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Host response modulation in the management of periodontal diseases

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Abstract

Objective: To review the biological mechanisms and clinical utility of therapeutic modulation of the host response in the management of periodontal diseases.

Material and methods: A search of MEDLINE–PubMed was performed up to and including December 2004. The search was limited to in vitro, experimental animal and clinical studies published in English. The selection criteria included all levels of available evidence: systematic reviews, randomised-controlled clinical trials, controlled clinical trials, prospective and retrospective cohort studies and case reports of human and experimental animal studies.

Results: Six targets for non-microbial chemotherapeutic intervention were identified. Clinical trials have demonstrated the ability of non-steroidal anti-inflammatory drugs to slow periodontal disease progression. However, recently reported serious adverse effects preclude the use of cyclooxygenase-2 inhibitors as an adjunct to periodontal therapy. Adjunctive use of subantimicrobial dose doxycycline to non-surgical periodontal therapy is beneficial in the management of chronic periodontitis over 12 months. Controversial data exist on the effects of bisphosphonate administration as an adjunct to periodontal therapy. Evidence on modulation of other host mediators including lipoxins, cytokines and nitric oxide synthase is limited to animal research.

Conclusion: After validation in long-term clinical trials, adjunctive host modulation therapy may prove advantageous in the management of periodontal diseases.

Key words: cytokines; gingivitis; host modulation; inflammation; periodontitis; prostaglandins

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Periodontal diseases encompass multifactorial diseases involving bacterial biofilms and the generation of an inflammatory response, including the production of cytokines, eicosanoids and matrix metalloproteinases (MMPs). Bacterial biofilms have been shown to be the primary aetiological factor in the initiation of gingival inflammation and subsequent destruction of periodontal tissues (Haffajee & Socransky 1994). Although chronic bacterial and endotoxin exposure is a prerequisite for gingival inflammation and periodontal tissue destruction to occur, its presence alone accounts for a relatively small proportion (i.e. 20%) of the variance in disease expression (Grossi et al. 1994). According to a novel model of pathogenesis (Offenbacher 1996, Page & Kornman 1997), this is not sufficient to explain disease initiation and progression. The major component of soft- and hard-tissue destruction associated with periodontal disease is the result of activation of the host's immuno-inflammatory response to the bacterial challenge. The underlying biological mechanisms of this response are characterized by the expression of endothelial cell and intercellular adhesion molecules and by the production of host-derived inflammatory mediators including cytokines and lipids by neutrophils, monocytes, lymphocytes and fibroblasts. Acquired and environmental risk factors, such as diabetes mellitus, cigarette smoking and stress, as well as genetically transmitted traits, such as interleukin-1 (IL-1) gene polymorphisms, may accentuate the host inflammatory response to the bacterial challenge and, eventually, the susceptibility to the disease (Kornman & Di Giovine 1998, Salvi et al. 1998, Kinane & Chestnutt 2000, Albandar 2002). The mechanisms of interaction between bacteria, host cells and the extracellular matrix components have been reviewed by Offenbacher (1996). Based upon histopathological features, inflammatory processes may be divided into three phases: an acute phase, an immune response and a chronic phase. The transition process from gingival health to early inflammatory changes is characterized by a local increase in vascular permeability, redness, swelling and by

the recruitment and activation of polymorphonuclear granulocytes (PMNs) (Delima & Van Dyke 2003). In the course of this acute phase, several products modulate vasodilatation (e.g. bradykinin and prostaglandins), vascular permeability (e.g. histamine and leukotriene (LT)) and additional recruitment of inflammatory cells through chemotaxis (e.g. complement products, LT, IL-8). For example, LTB_4 is a product of the lipoxygenase (LO) pathway and is a marker of neutrophil activation. This potent chemotactic mediator plays a central role in the recruitment of PMNs and monocytes to sites of developing gingival inflammation. Moreover, LTB_4 has been associated with inflammatory processes involving bone resorption (Meghji et al. 1988, Gallwitz et al. 1993, Garcia et al. 1996). The subsequent immune response starts when antigen-presenting cells become involved presenting the foreign microorganisms or antigens to immunocompetent cells such as T lymphocytes. This leads to the expansion of antibody-secreting plasma cells and the development of a chronic lesion. In homeostasis, neutralization and phagocytosis of the invading microorganisms and secretion of appropriate cytokines may lead to a successful outcome of the inflammatory response. However, the outcome may be destructive if, during the chronic process, elevated amounts of inappropriate cytokines are released in the surrounding tissues (Gemmell & Seymour 2004). These mediators include cytokines such as IL and tumour necrosis factor (TNF) and are secreted in addition to the acute-phase products such as complement components and metabolites of the arachidonic acid (AA) pathway (e.g. prostaglandins, prostacyclin, LTs and thromboxanes). For example, the changes in gingival crevicular fluid (GCF) levels of PGE_2, TxB_2, IL-1β TNF-α and LTB_4 were monitored over a period of 6 months using the ligature-induced periodontitis model in rhesus monkeys (Smith et al. 1993). In a split-mouth design, eight teeth were ligated to promote plaque accumulation and eight contra-lateral teeth were exposed to natural plaque accumulation, eventually leading to periodontal tissue destruction. After 1 month, the GCF-LTB_4 concentrations reached a three-fold peak over the baseline levels, together with a parallel rise in clinical signs of gingival inflammation, suggesting that neutrophil activation played an important role at this stage. After 2 months in the ligated sites, a significant three-fold increase in GCF-PGE_2 could be observed when compared with baseline levels, indicating that at a more advanced stage of the lesion other cell types (e.g. monocytes and lymphocytes) were activated, leading to an elevated secretion of cytokines and PGE_2 with consequent loss of periodontal attachment and alveolar bone. On the other hand, findings from recent studies have indicated that endogenous lipid-derived mediators (e.g. lipoxins (LXs)) are released in the course of the host response to dampen the inflammatory process and modulate resolution of inflammation (Van Dyke & Serhan 2003). In this context, LXs were identified and recognized as endogenous blocking signals for PMN (Diamond et al. 1999, Pouliot et al. 2000).

Based on these and other pathways of inflammation, the aim of this review is to elucidate the biological mechanisms and clinical applications of host response modulation including regulation of (i) AA metabolites, (ii) LXs, (iii) MMPs, (iv) bone remodeling, (v) cytokine receptors and (vi) nitric oxide synthase (NOS) activity.

Modulation of AA Metabolites

The AA cascade

The characteristics of a vasoactive fatty acid from human seminal vesicle fluid capable of decreasing blood pressure in rabbits were first described by Von Euler (1939). This product was named ''prostaglandin'' because it was assumed to originate from the prostate gland. In the following decades, the implication of prostaglandins as mediators of inflammation increased continuously (Kuehl & Egan 1980, Oates et al. 1988).

AA is a 20-carbon polyunsaturated fatty acid (eicosanoid) liberated from membrane phospholipids by the action of phospholipase A_2. Free AA is metabolized via either the cyclooxygenase (COX) or the LO pathways. AA is enzymatically oxidized by either COX to form unstable cycloendoperoxide intermediates (PGG_2 and PGH_2) leading to prostanoid synthesis (prostaglandins, prostacyclin and thromboxane) or by the action of LO to form the LTs and other monohydroxy-eicosatetraenoic acids. This process is referred to as the AA cascade (Fig. 1).

Two isoforms of the enzyme COX with approximately 64% overall identities have been characterized (Futaki et al. 1994, Gierse et al. 1995). COX-1 is a constitutive enzyme expressed in most cells and tissues, and appears to represent an essential component of tissue homeostasis (e.g. gastric cytoprotection, vascular and renal homeostasis). In contrast, COX-2 represents an inducible isoform localized primarily in inflamed tissues (Morton & Dongari-Bagtzoglou 2001) and is upregulated by IL-1β, TNF-α, bacteria and lipopolysaccharide (LPS) (Noguchi et al. 1996, 2000, 2001, Ueda et al. 1998, Pouliot et al. 2000, Yumoto et al. 2001, Miyauchi et al. 2004), nicotine (Chang et al. 2003), cell-to-cell contact (Yucel-Lindberg et al. 2001) and mechanical stress (Shimizu et al. 1998). Cells stimulated with cytokines such as IL-1β or LPS are able to rapidly synthesize COX-2 from pre-existing mRNA and translate new COX-2 transcripts, leading to a prolonged COX-2 release without inducing COX-1 biosynthesis.

