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Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis

A review

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Abstract

Background: Subantimicrobial dose doxycycline (SDD – 20 mg doxycycline twice daily) is indicated as an adjunctive treatment for periodontitis. Doxycycline downregulates the activity of matrix metalloproteinases (MMPs), key destructive enzymes in periodontal disease. Current understanding of periodontal pathogenesis suggests that MMPs play a major role in the destruction of periodontal tissues, leading to the clinical signs of periodontitis. Research supports that downregulation of MMPs by SDD confers benefit to patients with periodontitis.

Method: We review the clinical, microbiological and safety data relating to the use of SDD in patients with periodontitis, and consider the historical events that led to the development of adjunctive SDD as a treatment for periodontitis.

Results: Studies have shown that SDD, when prescribed as an adjunct to scaling and root planing (SRP), results in statistically and clinically significant gains in clinical attachment levels and reductions in probing depths over and above those that are achieved by SRP alone. SRP must be thorough and performed to the highest standard to maximise the benefits of adjunctive SDD. SDD does not result in antibacterial effects, or lead to the development of resistant strains or the acquisition of multiantibiotic resistance. The frequency of adverse events is low, and does not differ significantly from placebo.

Conclusions: Adjunctive SDD confers clinical benefit to patients with periodontitis. A comprehensive treatment strategy is suggested, involving patient education and motivation, reduction of the bacterial burden by SRP, host response modulation with SDD, and periodontal risk factor modification.

Key words: adjunctive treatment; doxycycline; host response modulation; periodontitis; risk factors; scaling and root planing; subantimicrobial dose doxycycline

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The mechanical removal of dental plaque and calculus from tooth surfaces is considered the standard treatment for chronic periodontitis. It is sobering to reflect that, despite spectacular advances in medical sciences, treatment of periodontal diseases has changed very little, in principle, over the years. A large evidence base now exists to demonstrate the efficacy of non-surgical periodontal therapy (as reviewed by Cobb 2002), and despite debate relating to the merits of manual versus ultrasonic instrumentation or the degree of root surface smoothness/hardness to be achieved, scaling and root planing (SRP) remains the ''gold standard'' treatment for periodontitis against which other treatments are compared. Indeed, the standards of periodontal care and associated treatment philosophies are relatively homogeneous throughout Europe, as evidenced by the consensus agreements reached at various European Workshops in Periodontology. Moreover, the clinical improvements that can be expected to occur following SRP are remarkably consistent across studies. For example, for those pockets initially 4–6 mm deep, data from various clinical studies indicate mean probing-depth reductions of 1.29 mm and mean attachment gains of 0.55 mm following SRP. Deeper periodontal pockets (7 mm or greater) demonstrate mean probing-depth reductions of 2.16 mm

and attachment gains of 1.19 mm following SRP (Cobb 1996).

Thus, periodontal treatment through the ages has focussed on the reduction of bacterial infection by mechanical removal of infectious agents (i.e. SRP). However, recent research into the pathogenesis of periodontal diseases has led to an important paradigm shift in the way we view periodontal disease progression. That is to say, it is now recognised that the major component of the soft and hard tissue destruction seen in periodontitis occurs as a result of activation of the host's immune-inflammatory defence mechanisms in response to the presence of bacterial plaque (Offenbacher 1996). The precise nature of the host inflammatory response is still an area of intense research, but it is clear that host-derived pro-inflammatory mediators and cytokines, together with proteolytic enzymes such as matrix metalloproteinases (MMPs), play a significant role in the changes in connective tissue and bone metabolism that lead to the breakdown of periodontal ligament (PDL) and alveolar bone resorption. For example, studies have shown that the predominant MMPs found in inflamed human gingiva and gingival crevicular fluid (GCF) derive from human cells rather than bacteria (Sorsa et al. 1988). The importance of the host inflammatory response in periodontal pathogenesis presents the opportunity for exploiting new treatment strategies for periodontitis by means of host response modulation. Host modulatory therapy (HMT) can be combined with traditional periodontal therapies that reduce the bacterial burden (e.g. SRP) and also risk factor modification (e.g. smoking cessation therapy) to constitute a comprehensive treatment strategy for periodontitis. To date, there is one approved, systemic therapy that is prescribed as a host response modifier in the treatment of periodontal disease, and that is adjunctive subantimicrobial dose doxycycline (SDD) (Periostat®, CollaGenex Pharmaceuticals Inc., Newtown, PA, USA), which downregulates the activity of MMPs.

The MMPs

The MMPs comprise a family of zinc-dependent proteolytic enzymes. Both secreted and membrane-bound MMPs catalyse the breakdown of proteins located either on the cell plasma membrane or within the extracellular matrix, including collagen, gelatin, proteoglycan core protein, fibronectin, laminin and elastin (Birkedal-Hansen 1993, Ryan et al. 1996). MMPs are primarily responsible for degrading the extracellular matrix in a variety of pathological conditions including rheumatoid arthritis, osteoarthritis, autoimmune ulcerative skin lesions and also tumour cell invasion and metastasis (Birkedal-Hansen et al. 1993). MMPs also play a key role in periodontitis, and are produced by each of the major cell types found in human periodontal tissues including fibroblasts, keratinocytes, macrophages, PMNs (neutrophils) and endothelial cells. 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 MMP genes is upregulated by pro-inflammatory mediators known to be important in periodontal disease progression, including interleukin-1α and β (IL-1α and β) and tumour necrosis factor-α (TNF-α) (MacNaul et al. 1990). Regulation of MMP activity involves specific, endogenous tissue inhibitors of MMPs (TIMPs) and α-macroglobulins, which form complexes with active MMPs, and in some cases with latent MMP precursors (Ryan et al. 1996, Reynolds & Meickle 1997). TIMPs are produced by various cell types including fibroblasts, keratinocytes, macrophages and endothelial cells and are widely distributed in body fluids and tissues (Birkedal-Hansen 1993).

