pathways. Thus, polypharmaceutical approaches may be developed that modify a number of different pathways associated with inflammation and tissue destruction. Alternatively, targeting of mediators that play a particularly important role in periodontal pathogenesis, such as interleukin-1β or tumor necrosis factor-α, may constitute a rational therapeutic strategy. Thus, cytokine antagonists, such as interleukin-1 receptor antagonist or soluble tumor necrosis factor-α receptors, which competitively inhibit receptor-mediated signal transduction, may offer potential in the treatment of periodontal disease (20, 59). The effects of soluble receptors and receptor antagonists of interleukin-1 and tumor necrosis factor-α have been studied in experimental periodontitis models in nonhuman primates (90), and collectively demonstrate a reduction in the progression of the inflammatory cell infiltrate towards the alveolar bone crest, reduced recruitment of osteoclasts, and decreased attachment loss and alveolar bone loss (2, 18, 19, 39, 64, 72). However, a degree of caution is required, as, for example, the monoclonal antibody to tumor necrosis factor (infliximab), which has been successfully used over recent years in the treatment of rheumatoid arthritis, has also been associated with the re-emergence of latent tuberculosis infection in a small percentage of patients (71). Thus, we must carefully evaluate new agents that, while modifying inflammatory responses, may also have unexpected effects on host defences.

Interleukin-11 has anti-inflammatory effects including inhibition of tumor necrosis factor-α (101) and recombinant human interleukin-11 has been shown to result in significant reductions in the rate of attachment and bone loss over an 8-week period in experimental periodontitis in dogs (64). Blockade of cytokine receptors, soluble cytokine blockers and anti-inflammatory cytokines therefore hold promise for the future. Other agents that block transcriptional pathways (e.g. the nuclear factor-κB and mitogen-activated protein kinase pathways), such as the protein kinase inhibitors, may be useful because genes

that are regulated by nuclear factor-κB (for example) include many cytokines, chemokines, cell-adhesion molecules, acute-phase proteins and anti-apoptotic proteins (71).

Vasoactive intestinal peptide has a role in immunoregulation and has been identified as a molecule with therapeutically beneficial immunosuppressive effects in inflammatory and autoimmune conditions (17). We have recently demonstrated that this immunologically active peptide significantly reduces tumor necrosis factor-α production in human monocytes stimulated with *Porphyromonas gingivalis* lipopolysaccharide and also inhibits nuclear translocation of nuclear factor-κB and c-Jun (25). We have also shown that vasoactive intestinal peptide inhibits lipopolysaccharide-induced differentiation of monocytes with a concomitant reduction in the expression of Toll-like receptor-2 and -4. It also blocks lipopolysaccharide-induced differentiation of monocytes to macrophages, possibly via inhibition of the transcription factor PU.1 (26).

However, no agents that block intracellular signalling pathways have been (as yet) the subject of clinical trials for the treatment of periodontal disease, but as the pilot data reviewed above indicate, they may ultimately prove to be of benefit. Improved knowledge of pro-inflammatory signal transduction pathways has suggested new therapeutic targets, and as these pathways are common to various cytokines, their blockade may be more efficacious than targeting specific cytokines (51). However, it should be remembered that these pathways are important in physiological processes and therefore their inhibition could also result in adverse effects, such as increased susceptibility to infection, and the development and investigation of such agents require careful monitoring.

Lipoxins are another group of compounds that may ultimately be of benefit in modifying inflammatory responses in periodontal tissues. These lipid-derived mediators are released during inflammatory responses and have the effect of damping inflammation and modulating resolution of inflammation (102). Resolution of inflammation is now considered an active process, and failure of resolution of periodontal inflammation contributes to ongoing tissue breakdown. Lipoxins block interleukin-1β secretion from human neutrophils stimulated with tumor necrosis factor-α (79) and block neutrophil migration following exposure to *P. gingivalis* in a murine air pouch model (78). In an experimental periodontitis study of transgenic rabbits overexpressing 15-lipoxygenase and in nontransgenic animals receiving

topical application of 15-epi-lipoxin A4, enhanced expression of 15-lipoxygenase as well as topical 15-epi-lipoxin A4 significantly reduced bone loss and gingival inflammation (91). These results suggest that lipoxins can be targets for novel approaches in diseases such as periodontitis in which inflammation and bone destruction occur.

To summarize, host response modulators must be viewed as comprising part of the overall management strategy for patients with periodontitis. Thus, they should form part of an integrated treatment approach, together with hygiene therapy, plaque control, root surface instrumentation, maintenance care and risk factor modification. Periodontal disease is an unfortunate and distressing condition, and many patients are relieved to realize that multifaceted treatment strategies are possible, including, for example, root surface instrumentation, enzyme suppression and modification of local and systemic risk factors. Thus, periodontal therapy in the 21st century should not only involve a high standard of clinical treatment and monitoring, but should also focus on patient involvement and improving the patient experience. The future will see a range of host response modulators developed as adjunctive treatments for periodontitis. At present, subantimicrobial dose doxycycline provides improvements in probing depth reductions and attachment gains compared with root surface instrumentation alone (49), and is the only licensed and approved host response modulator available to dentists to date. Although the use of nonsteroidal anti-inflammatory drugs has been associated with reduced alveolar bone loss, the unwanted effects of these drugs precludes their use. Similarly, although data supporting the use of bisphosphonates to improve clinical periodontal status have been published, given the association with osteonecrosis, further studies are warranted to determine the risks and benefits of these drugs. Lipoxins and compounds that block cytokine receptors have been shown to reduce gingival inflammation and bone loss in animal models and may represent the future of host response modulation for treating periodontal disease, although this remains to be demonstrated in clinical trials in humans.

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