AA metabolites as mediators of bone resorption

While studying mechanisms of bone resorption of mouse calvaria in tissue culture, Goldhaber (1971) found that the resorptive process could be stimulated by prostaglandins produced by human gingival extracts or by prostaglandins secreted into the bone culture upon stimulation by an unidentified factor released from the gingival tissue. Almost contemporarily, Klein & Raisz (1970) reported that prostaglandins and PGE_2 specifically had the potential to induce bone resorption directly in organ culture. Goodson et al. (1974) demonstrated with in vivo experiments that prostaglandins were implicated in the bone resorption process. A rapid bone resorption could be induced within 7 days after injection of a PGE_1-containing solution under the skin of rat calvaria. In addition to prostaglandins, other AA metabolites such as prostacyclin and LT appeared to be actively involved in bone resorption. Prostacyclin (PGI_2) is an endothelial cell product capable of preventing platelet aggregation and platelet adhesion to vessel walls (de Leval et al. 2004). Findings from tissue culture experiments demonstrated that PGI_2 stimulated bone resorption (Raisz et al. 1979, Neuman & Raisz 1984). PGI_2 rapidly and spontaneously hydrolysed to the inactive metabolite 6-keto $PGF_{1\alpha}$, which enzymatically oxidized

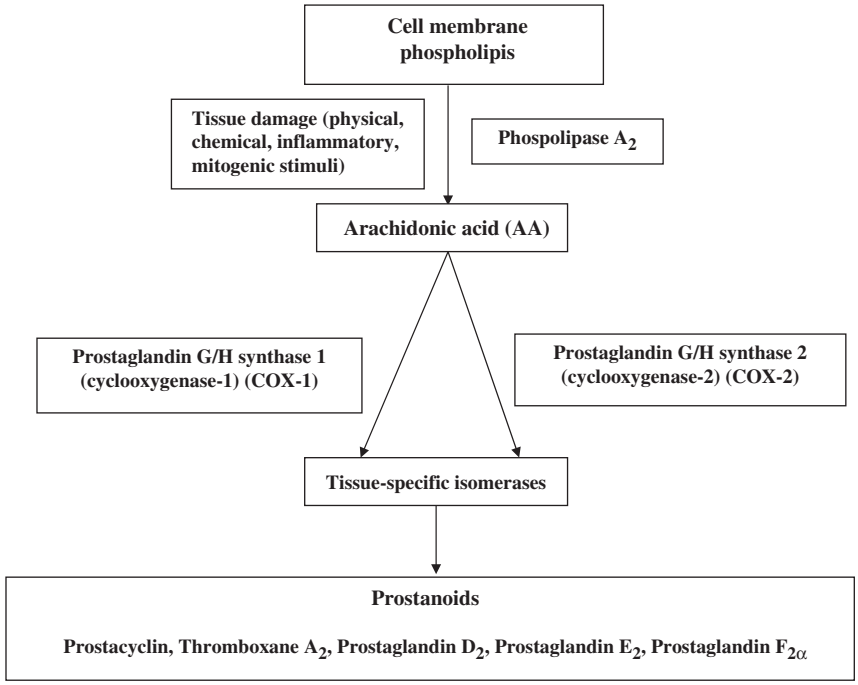

Fig. 1. Arachidonic acid (AA) metabolic transformation leading to prostanoids synthesis through the cyclooxygenase-1 (COX-1) and cycloxygenase-2 (COX-2) pathways.

to 6-keto $PGE_{1\alpha}$. This latter metabolite of PGI_2, 6-keto $PGE_{1\alpha}$, stimulated significantly more bone resorption in tissue culture compared with 6-keto $PGF_{1\alpha}$, but its potency was about one-twelfth of that of PGE_2 (Dewhirst 1984). On the other hand, the two main metabolites of PGI_2 stimulated bone resorption with a similar potency (Neuman & Raisz 1984). Similarly, LTB_4 has been shown to stimulate bone resorption both in vitro and in animal models (Meghji et al. 1988, Gallwitz et al. 1993, Garcia et al. 1996, Traianedes et al. 1998).

AA metabolite levels in periodontal tissues and GCF

Animal experiments and clinical studies have provided evidence that prostanoid levels within periodontal tissues and the GCF correlate with the clinical expression of periodontal disease severity (Offenbacher et al. 1986).

A summary of clinical studies associating the levels of AA metabolites in gingival tissues or GCF and periodontal disease severity is provided in Table 1.

Modulation of AA metabolites with non-steroidal anti-inflammatory drugs (NSAIDs)

Over decades, AA metabolites have been established as mediators of tissue destruction in various inflammatory diseases including rheumatoid arthritis and periodontal diseases (Offenbacher et al. 1993, O'Dell 2004). The fact that NSAIDs can suppress alveolar bone resorption suggests that the synthesis of AA metabolites may represent a critical regulatory pathway for potentially blocking periodontal disease progression. The majority of NSAIDs are weak organic acids that selectively (COX-2) and non-selectively (COX-1) inhibit the synthesis of AA metabolites, thereby blocking the production of prostaglandins, thromboxane and prostacyclin (Fitzgerald & Patrono 2001).

In vitro experiments

After the first report (Vane 1971) that aspirin and aspirin-like drugs inhibited the production of prostaglandins by inhibiting the COX enzyme, additional experiments followed (Ferreira et al. 1971, Smith & Willis 1971). In another series of experiments, Goldhaber et al. (1973) added indomethacin, a known inhibitor of COX, to the culture media, observing a decrease in bone resorption of up to 50%. Other researchers (Gomes et al. 1976, Heijl et al. 1976) suggested that prostaglandins synthesized during an acute inflammatory response may be important mediators involved in bone resorption. The amount of prostaglandins released from gingival monkey fragments into the culture medium could be reduced by at least 90% by indomethacin, indicating that over 90% of the released prostaglandins were synthesized during tissue culture (Gomes et al. 1976).

Findings from in vitro experiments showed that selective COX-2 inhibitors blocked prostaglandin production. The effect of cyclic tension forces applied to human periodontal ligament (PDL) cells was investigated with respect to PGE_2 production and COX-2 mRNA and protein expression (Shimizu et al. 1998). PGE_2 release into the culture medium and COX-2 mRNA and protein expression significantly increased in a time-dependent manner. When the NSAID NS-398, a selective COX-2 inhibitor, was added to the medium, PGE_2 synthesis was completely inhibited, indicating that tension force stimulated COX-2 expression in human PDL cells (Shimizu et al. 1998). Epithelial and connective tissue cell cultures from inflamed gingival biopsies challenged with IL-1β or bacterial cells yielded COX-2 expression as well as an upregulated COX-2 activity (Morton & Dongari-Bagtzoglou 2001). This resulted in a sustained release of PGE_2 into the culture supernatants. Administration of NS-398 almost completely suppressed PGE_2 synthesis by these cells. Recently, the role of COX-2 expression in human gingival fibroblasts (HGF) stimulated with nicotine was evaluated (Chang et al. 2003). The findings of this experiment showed that nicotine was capable of stimulating COX-2 mRNA and protein expression in HGFs. This suggested that one of the mechanisms involved in smoking-associated periodontal tissue destruction in vivo was the synthesis of COX-2 by resident periodontal cells. Administration of NS-398 to cultured HGFs, however, did not show any protective effects on nicotine-induced cytotoxicity, indicating that nicotine cytotoxicity was not directly related to the induction of COX-2 expression.

Animal experiments

The fact that elevated levels of PGE_2 levels stimulated bone resorption and that this process could be inhibited with NSAIDs prompted Nyman et al. (1979) to test the efficacy of systemic doses of indomethacin on the suppression of alveolar bone resorption and gingival inflammation in a ligature-induced periodontitis model in beagle

Table 1. Clinical studies associating arachidonic acid metabolites levels in gingival tissues or GCF with periodontal disease severity

References	Study design	Outcome
Goodson et al. (1974)	Measurement of PGE_2 in biopsy samples from six healthy, four gingivitis patients and three suppurative lesions	High PGE_2 levels in inflammation, very high PGE_2levels in suppurative lesions
El Attar (1976)	Measurement of tissue levels of PGE_2 in 12 healthy and 24 gingivitis samples	PGE_2 in gingival health was 16 pmol/g and 285 pmol/g in inflamed tissues.
Holmes & El Attar (1977)	Measurement of tissue levels of PGE_2 in seven healthy and seven gingivitis samples	Healthy PGE_2 = 3.8 pmol/g tissue Disease PGE_2 = 44.7 pmol/g tissue
Offenbacher et al. (1981)	Measurement of GCF levels in five patients with CP and in seven patients with gingivitis	CP patients had significantly higher GCF-PGE_2 levels compared with gingivitis patients Wide range of GCF-PGE_2 levels in the CP group
Dewhirst et al. (1983)	PGE_2, TxB_2 and 6-keto-$PGF_{1\alpha}$ measurement in gingival biopsies adjacent to shallow and deep inflamed periodontal pockets. Control sites without clinical inflammation	Detectable PGE_2 and TxB_2 levels in most of the deep sites No detectable PGE_2 and TxB_2 levels in control sites
Ohm et al. (1984)	PGE_2 in gingival biopsies from seven healthy, 17 gingivitis and 26 periodontitis patients	Healthy PGE_2 = 1.7 ng/g tissue Gingivitis = 5.7 ng/g tissue Periodontitis 23.2. ng/g tissue
Offenbacher et al. (1984)	GCF and tissue analyses of PGE_2 in seven CP and 12 AP patients	Chronic GCF-PGE_2 = 57.5 ng/ml Aggressive GCF-PGE_2 = 144.0 ng/ml Local GCF levels correlated with local tissue levels
Offenbacher et al. (1986)	Measurement of GCF-PGE_2 levels in 41 CP patients over a period of 18 months	Mean GCF-PGE_2 levels higher than 66.2 ng/ml placed the patient at risk for ALOSS at one or more sites within the subsequent 6-month period
Offenbacher et al. (1993)	Cross-sectional evaluation of GCF-PGE_2 in 27 CP, 12 AP and four refractory periodontitis patients	GCF-PGE_2 levels elevated two- to threefold in AP patients compared with those in CP patients
Nakashima et al. (1994)	Measured GCF-PGE_2 in healthy, gingivitis and periodontitis sites in 17 CP patients	GCF-PGE_2 levels positively correlated with disease severity (PPD)
Zhou et al. (1994)	Measured GCF-PGE_2 levels in 46 healthy and 90 CP patients	GCF-PGE_2 levels positively correlated with disease severity