In healthy tissues, collagen turnover is a controlled intracellular event that is mediated extracellularly by fibroblast-derived collagenase (MMP-1) and intracellularly by a variety of lysosomal acid-dependent enzymes. In inflamed periodontal tissues, the balance between MMPs and TIMPs is disrupted as a result of pathological alterations in the types and quantities of MMPs present. This leads to excessive breakdown of extracellular collagen and inappropriate destruction of periodontal tissues. MMP expression alters in inflamed tissues relative to non-inflamed tissues because each of the major cell types in the periodontium expresses, when appropriately stimulated, a unique combination of MMPs (Birkedal-Hansen 1993, Tervahartiala et al. 2000). For example, there is increased secretion of MMP-8 (neutrophil-derived collagenase, collagenase-2) and MMP-9 (neutrophil-derived gelatinase, gelatinase-B) by infiltrating PMNs, the primary defence cell type in the periodontal tissues. The PMN has evolved to respond rapidly and aggressively to external stimuli, and surpasses all other cell types in its ability to release or ''dump'' large quantities of destructive enzymes very rapidly (Birkedal-Hansen 1993). As a result of interactions between bacteria and their products with the junctional epithelium and underlying connective tissues, vascular endothelial responses occur that involve PMNs migrating from the capillaries, through the gingival tissues, to the gingival sulcus or periodontal pocket, releasing MMPs to facilitate their passage through the connective tissue matrix (Page et al. 1997).

It has been identified that the predominant MMPs (in particular MMP-8 and MMP-9) in GCF in periodontitis patients derive from PMNs (Table 1) (Golub et al. 1995, 1998b). These MMPs are particularly effective in degrading type-1 collagen, which is the predominant collagen type in gingiva and PDL (Mariotti 1993). Markedly increased expression of MMP-13 (collagenase-3) in basal epithelial cells has also been demonstrated in gingival sections taken from subjects with chronic periodontitis, suggesting that MMP-13 expression is important in the proliferation of the activated epithelium into the connective tissues seen in periodontitis (Uitto et al. 1998).

Thus, the predominant MMPs in inflamed gingival and periodontal tissues are PMN-type MMPs (MMP-8 and MMP-9) and bone-derived MMP-13, rather than fibroblast-type MMP (MMP-1) (Ejeil et al. 2003). Levels of PMN-type MMPs have been shown to increase with increasing periodontal disease severity and decrease following therapy (Golub et al. 1995, Kinane et al. 2003). These MMPs are secreted in large quantities by infiltrating PMNs and the resultant degradation of collagen bundles within the tissues accommodates further infiltrating defence cells that migrate from the vasculature. The significance of the excessive release of MMPs during the inflammatory response becomes clear when considering the composition of the gingival and periodontal structures (Table 2) (Golub et al. 1998b). MMPs also facilitate bone resorption, by degrading unmineralised osteoid and the collagen matrix that remains after demineralisation of bone

by osteoclasts. Thus, the breakdown of collagen fibres in the periodontal soft and hard tissues by MMPs, together with osteoclast-mediated bone resorption, upregulated by proresorptive cytokines such as IL-1β, contributes to the clinical signs of periodontitis including pocket formation, attachment loss, bone resorption, gingival recession, tooth mobility and tooth loss. Clearly, therefore, a systemic pharmaceutical that could downregulate the pathologically elevated levels of MMP activity could be extremely useful as an adjunctive treatment in periodontitis.

Doxycycline as an inhibitor of MMPs

Doxycycline possesses the ability (an ability shared by all members of the tetracycline family) to downregulate MMP activity. This property was first identified in the early 1980s, during experiments in diabetes. It was noted that there was abnormally elevated collagenase activity in the gingiva of diabetic rats (Ramamurthy & Golub 1983), and it was initially hypothesised that this may be a result of a change in the microflora in the gingival crevice. Thus, an experiment was performed in which minocycline was administered to the diabetic rats (the hypothesis being that minocycline would result in a decrease in collagenase levels by inhibiting the microflora), and, indeed, a fall in gingival collagenase levels was observed (Golub et al. 1983). More notably, however, minocycline treatment also suppressed gingival collagenase levels in germ-free diabetic rats, indicating that this ability was not related to any effect of the drug on the microbial flora. Minocycline was further shown to inhibit PMN collagenase activity in vitro, and retard alveolar bone loss in diabetic rats.

This remarkable discovery (that tetracyclines possess the ability to inhibit collagenolytic activity distinct from any antimicrobial or antibiotic properties) was confirmed in follow-up studies. Minocycline, doxycycline and tetracycline were all shown to inhibit collagenolytic activity, whereas non-tetracycline antibiotics had no effect on collagenase levels (Golub et al. 1984). In a case-study of a diabetic patient with aggressive periodontitis, doxycycline treatment produced a long-term reduction in collagenolytic activity in the patient's GCF (Golub et al. 1985). It was recognised in the mid-1980s that the inhibition of tissue collagenolysis by tetracyclines represented a new therapeutic modality in the management of periodontal disease, and intense research began to identify the most effective dosing regimens.

