Redox Status in Periodontal and Systemic Inflammatory Conditions Including Associated Neoplasias: Antioxidants as Adjunctive Therapy?

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Abstract: The aetiopathogenesis of periodontal diseases is initiated by microbial biofilm which could lead to a hyperinflammatory status in aggressive forms of the disease; and cause an imbalance in the redox status, resulting in oxidative stress-induced damage. There is increasing documentation of the link between periodontal and other inflammatory diseases driven by a pro-oxidant profile. This could impose a significant systemic loading of reactive oxygen species relevant to metabolic, arthritic, age related and neoplastic diseases. They demonstrate similar markers of risk / oxidative stress induced injury during disease progression which abate in response to treatment. In certain aggressive forms of periodontal diseases resulting in a substantial systemic pro-oxidant profile, the co-existence of systemic diseases with a similar inflammatory pathogenesis could lead to progressive tissue / organ damage fuelled by the same process. Some of the common mechanisms involved are discussed, relevant to periodontal, metabolic and rheumatoid diseases, pregnancy and the foetus, age related changes and certain neoplasias which have been recently linked to periodontal disease progression. In view of a distinct pro-oxidant profile in severe cases there may a role for selective use of antioxidant adjuncts with suitable therapeutic targeting. It is relevant that periodontal diseases are associated with the above diseases and a small but significant overall cancer risk which persists in non-smokers. Periodontal disease may be a useful marker of a susceptible immune system, or directly affect the progression of systemic diseases due to inflammatory loading. Formulation of therapeutic agents shown to have efficacy in this context, with accurate targeting is a challenge.

Keywords: Periodontitis, systemic diseases, oxidative stress, lipid peroxidation, risk markers, antioxidants, therapeutic targets.

INTRODUCTION

It is relevant that although the primary agent in the aetiology of periodontal disease is bacterial plaque, it can contribute to a hyper-responsive inflammatory state. Associated pathways leading to the formation of reactive oxygen species play an important role in the initiation and progression of periodontal destruction. There is unexpected diversity amongst species of periodontal pathogens; certain evolutionary types are distinctly linked to disease progression. Genetically distinct types of *Porphyromonas gingivalis* (*Pg*) have greater association with the aetiopathogenesis of periodontal disease than others; but needs further clarification [1]. *Pg* contributes to destruction of connective tissue and supporting bone around teeth; during tissue invasion *Pg* initiates the release of cytokines [2] such as interleukin-8 (IL-8) and tumour necrosis factor (TNF)-α, leading to increased numbers and activity of polymorphonuclear leucocytes (PMNs). In response to bacterial antigenic stimulation, PMNs produce reactive oxygen species. Heightened PMN activity and high levels of superoxide production during phagocytosis of periodontal pathogens leads to oxidative damage to gingivae, periodontal ligament and alveolar bone [3]. Antioxidants could play a role in minimizing oxidative damage caused by an excessive inflammatory response akin to an autoimmune response to plaque antigen, seen in certain categories of aggressive forms of periodontal diseases.

Lipopolysaccharides (LPS) which are glucosamine-based phospholipids form the outer layer of surface membranes of gram-negative bacteria, implicated in the aetiopathogenesis of periodontal diseases. Pro-atherogenic properties of LPS derived from serotypes b and d of a significant periodontal pathogen *Aggregatibacter actinomycetemcomitans (Aa)* have been investigated in macrophages [4]. These LPS preparations induced the release of TNF-α and IL-1β; foam cell formation and accumulation of cholesterol ester from low density lipoprotein (LDL) were demonstrated. The pro-atherogenic potential of *Aa* may be dependent on the strain present in periodontal lesions and correlates with the pathogenic potential of the lesion. LPS-induced release of inflammatory mediators, lipoprotein profile changes and an imbalance in cholesterol homeostasis could contribute to an association between periodontal and cardiovascular diseases. Tyrosine phosphorylation in response to LPS in monocytes and macrophages is an early step in signal transduction [5]. LPS purified from *Aa*, has also been shown to increase protein tyrosine phosphorylation in human gingival fibroblasts. This increased phosphorylation was dose-dependent at 10ng- 10µg/ml. Pre-treatment with the tyrosine kinase inhibitors herbimycin A and genistein inhibited phosphorylation of MAP kinases in a dose-dependent manner. Pre-treatment of HGF with antibodies to CD-14 or toll-like receptor-4 (TLR-4), but not TLR-2, prevented LPS-induced tyrosine phosphorylation, confirming cluster of differentiation-14 (CD-14) and TLR-4 receptor involvement in *Aa*-LPS mediated effects on fibroblasts.

An association between the periodontal pathogen *Porphyromonas gingivalis* (*Pg*) and atherogenesis has also been documented. The potential role of *Pg*- LPS on

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monocyte-endothelial interactions has been investigated recently [6]. Human umbilical vein endothelial cells (HUVECs) were incubated with *Pg* or *Escherichia coli* (*E coli*) LPS for various periods and mononuclear cell adhesion assays were done. The adhesion of mononuclear cells to HUVECs peaked at 24h of incubation in response to *Pg*-LPS fraction and at 4h with *E coli*- LPS fraction. There was significant up-regulation of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in HUVECs in response to the *Pg*-LPS fraction. This was attenuated by antibodies against ICAM-1 and TLR-2, but not TLR-4. These results indicate that chronic infection with *Pg,* as in periodontal diseases could contribute to monocyte recruitment to vascular endothelium and atherogenesis via the TLR-2 pathway of *Pg*-LPS signal transduction.