GCF, gingival crevicular fluid; PGE_2, prostaglandin E_2; CP, chronic periodontitis; AP, aggressive periodontitis.

dogs. The results showed that indomethacin delayed the onset and suppressed the magnitude of the acute inflammatory response and decreased the amount of alveolar bone resorption. This report represented the beginning of a long series of animal experiments investigating the effects of NSAIDs on periodontal disease progression. The effects of systemic and topical NSAIDs administrations on GCF AA metabolites levels, gingival inflammation, periodontal tissue and alveolar bone destruction are summarized in Table 2.

Clinical studies

The effects of systemic and topical NSAIDs administration to prevent gingival inflammation and as an adjunct to surgical and non-surgical periodontal therapy are summarized in Tables 3 and 4, respectively.

Adverse systemic effects of NSAIDs

COX-1 inhibitors seem to damage the mucosa of the gastrointestinal tract predominantly by interfering with the physiological synthesis of prostaglandins. PGE_2 and PGI_2 reduce gastric acid secretion, increase blood flow through the gastric mucosa and stimulate the production of cytoprotective mucus. Because of suppression of prostaglandin production, COX-1 inhibitors may cause gastric ulceration by producing mucosal ischaemia and by impairing the protective function of the mucous barrier, thus exposing the gastric mucosa to the effects of acid attacks (Hawkey 1993). Moreover, PGE_2 and PGI_2 are synthesized in the renal medulla and glomeruli, respectively. Both are powerful vasodilators involved in the control of renal blood flow and salt and water excretion. Prolonged NSAIDs intake leading to the suppression of renal prostaglandin synthesis may result in increased sodium retention, reduced renal blood flow and eventually renal failure (Lindsley & Warady 1990). Moreover, recent studies have postulated that some COX-2 inhibitors may affect cardiovascular risks through various mechanisms (Couzin 2004, Wardle 2004). While our understanding of the role of COX-2 in the pathogenesis of periodontitis suggests that inhibition of COX-2 might be a desirable target for therapeutic intervention, serious adverse effects of current formulations preclude

Table 2. Animal studies on the effects of topical and systemic NSAIDs administration on GCF arachidonic acid metabolites levels, gingival inflammation, periodontal tissue and alveolar bone destruction

References	Study design	NSAID	Observation period	Outcome
Nyman et al. (1979)	Effects of NSAID administration on experimental periodontitis in beagle dogs	Systemic indomethacin	28 days	Indomethacin administration reduced inflammation and bone loss
Nuki et al. (1981)	Effects of NSAID administration on experimental periodontitis-induced bone loss in beagle dogs	Systemic indomethacin	21 days	No effect of indomethacin administration on ligature-induced bone-resorbing activity of gingival extracts
Weaks-Dybvig et al.(1982)	Effects of NSAID administration on bone loss in experimental periodontitis in squirrel monkeys	Systemic indomethacin	14 days	Indomethacin eliminated the loss of alveolar bone height and mass and suppressed the increase in osteoclast density
Lasfargues & Saffar (1983)	Effects of NSAID administration compared with those of calcitonin on bone resorption during experimental periodontitis in hamsters	Systemic indomethacin	12 weeks	Although not significantly, indomethacin induced a decrease in bone loss and osteoclast density
Williams et al. (1984)	Effects of NSAID administration combined with surgical or non-surgical treatment of naturally occurring periodontal disease in beagle dogs	Systemic flurbiprofen	12 months	Flurbiprofen significantly decreased the rate of alveolar bone loss
Jeffcoat et al. (1986)	Follow-up of above study after withdrawal of the drug	Systemic flurbiprofen	6 months	The inhibitory effect of flurbiprofen on disease progression was lost 6 months after withdrawal of the drug
Vogel et al. (1986)	Effects of NSAID administration compared with those of a topical substituted oxazolopyridine derivative during experimental periodontitis in squirrel monkeys	Systemic indomethacin	14 days	The topical NSAID inhibited inflammation and ALOSS when compared with indomethacin Both NSAIDs inhibited bone loss
Offenbacher et al. (1987)	Effects of NSAID administration on bone loss and clinical parameters during experimental periodontitis in *Macaca mulatta* monkeys	Systemic flurbiprofen	6 months	Flurbiprofen inhibited ALOSS, gingival redness and BOP
Williams et al. (1988a)	Effects of NSAID administration on bone loss in experimental periodontitis in beagle dogs	Systemic ibuprofen	13 months	Ibuprofen significantly reduced bone loss
Williams et al. (1988c)	Effects of NSAID administration on bone loss during experimental periodontitis in beagle dogs	Systemic flurbiprofen and indomethacin	12 months	Flurbiprofen and indomethacin reduced bone loss but the effect of indomethacin was not significant. Both drugs reduced GCF arachidonic acid metabolites
Williams et al. (1988b)	Effects of NSAID administration on bone loss during experimental periodontitis in beagle dogs	Topical flurbiprofen	13 months	Topical flurbiprofen significantly decreased the rate of alveolar bone loss
Offenbacher et al. (1989)	Effects of NSAID administration on GCF levels of cyclooxygenase metabolites in *Macaca mulatta* monkeys	Systemic flurbiprofen	6 months	Flurbiprofen did not affect the elevation of GCF-PGE_2 at 3 months. However, at 6 months, flurbiprofen caused a dose-dependent inhibition of GCF-PGE_2
Kornman et al. (1990)	Effects of NSAID administration on gingivitis and PMN response in monkeys	Topical ibuprofen and meclofenamic acid	20 weeks	Both NSAIDs inhibited bone loss. All changes were in the absence of any effect on gingivitis
Howell et al. (1991a)	Effects of NSAID administration on bone loss during experimental periodontitis in beagle dogs	Systemic naproxen	7 months	Naproxen significantly reduced alveolar bone resorption
Howell et al (1991b)	Effects of NSAID administration on gingivitis in beagle dogs	Topical piroxicam	16 weeks	Piroxicam significantly reduced gingival and BOP scores. No data on bone metabolism were available

Offenbacher et al. (1992)	Clinical and biochemical effects of three different NSAIDs and routes of application during experimental periodontitis in beagle dogs	Systemic ibuprofen Systemic naproxen Topical flurbiprofen	6 months	All three NSAID treatments significantly reduced bone loss. A significant decrease in the GCF levels of both PGE_2 and TxB_2 was noted in all NSAID-treated groups
Li et al. (1996)	Effects of NSAID administration on GCF $PGE_2$and LTB_4 levels during experimental periodontitis in monkeys	Topical ketoprofen	6 months	Significant reductions in GCF $PGE_2$and LTB_4 levels compared with control animals
Paquette et al. (1997)	Effects of NSAID administration on alveolar bone loss during experimental periodontitis in beagle dogs	Topical ketoprofen Systemic ketoprofen	2 months	Lower rates of alveolar bone loss in ketoprofen-treated animals compared with those of placebo-treated animals
Bezerra et al. (2000)	Effects of NSAID administration on alveolar bone loss during experimental periodontitis in rats	Systemic indomethacin Systemic meloxicam	7 days	Both NSAIDs regimens significantly inhibited alveolar bone loss and inflammation compared with non-treated animals. Meloxicam administration resulted in less gastric damage compared with indomethacin treatment
Holzhausen et al. (2002)	Effects of NSAID administration on alveolar bone loss during experimental periodontitis in rats	Systemic celecoxib	30 days	Celecoxib administration resulted in significant reduction of alveolar bone loss compared with controls. Progression of bone loss, however, was not completely stopped in celecoxib-treated rats. Infiltration of neutrophils, monocytes and lymphocytes into the connective tissue of celecoxib-treated rats was significantly reduced compared with that of controls
Gurgel de Vasconcelos et al. (2004)	Effects of NSAID administration on alveolar bone loss during experimental periodontitis in rats	Systemic meloxicam	15 and 45 days	Meloxicam administration resulted in significant reduction of alveolar bone loss compared with controls. After drug withdrawal, no remaining effect was observed

NSAID, non-steroidal anti-inflammatory drugs; GCF, gingival crevicular fluid; PMN, polymorphonuclear granulocytes; BOP, bleeding on probing.

their use as an adjunct to periodontal therapy.