Research focussed on doxycycline, as it possesses the most potent anti-collagenase properties of commercially available tetracyclines (Burns et al. 1989, Golub et al. 1991). Doxycycline has a much lower inhibitory concentration ($IC_{50} = 15\,\mu M$) than minocycline ($IC_{50} = 190\,\mu M$) or tetracycline ($IC_{50} = 350\,\mu M$), indicating that a much lower dose of doxycycline is necessary to reduce a given collagenase level by 50% compared with minocycline or tetracycline (Burns et al. 1989). Furthermore, doxycycline has been found to be more effective in blocking PMN-type collagenase activity (MMP-8) than fibroblast-type collagenase activity (MMP-1) (Golub et al. 1995, Smith et al. 1999), suggesting that doxycycline can provide a safe therapeutic method for reducing pathologically elevated collagenase levels without interfering with normal connective tissue turnover.

Doxycycline downregulates collagenolytic activity by several synergistic mechanisms. For example, doxycycline inhibits active MMPs directly by a mechanism that is dependent on its calcium- and zinc-binding properties (Golub et al. 1998a). In addition, tetracyclines are known to scavenge for, and inhibit, the production of PMN-derived reactive oxygen metabolites, including hypochlorous acid (HOCl) (Wasil et al. 1988). This ability may further contribute to the non-antimicrobial, anti-inflammatory properties of doxycycline by inhibiting HOCl from activating latent pro-MMPs (Ramamurthy et al. 1993, Ryan & Ashley 1998). Furthermore, HOCl oxidises and inactivates host-derived proteinase inhibitors α_1-PI and α_2-macroglobulin (inhibitors of MMPs) (Nagase et al. 1994). Thus, the ability of tetracyclines to directly inhibit MMP activity and also scavenge for, and inhibit, reactive oxygen metabolites such as HOCl, represents an important pathway for modulation of the destructive connective tissue events that occur in periodontitis.

Additional mechanisms of MMP inhibition by tetracyclines were also identified. For example, tetracyclines inhibit osteoblast- and osteoclast-derived MMPs, thereby inhibiting bone resorption (Rifkin et al. 1994). Doxycycline can inhibit production of epithelial cell-derived MMPs by inhibiting intracellular expression or synthesis of these enzymes (Nip et al. 1993, Uitto et al. 1994). Doxycycline also contributes to decreased connective tissue breakdown by downregulating the expression of pro-inflammatory mediators and cytokines (including IL-1 and TNF-α) (Milano et al. 1997), and increasing collagen production, osteoblast activity and bone formation (Golub et al. 1998a). Tetracyclines have also been shown to normalise collagen formation in diabetic rats with previously suppressed collagen synthesis (Schneir et al. 1990, Sasaki et al. 1992).

SDD as an adjunctive treatment for periodontitis

It was realised that the ability of doxycycline to downregulate collagenolytic activity presented an opportunity for novel treatment strategies in the management of patients with periodontitis. A major concern, however, was that the long-term administration of doxycycline might be associated with the development of antibiotic resistance. Indeed, when antibiotic doses of tetracycline (250 mg daily for 2–7 years) had previously been given to patients with refractory periodontitis, up to 77% of the patients' cultivable subgingival microflora exhibited tetracycline resistance (Kornman & Karl 1982). In light of this concern, a low, SDD preparation was introduced, containing 20 mg doxycycline, as opposed to the 50 or 100 mg dose that is available for antibiotic purposes (Golub et al. 1990). One of the preliminary experiments to be conducted with this new formulation demonstrated clearly that SDD (20 mg twice daily) administered for just 2 weeks inhibited collagenase activity by 60–80% in the gingival tissues of patients with chronic periodontitis (Golub et al. 1990). Collagenase activity was also significantly reduced in GCF collected from these patients. Subsequent studies of relatively short duration (1–3 months) indicated that this dosing regimen could prevent periodontitis progression without the emergence of doxycycline-resistant microorganisms or other typical antibiotic side-effects (Golub et al. 1994).

Table 1. Destructive matrix metalloproteinases (MMPs) in periodontitis (Golub et al. 1995, 1998b)

Enzyme	Primary cellular source	Description
MMP-8	Polymorphonuclear leucocyte (PMN)	Collagenase. A dominant MMP in gingival crevicular fluid in periodontitis
MMP-9	PMN	Gelatinase. Also dominant in gingival crevicular fluid
MMP-13	Bone and epithelium	Collagenase. Dominant MMP in diseased gingival tissues. Mediates pathological bone loss

Table 2. Abundance of collagen in periodontal support structures (Golub et al. 1998b)

Structure	Estimated % of collagen
Gingiva	60
Periodontal ligament	70–80
Cementum	
organic and mineral phases	21
organic matrix only	90
Alveolar bone	
organic and mineral phases	21
organic matrix only	90

Thus, the concept was born that SDD (20 mg twice daily) could be used as an adjunct for treatment of chronic periodontitis. A number of early studies were published supporting the clinical benefits of SDD in the management of periodontal disease. In a study of 14 patients with chronic periodontitis, after removal of subgingival plaque and calculus, patients were randomised to receive either SDD for 2 months, then no drug for 2 months, then SDD for 2 months ($n = 7$) or placebo for 2 months, then no drug for 2 months, then placebo for 2 months ($n = 7$) (Crout et al. 1996). SDD resulted in significantly improved probing depths and attachment levels compared with placebo, but did not affect plaque index or gingival inflammation (as measured by the gingival index). This same study also demonstrated that GCF collagenase activity was significantly reduced by SDD therapy, as was GCF α_1-PI degradation (a substrate for collagenase), indicating that SDD may inhibit connective tissue destruction in periodontal disease. In another larger, double-blind, placebo-controlled study of 12 months duration involving 437 patients with chronic periodontitis, SDD therapy resulted in statistically significant attachment gains and probing-depth reductions compared with placebo (Caton et al. 1997). In a 36-week study of 66 patients undergoing a treatment regimen involving two episodes of SRP followed by 12 weeks of SDD or placebo and separated by a 12-week period of no drug, statistically significant reductions in GCF collagenase levels were observed after treatment with SDD, effects that were not seen in patients treated with placebo (Golub et al. 2001).