LXs: Endogenous Modulators of Inflammation

Recent evidence suggests that LXs are a class of both structurally and functionally unique eicosanoids involved in counter-regulation of inflammatory responses (Kantarci & Van Dyke 2003). These lipid mediators also appear to facilitate the resolution of the acute inflammatory response (Van Dyke & Serhan 2003). In short, resolution of inflammation is an active process. LX and aspirin-triggered lipoxin (ATL) are bioactive lipid mediators involved in the AA cascade and are formed by the interaction of 5- and 15-LOs (Claria & Serhan 1995). Like most lipid mediators, lipoxins are rapidly synthesized, act within a local environment and are rapidly enzymatically degraded. Metabolically stable analogues of both LXA_4 and its epimer 15-epi LXA_4 are active in the nanomolar range and inhibit PMN adhesion and diapedesis, thus representing counter-regulatory signals involved in the resolution of inflammatory processes (Serhan et al. 1995, Takano et al. 1997).

In vitro and in vivo experiments investigated the impact of metabolically stable LX and ATL analogues on TNF-α-induced neutrophil responses (Pouliot & Serhan 1999). At nanomolar concentrations, these LX analogues blocked IL-1β secretion from human PMNs stimulated with TNF-α. Furthermore, application of LX analogues to murine air pouches drastically reduced TNF-α-stimulated leucocyte transmigration, concomitantly stimulating IL-4 in pouch exsudates. These findings indicated that both LX and ATL analogues downregulated (e.g. IL-1β) and stimulated (e.g. IL-4) the release of cytokines involved in the pathogenesis of periodontitis.

To counteract the known proinflammatory effects of PGE_2 in periodontal disease, the potential protective contribution of lipoxins was investigated in the murine air pouch model (Pouliot et al. 2000). *Porphyromonas gingivalis* was introduced in the dorsal air pouch eliciting leucocyte infiltration concomitant with an upregulated expression of COX-2 mRNA in recruited leucocytes and elevated levels of PGE_2. The administration of stable analogues of LX and of ATL blocked neutrophil migration

Table 3. Studies in humans on the effects of NSAID administration during the development and resolution of gingivitis

References	Study design	NSAID	Observation period (days)	Outcome
Vogel et al. (1984)	Effects of topical fluocinonide (steroid) appplication compared with those of NSAID administration during experimental gingivitis	Systemic sulindac	22	Only the topical steroid drug was able to significantly inhibit gingival inflammation when compared with the systemically administered sulindac. Sulindac had no significant effect on GCF flow and bleeding index
Heasman et. al. (1989)	Effects of NSAID administration on experimental gingivitis	Topical flurbiprofen	17	Experimental gingivitis developed in all subjects, and no significant differences between treatments with respect to Gingival Index and PPD were noted
Johnson et al. (1990)	Effects of NSAID administration on the resolution of established gingivitis	Systemic naproxen	30	Naproxen had no significant effect on plaque, gingival and bleeding index scores. A significant effect was only seen in resolution of gingival inflammation after plaque was removed
Abramson et al. (1992)	Effects of NSAID administration on GCF-PGE_2 and TxB_2 levels in subjects with gingivitis and mild periodontitis	Systemic flurbiprofen	56	GCF-PGE_2 and GCF-TxB_2 levels were significantly reduced in the flurbiprofen-treated patients. One week after drug administration was discontinued, GCF levels of PGE_2 and TxB_2returned to pre-treatment levels
Heasman et al. (1993b)	Effects of NSAID administration during experimental gingivitis	Systemic flurbiprofen	28	GCF-PGE_2 and GCF-TxB_2 levels were significantly reduced in the flurbiprofen-treated patients. This coincided with clinically reduced bleeding on probing scores
Heasman et al. (1994)	Effects of NSAID administration in conjuction with toothbrushing on the resolution of experimental gingivitis	Systemic flurbiprofen	7	No significant differences between flurbiprofen- or placebo-treated groups were found with respect to Plaque Index and GCF flow rate. The flurbiprofen group demonstrated significantly greater resolution of gingival inflammation compared with the placebo group
Jones et al. (1999)	Effects of NSAID administration during experimental gingivitis	Topical flurbiprofen	7	Compared with placebo treatment, subjects receiving topical flurbiprofen application exhibited a significant reduction in Gingival Index scores and GCF volume rates
Sekino et al. (2005)	Effects of NSAID administration during experimental gingivitis	Systemic ibuprofen	14	Systemic ibuprofen administration had a significant beneficial effect on the development of gingival bleeding sites ($GI \geqslant 2$) but not on de novo plaque formation

NSAID, non-steroidal anti-inflammatory drugs; GCF, gingival crevicular fluid; PPD, probing pocket depth.

into the air pouch cavity and decreased PGE_2 levels within cellular exsudates.

The response to ligature-induced periodontitis was assessed both radiographically and morphometrically over a 6-week period in transgenic rabbits overexpressing 15-LO and in non-transgenic animals receiving topical application of 15-epi-LXA_4 (Serhan et al. 2003). Periodontitis was induced with silk ligatures alone and with topical application of *P. gingivalis* mixed in a carboxymethylcellulose vehicle. In the absence of ligature, bone loss was not detected. Enhanced expression of 15-LO in transgenic rabbits as well as topical application of 15-epi-LXA_4 in non-transgenic animals significantly reduced bone loss and gingival inflammation induced by *P. gingivalis* as well as by ligatures alone.

Investigations have focused on neutrophil abnormalities in subjects with localized aggressive periodontitis and their role in tissue destruction (Pouliot et al. 2000, Kantarci et al. 2003). Both studies provided evidence that LXA_4 was present in the GCF and was generated by *P. gingivalis*-exposed neutrophils from subjects with localized aggressive periodontitis, suggesting that this lipid mediator may be involved in inflammatory responses associated with specific forms (e.g. aggressive) of periodontitis.

Collectively, data have shown that lipoxins are capable of preventing gingival inflammation and bone loss in animal experimental periodontitis.

Modulation of MMPs

Role of MMPs in connective tissue breakdown and periodontal disease

MMPs encompass a family of zinc-dependent membrane-bound and secreted proteolytic enzymes. Their main function is to catalyse the breakdown of proteins in the cell plasma membrane or within the extracellular matrix (Birkedal-Hansen et al. 1993, Ryan & Golub 2000). The extracellular matrix consists of collagenous and non-collagenous (e.g. glycoproteins and proteoglycans) proteins. In order for the collagenases to have access to the collagen substrate, proteoglycans and fibronectin must be removed first by the action of specific MMPs such as stromelysin (MMP-3). Deregulation of MMPs activity is involved in a variety of pathological conditions such as rheumatoid arthritis, tumour cell metastasis

Table 4. Studies in humans on the effects of adjunctive NSAIDs administration on periodontal, biochemical and radiographic parameters