In a key study, SDD was given to 12 patients with chronic periodontitis for 2 months following a course of subgingival instrumentation (Golub et al. 1997). Six patients were prescribed placebo. At baseline, months 1 and 2, GCF samples were collected and analysed for MMP-8, MMP-13 and ICTP (carboxyterminal peptide, a pyridinoline-containing fragment of type-1 collagen). The 2-month regime of SDD resulted in statistically significant reductions in GCF concentrations of ICTP, MMP-8 and MMP-13 compared with placebo. This was the first study that demonstrated in human subjects that SDD results in a simultaneous reduction of elevated MMP activity with a concomitant reduction in levels of collagen degradation fragments. SRP alone has no effect on GCF ICTP levels (Al-Shammari et al. 2001).

These early clinical studies provided preliminary data and paved the way for large multi-centre clinical trials to evaluate the efficacy of SDD as an adjunctive treatment for periodontitis. The principal findings from these clinical studies are summarised in Table 3, demonstrating an overall benefit of treatment with adjunctive SDD compared with SRP alone.

When deciding whether or not these improvements also can be considered *clinically significant* (Jeffcoat 2002), it is useful to review the actual data. SRP is considered the standard of care for treating moderate–severe chronic periodontitis. We know from previous studies that the mean attachment gain that can be expected when performing SRP in sites with advanced disease (non-molar sites only) is of the order of 1.19 mm (Cobb 1996). In the studies cited in Table 3, the mean attachment gains that were achieved by SRP+ placebo in advanced sites (including molars) were very similar. By comparison, SRP+adjunctive SDD resulted in mean attachment gains that significantly exceeded those achieved in the placebo groups. One criticism that can be levelled at the use of mean changes in probing depths or attachment levels is that such means (although very useful summary statistics) may not directly reflect what is measurable at individual tooth sites with a periodontal probe in the clinical setting (Hujoel et al. 1993). Another way to consider the data is to look at the percent of sites undergoing a particular threshold of change as a result of treatment (Table 3). Threshold changes $\geqslant 2$ and $\geqslant 3$ mm are clearly clinically significant and are readily detectable by a dentist using routine chairside diagnostic procedures. Table 3 reveals the consistently increased percentage of sites achieving these thresholds in the SDD groups compared with the placebo groups in reported studies, underscoring the clinical benefit of SDD therapy.

Clinical significance may also be determined by the numbers of sites that resolved following treatment. For example, in the Caton study, adjunctive SDD resulted in significantly greater numbers of sites that resolved: of pockets that were 4–6 mm deep at baseline, 46% were $\leqslant 3$ mm at month 9 in the SDD group compared with 34% in the placebo group ($p < 0.001$), representing an enhanced predictability of treatment outcomes (Caton et al. 2000). The Caton study also identified that adjunctive SDD prevented diseased sites from worsening during the course of the research. For example, when considering the most severely diseased sites (baseline probing depth $\geqslant 7$ mm), 3.6% of sites in the placebo group underwent further attachment loss of $\geqslant 2$ mm over the course of the study (these sites were exited from the study and underwent additional treatment). By contrast, in the adjunctive SDD group, only 0.3% of sites demonstrated further attachment loss of $\geqslant 2$ mm over the course of the study. This clear higher risk for additional attachment loss in the placebo group has been reported previously (Herrera et al. 2002), and attachment loss of $\geqslant 2$ mm in 9 months is certainly clinically significant as it is indicative of a very rapid progression of periodontal breakdown (Machtei et al. 1993). The demonstrated ability of SDD

Table 3. Clinical data reported in randomised clinical trials of subantimicrobial dose doxycycline

Year	Author	Disease	Study length	Study groups	*N*	Mean CAL change (mm)		Mean PD reduction (mm)		% sites with CAL gain		% sites with PD reduction	
						4–6 mm pockets	7+ mm pockets	4–6 mm pockets	7+ mm pockets	⩾2 mm†	⩾3 mm†	⩾2 mm†	⩾3 mm†
2000	Caton et al.	Chronic periodontitis	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
2001	Golub et al.	Chronic periodontitis	36 weeks	SRP+SDD‡	27	−0.15*							
				SRP+placebo	39	−0.80							
2002	Preshaw et al.	Chronic periodontitis	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
2002	Novak et al.	Severe generalised periodontitis	9 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
2003	Preshaw et al.§	Chronic periodontitis	9 months	SRP+SDD non-smokers	66	1.29**	2.12*	1.33**	2.35**	63*	37**	66*	42**
				SRP+placebo non-smokers	76	1.01	1.55	1.00	1.74	45	20	47	22
				SRP+SDD smokers	41	1.19*	2.02	1.19	2.25	50	27	56*	28
				SRP+placebo smokers	26	0.85	1.88	0.93	1.89	42	20	39	18

CAL, clinical attachment level. CAL change can be positive (CAL gain) or negative (CAL loss); PD, probing depth.
*$p<0.05$ compared with placebo.
**$p<0.01$ compared with placebo.
†Calculated for all pockets 6+ mm at baseline (outcomes not reported in original publications, but calculated from raw data).
‡SDD for two cycles of 12 weeks, with SRP at the beginning of each cycle, separated by a 12-week period of no drug.
§Same study population as Preshaw et al. (2002), stratified by smoking status.

to contribute to prevention of further attachment loss is likely to be clinically significant at a patient level, as this may translate into enhanced stability of the periodontium following treatment and a reduced requirement for further interventions (e.g. repeated non-surgical treatment or periodontal surgery), although this has yet to be established with certainty.

Following completion of the 9-month Caton study, patients stopped all study drug therapy (SDD or placebo) and were monitored for an additional 3 months (Caton et al. 2001). Cessation of SDD treatment was not associated with any accelerated regression of periodontal status, and there were no differences between the placebo and SDD groups relating to the incidence of adverse events or laboratory or microbiological parameters. The reductions in probing depths and gains in attachment level that were observed in the previous 9 months were maintained after completion of treatment.

The efficacy of adjunctive SDD has also been reported in smokers and non-smokers (Preshaw et al. 2003). The benefits of SDD, when used as an adjunct to SRP, were apparent in both smokers and non-smokers (Table 3). A hierarchical treatment response was observed such that non-smokers who had SDD tended to demonstrate the best treatment response, and smokers who received placebo demonstrated the poorest response.

Two of the larger clinical trials that investigated the efficacy of adjunctive SDD in the treatment of chronic periodontitis had similar study design and patient inclusion/exclusion criteria (Caton et al. 2000, Preshaw et al. 2002). This permitted us to perform a meta-analysis of the data gathered in the two studies. The adjusted least-square mean changes in probing depths and attachment levels from baseline for moderately diseased and severely diseased tooth sites are shown in Figs 1–4. Smoking status was included as a factor in the analyses. It is clear from this meta-analysis that at each time point, adjunctive SDD resulted in statistically significantly greater mean probing-depth reductions and attachment level gains compared with SRP alone ($p<0.05$ in all cases). The benefits of adjunctive SDD were apparent as early as 3 months after commencing treatment, and were maintained for the 9 months of the study.

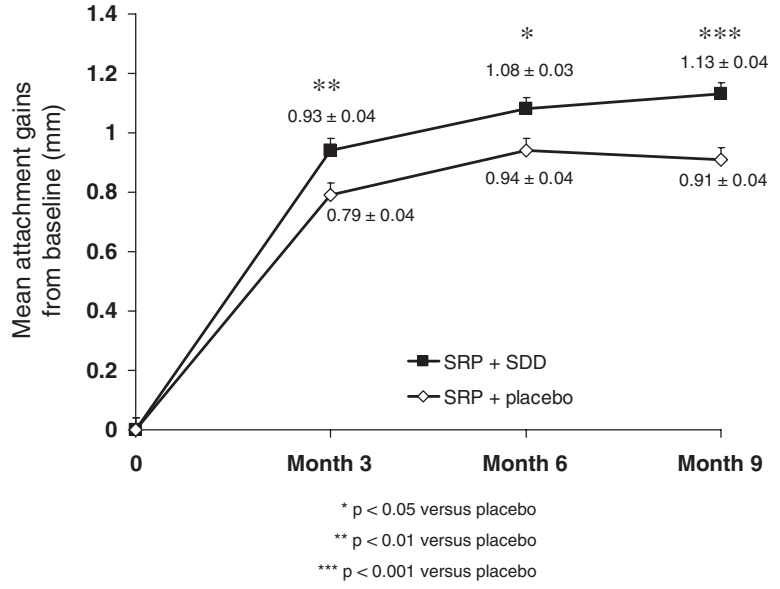

Fig. 1. Meta-analysis of data from Caton et al. (2000) and Preshaw et al. (2002). Effect of subantimicrobial dose doxycycline (SDD) on attachment gains in tooth sites with mild–moderate disease (baseline probing depths 4–6 mm). Patients received scaling and root planing (SRP) at baseline, and then received SDD 20 mg BID (■ , $n = 197$) or placebo BID (◇ , $n = 195$) for 9 months. The mean per patient changes from baseline, adjusted for smoking status, and standard errors are presented.

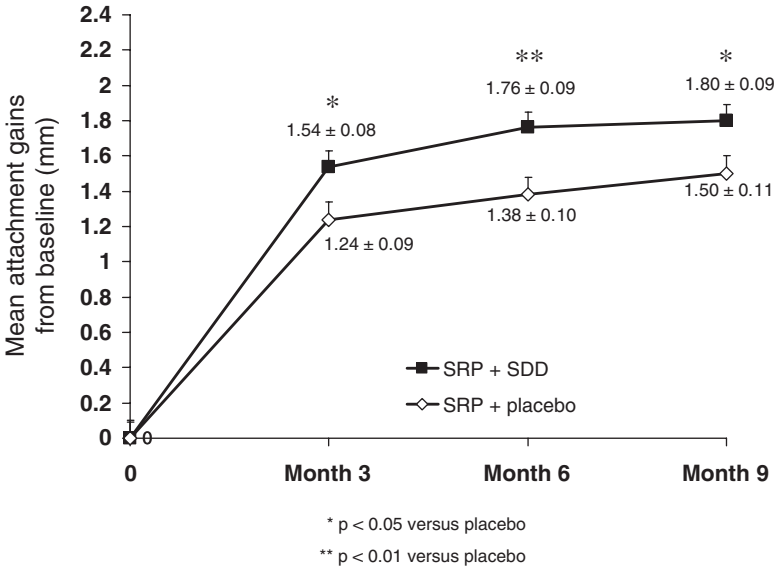

Fig. 2. Meta-analysis of data from Caton et al. (2000) and Preshaw et al. (2002). Effect of subantimicrobial dose doxycycline (SDD) on attachment gains in tooth sites with severe disease (baseline probing depths $\geqslant 7$ mm). Patients received scaling and root planing (SRP) at baseline, and then received SDD 20 mg BID (■ , $n = 197$) or placebo BID (◇ , $n = 195$) for 9 months. The mean per patient changes from baseline, adjusted for smoking status, and standard errors are presented.