References	Study design	NSAID	Observation period (months)	Outcome
Jeffcoat et al. (1988)	Periodontal treatment of 15 patients with refractory periodontitis. Placebo control group	Systemic Flurbiprofen 50 mg bid	2	Standardized radiographs showed significantly less alveolar bone loss in the flurbiprofen group compared with the placebo group
Williams et al. (1989)	Monitoring of 44 patients with advanced CP on the progression of alveolar bone loss. Placebo control group	Systemic flurbiprofen 50 mg bid	24	After 12 and 18 months, significantly less alveolar bone loss in flurbiprofen-treated patients compared with controls. At 24 months, rate of bone loss returned to pre-treatment levels in both patient groups
Jeffcoat et al. (1991)	Periodontal treatment of seven patients with RPP. Placebo control group	Systemic naproxen 500 mg bid	3	Subtraction radiography showed significant decrease in bone loss in the naproxen-treated group compared with the placebo group. Significant bone gain in the naproxen group
Heasman et al. (1993a)	Non-surgical periodontal therapy of CP patients with adjunctive NSAID. Placebo control group	Topical flurbiprofen	12	No adjunctive effect of topical flurbiprofen on plaque and bleeding scores, PPD reduction and CAL gain. Significantly more sites with bone gain in the flurbiprofen-treated patients compared with the control group
Reddy et al. (1993)	Non-surgical periodontal therapy of RPP patients with adjunctive NSAID. Placebo control group	Meclofenamate sodium 50 or 100 mg bid	6	Subtraction radiography showed significant bone gain for both NSAID dosages compared with the placebo group
Haffajee et al. (1995)	Surgical periodontal therapy in CP patients with adjunctive NSAID. Placebo control group	Systemic ibuprofen 400 mg tid	10	Adjunctive systemic ibuprofen did not have a beneficial effect on clinical and microbiological parameters compared with placebo
Jeffcoat et al. (1995), Cavanaugh et al. (1998)	Oral prophylaxis in CP patients with adjunctive NSAID. Placebo control group	Ketorolac rinse 0.1% bid versus systemic flurbiprofen 50 mg bid	6	Both adjunctive NSAIDs significantly reduced alveolar bone loss rate and GCF-PGE_2 levels compared with placebo. No significant effects of adjunctive NSAIDs on clinical parameters. No significant influence of ketorolac rinse on GCF-IL-1β
Flemmig et al. (1995)	Supportive periodontal therapy of CP patients with adjunctive NSAID. Placebo control group	Irrigation with 0.3% ASA	6	Supragingival irrigation with ASA failed to significantly reduce Gingival Index scores and PPD compared with control group
Flemmig et al. (1996)	Non-surgical periodontal therapy in 30 CP patients with adjuctive NSAID. Placebo treatment	Systemic ASA 500 mg qid	3	Adjunctive ASA administration resulted in synergistic reductions of gingival inflammation and PPD as well as CAL gain compared with placebo treatment
Brägger et al. (1997)	Surgical periodontal therapy in CP patients with adjunctive NSAID. Placebo control group	Systemic flurbiprofen 50 mg tid for 30 days	6	Standardized radiographs showed no significant crestal bone density changes comparing flurbiprofen- to placebo-treated patients
Lawrence et al. (1998)	Untreated CP patients	Systemic and topical ketoprofen	14.5 days	Systemic ketoprofen administration resulted in significantly higher plasma concentrations compared with four topical ketoprofen formulations
Ng & Bissada (1998)	Non-surgical periodontal treatment in CP patients with adjunctive NSAID. Control group	Systemic ibuprofen 800 mg/day for 45 days	45 days	Adjunctive ibuprofen administration showed no beneficial effects with respect to PPD reduction and CAL gain compared with scaling and root planing alone

Table 4. (Contd.)

References	Study design	NSAID	Observation period (months)	Outcome
Preshaw et al. (1998)	Untreated CP patients. Placebo control group	Ketorolac rinse 0.1% bid	15 days	No significant reduction of GCF-PGE_2 concentrations from baseline levels compared with control group
Bichara et al. (1999)	Bone healing in intra-bony defects after GTR therapy with barrier membrane and adjunctive NSAID. Control group	Systemic naproxen 500 mg bid for 7 days	9	No significant superior osseous healing with adjunctive naproxen administration compared with placement of barrier membrane alone
Paquette et al. (2000)	Untreated CP patients	Systemic and topical ketoprofen	22 days	Topical as well as systemic ketoprofen administrations reduced GCF-PGE_2 but not GCF-LTB_4 levels
Buduneli et al. (2002)	Non-surgical periodontal therapy in CP patients with adjunctive NSAID. Placebo control group	Systemic meloxicam 7.5 mg for 10 days	10 days	GCF-MMP-8 levels were significantly lower in the meloxicam compared with the placebo group
Vardar et al. (2003)	Non-surgical periodontal therapy in 30 CP patients with adjunctive NSAID. Placebo control group	Systemic nimesulide 100 mg bid or naproxen 275 mg bid	3	Adjunctive naproxen administration significantly reduced gingival tissue levels of both PGE_2 and $PGF_{2\alpha}$ compared with controls, whereas adjunctive nimesulide administration significantly reduced gingival tissue levels of $PGF_{2\alpha}$ but not PGE_2 compared with controls
Sculean et al. (2003)	Effect of postsurgical administration of NSAID on healing of intra-bony defects following treatment with EMD. Control group	Systemic rofecoxib 12.5 mg bid for 14 days	6	Systemic administration of rofecoxib following regenerative surgery with EMD did not result in additional clinical improvements when compared with treatment with EMD alone

NSAID, non-steroidal anti-inflammatory drugs; GCF, gingival crevicular fluid; CAL, clinical attachment level; ASA, acetylsalicylic acid; GTR, guided tissue regeneration; CP, chronic periodontitis; RPP, rapidly progressing periodontitis; EMD, enamel matrix derivative.

and periodontal disease (Yoon et al. 2003). Periodontal tissue cells including fibroblasts, keratinocytes, neutrophils, macrophages and endothelial cells constitute the primary source of MMPs. Under healthy periodontal conditions, collagen homeostasis is a tightly regulated process controlled extracellularly by fibroblast-derived collagenase (e.g. collagenase-1 or MMP-1). Inflammatory mediators such as IL-1, TNF-α and PGE_2 (Nakaya et al. 1997, Domeij et al. 2002, Ruvanpura et al. 2004) as well as bacterial products (DeCarlo et al. 1998, Choi et al. 2001, 2003) have been shown to upregulate MMP production in several in vitro models. For example, IL-1β-induced MMP-3 production was downregulated by PGE_2 in human fibroblasts from healthy gingiva and upregulated by PGE_2 in fibroblasts from periodontally diseased tissue (Ruvanpura et al. 2004). Moreover, production of MMP-1 and MMP-3 by gingival fibroblasts was downregulated by interferon-γ (IFN-γ) (Wassenaar et al. 1999). Experimental studies indicate that MMPs activation plays an important role in extracellular matrix degradation during periodontal tissue destruction (Achong et al. 2003, Cesar Neto et al. 2004). Regulation of MMP functions involves activation of endogenous tissue inhibitors of MMPs (TIMPs) and α-macroglobulins. The wide distribution of TIMPs in body tissues is related to their production by various cells including fibroblasts, keratinocytes, endothelial cells and macrophages (Birkedal-Hansen et al. 1993, Ryan & Golub 2000). Cytokines such as IL-4 and IL-11 have been shown to suppress expression of MMP-3 in HGF (Jenkins et al. 2004) and to induce TIMP-1 expression in synoviocytes and chrondrocytes (Hermann et al. 1998), respectively. In inflamed periodontal tissues, the imbalance between MMPs and TIMPs leads to excessive breakdown of extracellular matrix components, resulting in loss of clinical attachment and alveolar bone. Under different periodontal conditions (e.g. health, gingivitis, chronic or aggressive periodontitis) and presence of environmental factors (e.g. cigarette smoking), gingival tissue, GCF and saliva have been shown to express specific combinations of MMPs (Haerian et al. 1995, Ingman et al. 1996, Golub et al. 1997, 2001, Uitto et al. 1998, Chen et al. 2000, Tervahartiala et al. 2000, Alpagot et al. 2001, Soder et al. 2002, Smith et al.

Table 5. Matrix metalloproteinases (MMPs) produced by periodontal cells

MMP	Cell source	Function	References
MMP-1	Fibroblast	Collagenase	Ingman et al. (1996), Domeij et al. (2002), Ejeil et al. (2003)
MMP-2	Fibroblast	Gelatinase	Dahan et al. (2001)
MMP-3	Fibroblast	Stromelysin	Haerian et al. (1995), Ingman et al. (1996), Alpagot et al. (2001), Domeij et al. (2002), Jenkins et al. (2004), Ruvanpura et al. (2004)
MMP-7	Fibroblast, macrophage, epithelial cells	Matrilysin	Tervahartiala et al. (2000)
MMP-8	PMN	Collagenase	Golub et al. (1995, 1997), Ingman et al. (1996)
MMP-9	PMN	Gelatinase	Golub et al. (1995), Ingman et al. (1996)
MMP-13	Epithelial and bone cells	Collagenase	Golub et al. (1997), Uitto et al. (1998), Tervahartiala et al. (2000), Ejeil et al. (2003)

PMN, polymorphonuclear granulocytes.

Table 6. Animal studies on the effects of MMP inhibitors on MMP production and alveolar bone loss

References	Study design	Observation period	Outcome
Llavaneras et al. (2001)	Intra-gingival LPS-induced experimental periodontitis in rats. Oral administration of a CMT-8 and/or clodronate	7 days	CMT-8 administration alone showed slight reduction in MMP-8, MMP-9 and elastase activities and bone loss. Only combined administration of CMT-8 and clodronate significantly reduced MMPs activities and bone loss
Bezerra et al. (2002)	Ligature-induced experimental periodontitis in rats with subcutaneous injection of SDD	7 days	Significant dose-dependent reduction in alveolar bone loss and osteoclast numbers in animals treated with SDD compared with controls
Ramamurthy et al. (2002a)	Intra-gingival LPS-induced experimental periodontitis in rats. Comparison of oral administration of doxycycline and five different CMTs	6 days	Administration of CMTs decreased synthesis of MMP-2 and MMP-9, the number of immuno-positive stained cells for IL-1, IL-6 and TNF and alveolar bone resorption
Ramamurthy et al. (2002b)	Intra-gingival LPS-induced experimental periodontitis in rats. Oral administration of two low-molecular-weight MMP inhibitors	8 days	Both compounds reduced active and/or total activity of MMP-1, -2, -3, -7, -8, -9, -13, -14: Dose-dependent inhibition of bone loss with both drugs
Escartin et al. (2003)	High carbohydrate diet-induced experimental periodontitis in hamsters. Intra-muscular injection of RGTA (heparan sulphate analogue) at four dosages. Untreated control animals	28 days	Dose-dependent RGTA administration induced: reduction of gingival inflammation, increased thickness of pocket epithelium, increased collagen accumulation in gingiva, reduction in bone loss, stimulation of bone formation, decrease in MMP-2 and MMP-9 pro-forms in gingival tissue
Buduneli et al. (2004)	Intra-gingival LPS-induced experimental periodontitis in rats Systemic administration of LDD alone or in combination with alendronate	7 days	Significant reduction of gingival tissue levels of PGE_2, LTB_4 and PAF by LDD administration alone. Significantly more alveolar bone loss in the LPS+LDD group compared with the saline control group
Björnsson et al. (2004)	Ligature-induced experimental periodontitis in the rat model Intra-peritoneal injection of batimastat (hydroxamic acid-based MMP inhibitor)	31 days	Batimastat did not reduce the progression of experimental periodontitis in test animals. Significantly increased bone loss in animals treated with batimastat was observed compared with placebo and control animals

MMP, matrix metalloproteinase; LPS, lipopolysaccharide; CMT, chemically modified doxycycline; SDD, subantimicrobial dose doxycycline; IL, interleukin; RGTA, regenerating agents; PAF, platelet-activating factor; LDD, low-dose doxycycline.

2004). For example, it has been documented that the predominant MMPs in diseased human gingiva are of neutrophil (i.e. MMP-8 and MMP-9) and of bone (i.e. MMP-13) origin rather than fibroblast derived (i.e. MMP-1) (Golub et al. 1995, 1997, Ejeil et al. 2003) (Table 5). Furthermore, levels of neutrophil-derived MMPs have been shown to correlate with disease severity and decrease following non-surgical and surgical periodontal therapy (Larivee et al. 1986, Haerian et al. 1996, Pourtaghi et al. 1996, Golub et al. 2001, Kinane et al. 2003, Mantyla et al. 2003, Persson et al. 2003, Figueredo et al. 2004).

In vitro and animal evidence for pharmacological modulation of MMPs

The ability of tetracyclines and doxycycline, in particular, to inhibit MMP activity was first identified in the early 1980s (Golub et al. 1983, 1985, Ramamurthy & Golub 1983). The direct and indirect non-antimicrobial mechanisms by which tetracyclines inhibit MMPs activities, thus preventing connective tissue breakdown and bone resorption, have been described in detail elsewhere (Ryan & Golub 2000).

Recent studies using the experimentally induced periodontitis model in rats and hamsters have investigated the effects of MMP inhibition with subantimicrobial doses of tetracyclines (Llavaneras et al. 2001, Bezerra et al. 2002, Ramamurthy et al. 2002a) and other MMP inhibitors (Ramamurthy et al. 2002b, Escartin et al. 2003, Björnsson et al. 2004, Buduneli et al. 2004). A summary of the findings of these experiments is provided in Table 6.

Clinical studies on pharmacological modulation of MMPs

A subantimicrobial dose (20 mg twice daily) of doxycycline (SDD) with the purpose of long-term administration in patients suffering from periodontitis was introduced and shown to downregulate collagenase activity without the emergence of doxycycline-resistant microorganisms or typical adverse events (Golub et al. 1990, 1994, Thomas et al. 2000). The findings of the first clinical study prescribing SDD as an adjunct to mechanical debridement (i.e. scaling and root planing, SRP) showed statistically significant reductions in GCF concentrations of MMP-8, MMP-13 and ICTP (i.e. a fragment of type-1 collagen) compared with placebo (Golub et al. 1997). The clinical, biochemical and microbiological effects of SDD on the modulation of wound healing have recently been reported in a pilot study comparing access flap surgery with SDD (20 mg bid for 6 months) or placebo in patients with advanced chronic periodontitis (Gapski et al. 2004). The findings showed that postsurgical wound healing was significantly enhanced compared with placebo with respect to probing pocket depth reduction at sites of $\geqslant 7$ mm and that adjunctive SDD administration did not induce significant shifts on the periodontal microbiota beyond those attributed to surgery alone.

Table 7. Clinical data from RCTs of SDD

References	Diagnosis	Study length (months)	*N*	Study groups	% of sites with CAL gain		% of sites with PPD reduction	
					$\geqslant 2$ mm	$\geqslant 3$ mm	$\geqslant 2$ mm	$\geqslant 3$ mm
Caton et al. (2000)	Chronic periodontitis	9	90	SRP+SDD (20 mg bid)	46	22	47*	22*
			93	SRP+placebo	38	16	35	13
Novak et al. (2002)	Severe generalized periodontitis	9	10	SRP+SDD (20 mg bid)	29	15	48	26**
			10	SRP+placebo	21	11	21	6
Preshaw et al. (2004)	Chronic periodontitis	9	107	SRP+SDD (20 mg bid)	42**	15*	43**	15**
			102	SRP+placebo	32	10	31	9

*$p < 0.05$ compared with placebo.
**$p < 0.01$ compared with placebo.
RCT, randomized clinical trial; SDD, subantimicrobial dose doxycycline; SRP, scaling and root planing; CAL, clinical attachment level; PPD, probing pocket depth.

Table 8. Clinical and biochemical (i.e. GCF) data from RCTs of SDD

References	Diagnosis	Study length	*N*	Study groups	Outcome
Choi et al. (2004)	Chronic periodontitis	4 months	15	SRP+SDD (20 mg bid)	Significant reduction in PPD and gain of CAL in the SDD-treated group compared with the placebo-treated group
			17	SRP+placebo	Significant decrease in GCF-MMP-8 level in SDD-treated patients compared with placebo-treated patients
Emingil et al. (2004a, b)	Chronic periodontitis	12 months	10	SRP+SDD (20 mg bid)	Significant reduction in PPD and Gingival Index scores in the SDD-treated group compared with the placebo-treated group
			10	SRP+placebo	Significant decrease in MMP-8 and laminin-5 $\gamma 2$ chain levels in GCF of SDD-treated patients compared with placebo-treated patients
Lee et al. (2004)	Chronic periodontitis	9 months	24	SRP+SDD (20 mg bid)	Significant reduction in PPD and gain of CAL in the SDD-treated group compared with the placebo-treated group
			17	SRP+placebo	Significant decrease in MMP-8 and MMP-13 levels in GCF of SDD-treated patients compared with placebo-treated patients

GCF, gingival crevicular fluid; RCT, randomized clinical trial; SDD, subantimicrobial dose doxycycline; SRP, scaling and root planing; CAL, clinical attachment level; PPD, probing pocket depth; MMP, matrix metalloproteinase.

Table 9. Animal experiments on the effects of bisphosphonate administration on inflammatory mediator levels and alveolar bone loss

References	Study design	Animal model	Drug	Observation period	Outcome
Brunsvold et al. (1992)	Effects of bisphosphonate administration on clinical parameters and alveolar bone loss during ligature-induced experimental peridontitis	Monkey	Systemic alendronate	16 weeks	Significant reduction in bone density changes in the alendronate group compared with the placebo group. Clinical parameters (PLI, GI, PPD) were not significantly affected by alendronate administration compared with placebo
Weinreb et al. (1994)	Effects of bisphosphonate administration on alveolar bone loss during ligature-induced experimental peridontitis	Monkey	Systemic alendronate	16 weeks	Alendronate significantly reduced bone loss associated with experimental periodontitis compared with control animals
Shoji et al. (1995)	Effects of bisphosphonate administration on alveolar bone resorption during ligature-induced experimental periodontitis	Rat	Systemic risedronate	8 days	Systemic risedronate significantly reduced alveolar bone resorption and loss of bone mineral content compared with control animals
Ouchi et al. (1998)	Effects of bisphosphonate administration on the progression of alveolar bone loss during ligature-induced experimental periodontitis	Beagle dog	Systemic incadronate	25 weeks	Incadronate administration prevented alveolar bone loss by reducing the increased alveolar bone turnover in dogs with experimental periodontitis
Alencar et al. (2002)	Effects of bisphosphonate administration on bone resorption during ligature-induced experimental periodontitis	Rat	Systemic clodronate	11 days	Clodronate administration significantly reduced alveolar bone loss and inflammatory cell infiltrations compared with control animals
Mitsuta et al. (2002)	Effects of bisphosphonate administration on alveolar bone resorption during ligature-induced experimental periodontitis	Rat	Topical clodronate	7 days	Topical clodronate significantly reduced bone mineral density changes and the number of osteoclasts per alveolar bone surface compared with control animals
Tani-Ishii et al. (2003)	Effects of bisphosphonate administration on the progression of *Porphyromonas gingivalis*-induced experimental periodontitis	Rat	Systemic incadronate	8 weeks	Systemic incadronate significantly inhibited aleveolar bone resorption and PMN migration compared with control animals
Buduneli et al. (2004)	Effects of bisphosphonate administration on PGE_2, PGF2α, LTB_4 and PAF levels and alveolar bone loss in endotoxin-induced periodontitis	Rat	Systemic alendronate	7 days	Significant reduction in the gingival tissue levels of PGE_2 and LTB_4compared with control animals No significant reduction in alveolar bone loss compared with control animals

PAF, platelet-activating factor; PLI, plaque index, GI, gingival index, PPD, probing pocket depth; PMN, polymorphonuclear granulocytes.