Microbiological considerations

It is of critical importance for success or failure of SDD therapy to consider whether or not the long-term prescription of doxycycline at a dose of 20 mg twice daily is likely to lead to the development of antibiotic resistance (Walker 1996). Pharmacokinetic studies in human volunteers have demonstrated that 20 mg doxycycline twice daily resulted in peak serum concentrations of 0.7–0.8 μg/ml and steady-state concentrations of approximately 0.4 μg/ml (Caton 1999). This level is below the minimum inhibitory concentration (MIC) determined for doxycycline in vitro for the great majority of the bacteria isolated from subgingival plaque (Walker et al. 1985, Walker 1996), and is well below the blood levels of 3–4 μg/ml produced by antibiotic doses of 100–200 mg (Walker et al. 2000b). From this perspective, therefore, at a (subantimicrobial) dose of 20 mg twice daily, doxycycline does not appear likely to exert any significant selection pressure resulting in the development of resistant strains, or have any influence on periodontal bacteria. This notion was evident clinically from the first studies of SDD which, although of relatively short duration, demonstrated convincingly that this dosing regimen was not associated with the emergence of doxycycline-resistant microorganisms or other typical antibiotic side-effects (Golub et al. 1994).

In a study of 38 patients who were randomised to receive SRP and adjunctive SDD or placebo, subgingival plaque samples were collected throughout the 9 months of the study (Thomas et al. 1998). Susceptibility testing on the most prevalent organisms was performed with tetracycline, amoxicillin, doxycycline, minocycline, erythromycin and clindamycin, and MIC_{50} and MIC_{90} were calculated for each antibiotic-organism combination. SDD therapy did not result in any overgrowth or replacement by opportunistic oral flora. There was no significant shift or drift in the MIC_{50} and MIC_{90} data, and there was no development of multi-drug resistance to the antibiotics.

In another study, 76 patients with chronic periodontitis were treated by SRP in two quadrants at baseline and either adjunctive SDD or placebo was prescribed for 9 months (Walker et al. 2000b). Plaque samples were collected at baseline, and every 3 months for the 9 months of drug therapy, and also 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 treatment groups, indicating a return towards a

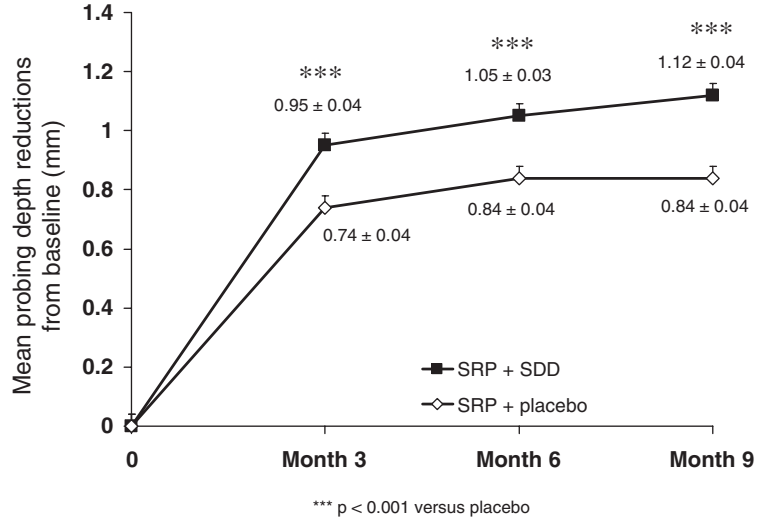

Fig. 3. Meta-analysis of data from Caton et al. (2000) and Preshaw et al. (2002). Effect of subantimicrobial dose doxycycline (SDD) on probing depth (PD) reductions in tooth sites with mild–moderate disease (baseline PD 4–6 mm). Patients received scaling and root planing (SRP) at baseline, and then received SDD 20 mg BID (■ , $n = 197$) or placebo BID (◇ , $n = 195$) for 9 months. The mean per patient changes from baseline, adjusted for smoking status, and standard errors are presented.