Thus far, one approved host modulation therapy (HMT) prescribed as systemic SDD (Periostat®, Colla-Genex Pharmaceuticals Inc., Newtown, PA, USA) in conjunction with mechanical periodontal therapy is available in some countries. A summary of clinical and biochemical (i.e. GCF) data reported in randomized clinical trials of non-surgical periodontal therapy with adjunctive SDD administration is provided in Tables 7 and 8.

Modulation of Bone Remodelling

The discovery of a novel receptor called osteoprotegerin (OPG) revealed a key regulatory mechanism in osteoclast differentiation and activity (Simonet et al. 1997). Briefly, OPG and receptor activator of NF-κ B ligand (RANKL) are two molecules that regulate osteoclast formation and bone resorption. RANKL induces osteoclast differentiation and activation, whereas OPG blocks this process by acting as a decoy receptor for RANKL. Factors regulating osteoblast and osteoclast activity have become important targets for developing pharmacological and clinical strategies to modulate the rate of bone formation and resorption. The identification of the interaction between RANKL and OPG has recently received attention in periodontal research (Teng et al. 2000, Liu et al. 2003, Valverde et al. 2004, Belibasakis et al. 2005). For the purpose of this review, however, the focus will be on modulation of bone remodelling with the use of bisphosphonates. Bisphosphonates represent a class of chemical compounds structurally related to pyrophosphate, a natural product of human metabolism present in the serum and urine with calcium-chelating properties (Rodan 1998, Rogers et al. 2000). Pyrophosphate regulates mineralization by binding to hydroxyapatite crystals in vitro but it is not stable in vivo, undergoing rapid hydrolysis of its labile P–O–P bond as a result of phyrophosphatase activity (Shinozaki & Pritzker 1996). The replacement of the linking oxygen atom with a carbon atom (e.g. P–C–P) results in the formation of a bisphosphonate molecule. This compound is chemically stable and completely resistant to enzymatic hydrolysis via pyrophosphatase and alkaline phosphatase. Given their affinity to bind to hydroxyapatite crystals and prevent their growth and dissolution and to their ability to increase osteoblast differentiation and

Table 10. Clinical studies on the effects of bisphosphonate administration

References	Study design	Subjects	Drug	Periodontal treatment	Observation period	Outcome
Jeffcoat & Reddy (1996)	Parallel arm, randomized, placebo-controlled trial	40 with chronic periodontitis	Systemic alendronate 10 mg/day for 6 months	SRP	9 months	Placebo group: 40% of sites lost bone height Test group: 20% of sites lost bone height ($p = 0.04$)
Rocha et al. (2001)	Parallel arm, randomized, placebo-controlled trial	40 with chronic periodontitis and Type 2 diabetes	Systemic alendronate 10 mg/day for 6 months	SRP	6 months	Test group: 1.3 ± 1.3 mm difference in bone height ($p = 0.003$) and 0.52 ± 0.85 mm CAL gain ($p = 0.013$) compared with placebo
El-Shinnawi & El-Tantawy (2003)	Parallel arm, randomized, placebo-controlled trial	24 with chronic periodontitis	Systemic alendronate 10 mg/day for 6 months	SRP	6 months	Significant difference ($p < 0.001$) in bone mineral density of maxilla and mandible as assessed by DEXA. No significant difference in GI, PPD and CAL
Takaishi et al. (2003)	Case report	4 women with chronic periodontitis	Systemic etidronate 200 mg/day for 2 weeks at intervals of 6 months	Ordinary dental treatment	4–5 years follow-up	Improvements in clinical parameters (PPD and tooth mobility) and in alveolar bone density

SRP, scaling and root planing; CAL, clinical attachment level; PPD, probing pocket depth; DEXA, dual-energy absorptiometer; GI, gingival index.

inhibit osteoclast recruitment and activity, bisphosphonates are widely used in the management of systemic metabolic bone disorders such as tumour-induced hypercalcaemia, osteoporosis and Paget's disease (Fleisch 1997).

In the management of periodontal disease-associated bone loss, administration of bisphosphonates may have potential applications. Findings from in vitro experiments demonstrated that bisphosphonates downregulated activity levels of several MMPs (Teronen et al. 1999) including MMP-3, MMP-8 and MMP-13 from human PDL cells (Nakaya et al. 2000).

Several animal studies have examined the effects of local or systemic bisphosphonate delivery on alveolar bone resorption by using the experimental periodontitis model (Brunsvold et al. 1992, Weinreb et al. 1994, Shoji et al. 1995, Ouchi et al. 1998, Alencar et al. 2002, Mitsuta et al. 2002, Tani-Ishii et al. 2003, Buduneli et al. 2004), the naturally occurring periodontitis model (Reddy et al. 1995) or after mucoperiosteal flap elevation (Yaffe et al. 1995, 1997, 2000, 2003, Binderman et al. 2000, Kaynak et al. 2000, 2003).

The findings of the two animal experiments (Brunsvold et al. 1992, Reddy et al. 1995) showed that, although bone loss was reduced by systemic delivery of alendronate, clinical signs of inflammation and pocket depth were not affected. This may be attributed to the fact that high-dose release of alendronate from hydroxyapatite in inflamed periodontal pockets upregulated the inflammatory host response by stimulating the secretion of cytokines such as IL-1 and IL-6 (Adami et al. 1987, Schweitzer et al. 1995). Collectively, controversial data from animal experiments on the use of topical or systemic bisphosphonates to prevent periodontal disease progression and alveolar bone loss have been reported (Table 9). Moreover, conflicting data on the effects of systemic bisphosphonate administration have been reported in human studies (Jeffcoat & Reddy 1996, Rocha et al. 2001, El-Shinnawi & El-Tantawy 2003, Takaishi et al. 2003) (Table 10).

Modulation of Host Cell Receptors

Cytokines are defined as regulatory proteins controlling the survival, growth, differentiation and functions of cells. Cytokines are produced transiently at

Table 11. Animal experiments on the effects of cytokine modulation

References	Study design	Animal model	Cytokine	Observation period	Outcome
Assuma et al. (1998)	Effects of soluble receptors to IL-1 and TNF during ligature-induced experimental periodontitis	Monkey	IL-1 TNF	6 weeks	Compared with control sites, local injection of soluble receptors to IL-1 and TNF inhibited the recruitment of inflammatory cells in close proximity to bone by 80%, the formation of osteoclasts by 67% and the amount of bone loss by 60%. All results statistically significant at $p < 0.01$
Graves et al. (1998)	Effects of soluble receptors to IL-1 and TNF during ligature-induced experimental periodontitis	Monkey	IL-1 TNF	6 weeks	Compared with control sites, local injection of soluble receptors to IL-1 and TNF inhibited osteoclast formation and progression of inflammatory cell infiltration towards alveolar bone
Martuscelli et al. (2000)	Effects of subcutaneous injection of rhIL-11 during ligature-induced experimental periodontitis	Beagle dog	IL-11	8 weeks	Statistically significant differences in clinical attachment level and radiographic parameters were found between treatment and placebo groups
Delima et al. (2001)	Effects of soluble receptors to IL-1 and TNF during ligature-induced experimental periodontitis	Monkey	IL-1 TNF	6 weeks	Compared with control sites, local injection of soluble receptors to IL-1 and TNF inhibited by 51% the loss of connective tissue attachment and by 91% the loss of alveolar bone height
Delima et al. (2002)	Effects of soluble receptors to IL-1 during ligature-induced experimental periodontitis	Monkey	IL-1	6 weeks	Compared with control sites, local injection of soluble receptors to IL-1 significantly reduced inflammation, connective tissue attachment loss and bone resorption
Oates et al. (2002)	Effects of soluble receptors to IL-1 and TNF during ligature-induced experimental periodontitis	Monkey	IL-1 TNF	6 weeks	Radiographic bone loss was reduced by 50% in the experimental group compared with the placebo group. No significant differences in GI, GCF amounts and GCF-bone alkaline phosphatase levels were observed comparing the experimental with the placebo groups

IL, interleukin; TNF, tumour necrosis factor; rhIL-11; recombinant human IL-11; GI, gingival index; GCF, gingival crevicular fluid.

generally low concentrations, act and are degraded in a local environment. This is documented by the fact that cytokine-producing cells are often physically located immediately adjacent to the responding cells. Moreover, the responding cell destroys the cytokine that it responds to in the process of receptor-mediated endocytosis. Several cytokines bind to elements of the extracellular matrix, thus restricting their spread beyond the site of action and increasing their bioavailability to the responding cells. Cytokines function as a network, are produced by different cell types and share overlapping features. This phenomenon is called biological redundancy. While very few biological responses are mediated by only one cytokine, many responses can be achieved by several different cytokines. Thus, important cellular functions are usually backed up in mechanisms where one cytokine can compensate for the loss of another. Consequently, blocking one inflammatory mediator or cytokine will not assure that a receptor-mediated response will not be activated by alternate pathways. This would require the development of polypharmaceutical approaches controlling all pathways associated with inflammation and tissue destruction.

Based upon the increased expression of IL-1 and TNF in inflamed gingiva and high levels in the GCF of periodontitis patients, several studies have suggested that increased production of these cytokines may play an important role in periodontal tissue destruction (for a review, see Graves & Cochran 2003). To counteract tissue destruction and maintain homeostasis, cytokine antagonists such as IL-1 receptor antagonist (IL-1Ra) or soluble TNF receptors can competitively inhibit receptor-mediated signal transduction (Dinarello 2004, Levine 2004). In vivo application of soluble receptors of IL-1 or TNF-α has been demonstrated to inhibit a number of pathologic processes including arthritis and septic shock. For example, IL-1ra is currently approved for treating rheumatoid arthritis.

In periodontal research, the effects of soluble receptors and receptor antagonists of IL-1 and TNF-α have been studied during experimentally induced periodontitis in a non-human primate model (e.g. *Macaca fascicularis*) (Assuma et al. 1998, Graves et al. 1998, Delima et al. 2001, 2002, Oates et al. 2002). Collectively, the clinical, radio-

graphic and biochemical findings of these experiments showed that IL-1 and TNF-α antagonists blocked (i) the progression of the inflammatory cell infiltrate towards the alveolar crest, (ii) the recruitment of osteoclasts and (iii) periodontal attachment and bone loss. Compared with control animals, intrapapillary injection of soluble receptor antagonists of IL-1 and TNF-α reduced the pattern of bone loss by approximately 50% as assessed by computer-assisted densitometric image analysis (CADIA).

To prevent an uncontrolled inflammatory response with rapid tissue destruction, the activities of IL-1 and TNF-α are naturally counteracted by the production of cytokines such as IL-4, IL-10 and IL-11 (Essner et al. 1989, De Waal Malefyt et al. 1991). More specifically, IL-11 has been shown to inhibit the production of IL-1β, TNF-α, IL-12 and nitric oxide (NO) in a variety of inflammatory conditions (Trepicchio et al. 1996, 1999, Leng & Elias 1997). The potential to downregulate mediators of inflammation associated with periodontal tissue destruction was investigated during experimental periodontitis in beagle dogs over an 8-week period (Martuscelli et al. 2000). The findings of this experiment indicated that subcutaneous injection of recombinant human IL-11 (rhIL-11) was able to alter periodontal disease progression measured by changes in attachment level and radiographic bone height. A summary of the findings of animal experiments is provided in Table 11.

In diabetics, the chronically elevated glucose levels result in an accelerated formation of advanced glycation end-products (AGEs). AGEs represent a heterogeneous class of non-enzymatically glycated proteins and lipids found in plasma, vessel walls and tissues. Endothelial cells and monocytes possess specific receptors for AGEs called RAGEs located on their cell surfaces (Hudson & Schmidt 2004). Studies have shown that the interaction of AGEs with their receptors (RAGEs) plays an important role in the development of diabetic complications (for a review, see Hudson et al. 2003). The interaction of macrophages with AGEs has been shown to stimulate increased secretions of cytokines such as TNF-α and IL-1 (Vlassara et al. 1988). In diabetic mice, blockade of RAGEs with soluble receptors (sRAGEs) suppressed periodontitis-associated bone loss and reduced the levels of IL-6, TNF-α and MMPs (Lalla et al. 2000).

In conclusion, blockade of cytokine receptors (IL-1ra, TNF-αR1, TNF-αR2) soluble cytokines (rhIL-11) and soluble receptor for advanced glycation end-products (sRAGEs) reduce periodontal attachment and bone loss in animal experimental periodontitis.

Modulation of NOS Activity

NO is a short-lived molecule implicated in a wide range of biological processes ranging from immune homeostasis to cancer (Brennan et al. 2003). It is synthesiszed in vivo from the substrate L-arginine by three isoenzymes called NOSs. While low levels of NO are present in tissue homeostasis, NO is produced at higher concentrations in response to inflammatory stimuli such as bacterial LPS via inducible forms of NOS (iNOS) (Southan & Szabo 1996). NO is a highly reactive free radical reacting with metal and thiol residues leading to lipid peroxidation, protein and DNA damages and stimulation of cytokine release (Brennan et al. 2003). An exaggerated production of NO has been implicated in the pathophysiology of several inflammatory processes such as arthritis, colitis and ileitis (Boughton-Smith et al. 1993, Middleton et al. 1993, Miller et al. 1995, Brahn et al. 1998). Animal experiments have shown that pharmacological inhibition of iNOS with mercaptoalkylguanidines was associated with decreased inflammation, haemorrhagic shock and arthritis scores (Zingarelli et al. 1997, Brahn et al. 1998, Cuzzocrea et al. 1998). This may be explained by the fact that this class of drugs (e.g. mercaptoethylguanidines (MEGs)) is able to (i) inhibit COX (Zingarelli et al. 1997), (ii) scavenge peroxinitrite (i.e. the product of NO and superoxide) (Szabo et al. 1997) and (iii) block iNOS (Szabo et al. 1996).

Since NO activity has not been detected in the gingival tissues of sterile animals (Lohinai et al. 2001), oral bacteria have been postulated to trigger iNOS upregulation in periodontal tissues (Lohinai et al. 1998, 2001). On the other hand, mice lacking iNOS demonstrated impaired killing of *P. gingivalis* inoculated into a subcutaneous chamber (Gyurko et al. 2003). Findings from in vitro experiments showed that HGF expressed elevated levels of iNOS and were able to modulate NO synthesis in response to cytokines such as TNF-α, IL-1β and IFN-γ (Daghigh et al. 2002).

The ligature-induced periodontitis model in rats was used in a proof-of-principle experiment to investigate the role of iNOS and the effects of its inhibition with MEG (Lohinai et al. 1998). Animals treated with intra-peritoneal injection of MEG exhibited significantly less plasma extravasation and bone loss at ligated sites compared with vehicle-treated controls. These preliminary results demonstrated that ligature-induced periodontitis increased NO production and MEG administration protected against bone loss, suggesting that NO and peroxynitrite played an important role in the pathogenesis of experimental periodontitis.

Recently, the role of activation and pharmacological inhbition of nuclear poly (ADP-ribose) polymerase (PARP) enzyme, a mediator of downstream NO toxicity, was investigated using the ligature-induced periodontitis model in rats and mice (Lohinai et al. 2003). After ligature placement around the neck of the first left mandibular molar, the rats were administered a potent PARP inhibitor (e.g. PJ34) or vehicle by intraperitoneal injection. Non-ligated right first mandibular molars served as controls. Immunohistochemical analysis revealed significantly increased PARP staining in the subepithelial connective tissue of ligated sites compared with non-ligated sites. Ligature-induced periodontitis resulted in marked plasma extravasation in the gingival tissue and alveolar bone loss compared with non-ligated sites. Pharmacological inhibition of PARP in rats as well as PARP-1 gene disruption in mice significantly reduced extravasation and alveolar bone resorption of ligated compared with non-ligates sites. These findings, however, contradicted previous in vitro reports on human and animal (e.g. monkey) cultured fibroblasts derived from periodontally diseased sites displaying reduced PARP synthase activity compared with that of periodontally healthy controls (Hussain et al. 1994, Ghani et al. 1996).

In conclusion, in animal experimental periodontitis, the use of pharmacological inhibitors of NO and PARP synthases reduces periodontal attachment and bone loss.

Conclusions

The recognized importance of the host inflammatory response in the pathogen-

esis of periodontal diseases presents the opportunity to explore new treatment strategies. The adjunctive use of HMT with mechanical periodontal therapy has been reported involving non-surgical and surgical approaches. Clinical trials have shown that inhibition of AA metabolites with NSAIDs reduces gingival inflammation and periodontal disease progression. However, recently reported serious adverse effects of some COX-2 inhibitors preclude their use as an adjunct to mechanical periodontal therapy. Evidence shows that non-surgical periodontal therapy with adjunctive 20 mg SDD twice daily for 9 months is beneficial in the management of chronic periodontitis over 12 months. Further research is needed to evaluate the added benefits of adjunctive SDD administration in high-risk periodontitis patients. Controversial findings on the use of systemic bisphosphonates to prevent periodontal disease progression and alveolar bone loss have been reported in human studies.

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