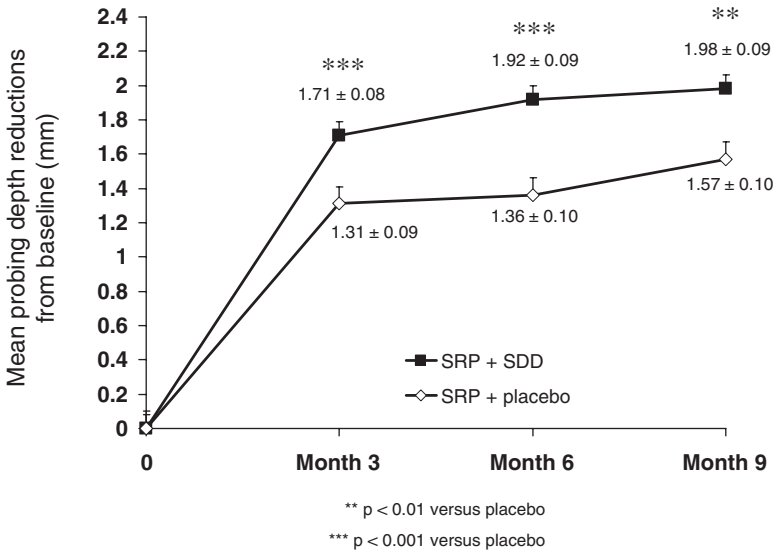

Fig. 4. Meta-analysis of data from Caton et al. (2000) and Preshaw et al. (2002). Effect of subantimicrobial dose doxycycline (SDD) on probing depth (PD) reductions in tooth sites with severe disease (baseline PD $\geqslant 7$ mm). Patients received scaling and root planing (SRP) at baseline, and then received SDD 20 mg BID (■ , $n = 197$) or placebo BID (◇ , $n = 195$) for 9 months. The mean per patient changes from baseline, adjusted for smoking status, and standard errors are presented.

flora more associated with periodontal health. No statistical or microbiological differences in any of the microbiological parameters were detected between SDD and placebo groups and between SRP-treated and non-SRP-treated quadrants, with the exception of small and large spirochetal groups. The spirochetal proportions present in the SDD group were significantly lower at certain periods in the SDD treatment than those in the placebo group and were preceded by a significant decrease in the number of sampled sites that bled on probing. It was felt that the decrease in spirochetal groups in the SDD population resulted from increasingly aerobic conditions in periodontal pockets as a result of clinical improvements derived from SDD therapy, as opposed to any effect of SDD on the spirochetes themselves. Reductions in probing depths favour a less anaerobic plaque composition (Kenney & Ash 1969). Since spirochetes are more sensitive to local oxygen tension than other periodontal pathogens and have relatively low redox requirements for growth (Mikx 1997), a reduction in the proportion of spirochetes is not unexpected in pockets that exhibit reduced probing depths. The distribution of spirochetes, but not other organisms, has been shown to directly correlate with the severity of periodontitis (Riviere et al. 1992). In summary, this study showed that no antimicrobial effect could be detected during or following a 9-month treatment with 20 mg doxycycline twice daily relative to placebo in terms of total bacterial counts, the normal flora, or in periodontal or opportunistic pathogens.

In a subsequent report that addressed the issue of changes in antibiotic susceptibility, patients with periodontitis taking different formulations of SDD or placebo were monitored for up to 27 months. Antibiotic susceptibility of bacteria in subgingival plaque samples was assessed by MIC levels, cross-resistance to non-tetracycline antibiotics, and the proportions of doxycycline-resistant isolates (Thomas et al. 2000). These studies demonstrated that organism MIC levels remained constant among treatment groups, there were no statistically significant differences in the proportion of doxycycline-resistant isolates among the treatment groups and no evidence of multi-antibiotic resistance or cross-resistance at any time point. The authors concluded that long-term SDD does not alter or contribute to changes in antibiotic susceptibility of the subgingival microflora compared with placebo.

The previous studies all demonstrated that adjunctive SDD does not exert any antimicrobial effects or result in changes in antibiotic susceptibility or resistance in the subgingival microflora. A follow-up study was therefore undertaken to identify whether SDD had any effect on the microflora in other body compartments, specifically the intestinal or vaginal microflora (Walker et al. 2000a). Seventy patients with periodontitis were randomised to receive either SDD or placebo over a 9-month

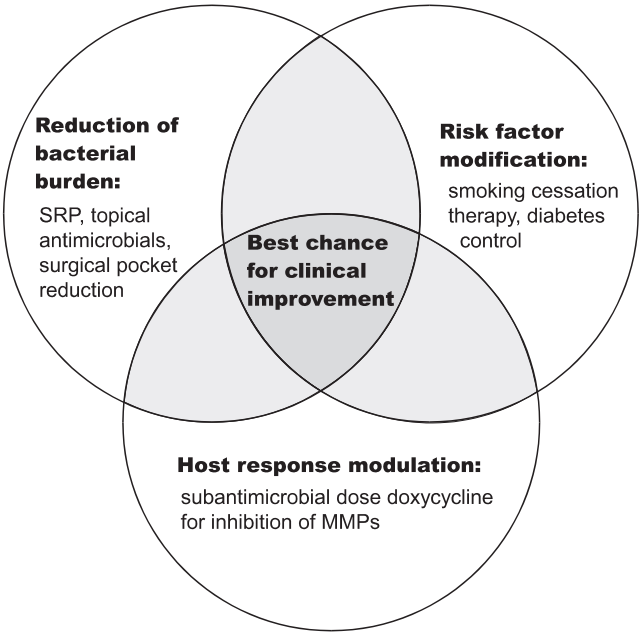

Fig. 5. Complementary strategies for treating periodontitis patients. SRP, scaling and root planing; MMP, matrix metalloproteinase.

period. Stool samples and vaginal swabs were collected at baseline, at months 3 and 9 and examined for total anaerobic counts, opportunistic pathogens and doxycycline resistance ($\geqslant 4\,\mu$g/ml). At 9 months, there were no significant differences in doxycycline-resistant bacteria among the predominant bacterial taxa recovered from either treatment group, or their antibiotic susceptibilities. There was no evidence that SDD exerted any effect on the composition or resistance level of either the faecal or vaginal microflora.

Safety data

Doxycycline at antibiotic doses ($\geqslant 100$ mg) is associated with adverse events including, among others, photosensitivity, hypersensitivity reactions, nausea, vomiting and oesophageal irritation. In the two large clinical trials conducted in the USA, SDD (20 mg doxycycline twice daily) was well tolerated, and the percentages of subjects who discontinued treatment because of adverse events of all causes was similar for both treatment groups (Caton et al. 2000, Preshaw et al. 2002). The most frequently reported adverse events were headache, common cold, and influenza symptoms, and there were no significant differences in the incidence of these adverse events between the treatment groups. The types of adverse events did not differ significantly between treatment groups and the typical side-effects of the tetracycline class of antibiotics were not observed (Caton et al. 2000, Preshaw et al. 2002). Furthermore, there was no evidence of adverse events that could be attributed to any antimicrobial effect of treatment, further supporting the premise that adjunctive SDD has no influence on bacteria in any body compartment. The overall reported adverse event rate with SDD is approximately 0.15% with the most common adverse events being dyspepsia (0.2%), rash (0.1%), headache (0.1%) and diarrhoea (0.1%) (data on file). In summary, SDD is well tolerated with a very low frequency of adverse events.

Limitations of available data

The data that have been described in this review indicate the clinical benefits that have been reported in clinical research populations, which tend to comprise highly motivated, compliant patient groups. Now, studies are required to investigate the adjunctive use of SDD as part of periodontal care in the dental/periodontal practice setting to consider, in particular, longer term outcomes such as prevention of tooth loss and stabilisation of the periodontium. Furthermore, patient-centred outcomes, such as satisfaction with the treatment provided and patient-perceived benefits must be considered. This would include cost/benefit determinations as clearly there is a cost implication with SDD therapy, which may be borne by either the patient, insurance providers or national healthcare systems. The cost of drug therapy may, however, be offset by reduced need for further periodontal treatment, but this clearly needs to be investigated.

A major issue when considering the use of SDD is the duration of therapy and the link between clinical and biological outcomes. There are presently very minimal data linking the clinical response to SDD treatment with changes in local inflammatory responses (e.g. reductions in gingival MMP levels). This is a deficiency in the literature and further elucidation of the links between clinical status and local MMP expression could be useful in terms of linking the duration of drug prescription to both clinical outcomes and direct measurement of the local inflammatory response. For the future, availability of testing systems (Mantyla et al. 2003) to assess the degree of inflammation in the tissues could be used to titrate the duration of therapy against predetermined end points in addition to desired clinical end points of treatment.

Finally, we do not know yet with certainty how non-responding or at-risk patients may benefit from adjunctive SDD therapy, or how SDD therapy may be combined with other treatment modalities such as the use of local delivery systems or periodontal surgery. Further studies are required to clarify these issues.

Summary: HMT as part of a comprehensive periodontal treatment strategy

To date, non-surgical periodontal treatment has primarily focussed on reducing the bacterial burden by mechanical disruption of the subgingival biofilm by SRP. Locally delivered topical antimicrobial agents are also used together with SRP to further reduce the bacterial burden. There is no doubt that plaque bacteria are necessary to initiate disease and drive the chronic inflammatory response in the periodontal tissues. There is strong evidence that destructive

processes occurring as part of the host inflammatory response are responsible for the majority of the hard and soft tissue breakdown leading to the clinical signs of periodontitis. Therefore, periodontists are now in a position to exploit complementary treatment strategies, namely reduction of the bacterial burden and HMT. Studies have shown that these complementary strategies result in significant clinical benefits for patients. The adjunctive use of SDD improves the clinical responses above and beyond the result of what is attainable by mechanical intervention and may lead to more cost-effective periodontal therapy (Ciancio & Ashley 1998). In order to maximise the potential for benefit when using adjunctive SDD, mechanical therapy must be undertaken to the very highest standard. This is the responsibility of the treating clinician who must also explain the rationale for prescribing adjunctive SDD to the patient. It takes time to explain the role of SDD to the patient, but this is important to ensure compliance. Furthermore, by spending time with patients in the educational and motivational phases of treatment, they are more likely to become interested in their condition and develop ownership of their periodontal management.

There is also another treatment strategy that should be undertaken when treating patients with periodontitis, and that is risk factor modification. For example, the deleterious effects of smoking on the periodontal tissues are well known to periodontists (Kinane & Chestnutt 2000), and successful smoking cessation therapy will likely be of major benefit to patients with periodontitis. Another known risk factor for periodontitis is poorly controlled diabetes (Mealey 1999). Periodontal therapy may also impact on diabetic control (Grossi & Genco 1998). Other possible risks include non-modifiable factors such as genetics, gender and race. Future studies will address the role of stress, socio-economic status and coping abilities in periodontal disease susceptibility and progression. As the relevance of different risk factors is established through epidemiological research, it is important that we remain aware of our responsibilities for informing and changing patients' behaviours in relation to modifiable risks.

There are therefore several complementary treatment strategies (patient education and motivation, reduction of the bacterial burden, host response modulation, risk factor modification) that can be implemented together when managing periodontitis patients (Fig. 5). Our improved understanding of the pathogenesis of periodontal diseases and the importance of risk factor management places us in the exciting position of being able to exploit different, but complementary, treatment strategies for treating periodontal disease. The precise treatment for the individual patient will be selected by the periodontist on an individual patient basis. We know from previous research that reducing the bacterial burden is clinically effective. The clinically significant benefits of subantimicrobial dose doxycycline when used in addition to high-quality SRP are also apparent. The development of host modulatory therapies, together with our better understanding of risk factors and disease processes allows us now to improve upon the age old treatment strategy of root surface debridement that was first described so many centuries ago (Ring 1985).

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