Interaction between LPS and TLR-4 results in its activation. LPS has been shown to activate macrophages via TLR-4 [7]. Adipose differentiation related protein (ADRP) is highly expressed in human atherosclerotic lesions and macrophages. TLR4-mediated signals which are involved in atherosclerotic changes, have been shown to enhance the expression of ADRP. Bacterial LPS has been shown to increase ADRP in peritoneal macrophages of wild type mice but not in those from toll-like receptor 4 deficient mice [8]. Enhanced expression of ADRP by LPS could occur at transcriptional level. LPS also induces the expression of IL-6, IL-1α and IFN-β mRNAs prior to increases in ADRP, which in turn stimulate expression of ADRP. Similarly exposure of aortic smooth muscle cells to LPS which is agonistic to TLR-4 resulted in induction of IL-8 gene transcription via promoter activation as well as enhanced release of IL-8 protein [9]. This was inhibited by the dietary antioxidant curcumin, which has implications in reducing inflammatory loading generated by cardiovascular and periodontal diseases.

ADRP mRNA levels are suppressed by antibodies against these cytokines or inhibitors of Jun N-terminal kinase (JNK) and nuclear factor- kappa B (NF-kappa B). It is relevant that the antioxidant pycnogenol an extract of French maritime pine bark suppressed the expression of the cytokines mentioned and ADRP. Pycnogenol may have useful applications in preventing atherosclerosis via inhibition of TLR-4 mediated ADRP expression induced by LPS from gram negative periodontal pathogens, emphasizing the link between periodontal diseases and atherosclerotic changes.

TLR-4 signalling in response to LPS in hepatic macrophages sensitized by chronic ethanol feeding increases the production of TNF-α and reactive oxygen species (ROS). Adiponectin normalizes TLR-4 mediated signaling in this model and likely to contribute to hepato-protective effects during the progression of alcoholic liver disease [10]. The mechanisms involved reveal a complex integrated response of macrophages to globular adiponectin resulting in an initial increase in TNF-α which subsequently triggers the expression of the potent anti-inflammatory cytokine IL-10, required for reduced TLR-4 mediated signalling by globular adiponectin. Adiponectin has implications for alleviation of progressive hepatic damage in patients with periodontal and

co-existing systemic diseases driven by bacterial LPS, cytokines and reactive oxygen species.

The pathogenesis of periodontal disease is associated with reactive oxygen species (ROS) resulting from an imbalance between oxidant /antioxidant activity. The extent of lipid peroxidation in gingival crevicular fluid (GCF) and saliva and the levels of glutathione (GSH) and glutathione peroxidase (GPx) in the saliva of patients with chronic periodontitis has been investigated in comparison with controls [11]. The extent of lipid peroxidation, GSH level and GPx activity were determined by spectrophotometric assay. Periodontal treatment was found to show significant reduction in periodontal disease parameters such as gingival index, probing attachment level, probing pocket depth and gingival crevicular fluid volume. Following treatment lipid peroxidation levels were decreased in gingival crevicular fluid and there were increased levels of GSH. Elevated levels of lipid peroxidation could contribute to sequential destruction during the progression of periodontal disease. The lowest levels of lipid peroxidation in saliva have been demonstrated in periodontally healthy non-smokers, using malondialdehyde (MDA) as a marker [12]. Patients with periodontal disease demonstrated significantly more lipid peroxidation [13] than controls and particularly smokers. Treatment consisting of thorough debridement of periodontal pockets reduced the inflammatory loading induced by periodontal pathogens; and resulted in decreased levels of MDA and glutathione peroxidase, comparable to those in periodontally healthy controls.

Complex interactions between periodontal pathogens and host modulated immune responses play a role in the progression of periodontal disease. An over-exuberant immune response results in the formation of excessive amounts of free radicals and their metabolites. Peripheral neutrophil hyper-responsiveness in chronic periodontal disease leads to the production of high levels of unstimulated neutrophil reactive oxygen species (ROS) production which continues after treatment [14] indicating that inherent and reactive mechanisms are responsible for neutrophil hyper-responsiveness in periodontal disease. The resultant oxidative stress drives several inflammatory diseases [15-18], including periodontitis.

The pro-inflammatory cytokine interleukin-1β is an important regulator of immune mediated inflammatory responses and free radical production. Modulators of redox defences such as glutathione peroxidase, lactoferrin and myeloperoxidase have been investigated [19]. Gingival crevicular fluid samples were collected from periodontitis subjects and healthy controls. Enzyme linked immunosorbent assays were done to analyze glutathione peroxidase, lactoferrin, myeloperoxidase and IL-1β in gingival crevicular fluid. The periodontally diseased sites showed significantly greater total amounts of glutathione peroxidase, lactoferrin, myeloperoxidase and IL-1β than healthy sites. These values showed a positive correlation with plaque index, gingival index, probing depth and probing attachment levels. Chronic insult from periodontal pathogens triggered by reactive oxygen species may be fuelled by the imbalance between the levels of myeloperoxidase/IL-1β and glutathione peroxidase/ lactoferrin resulting in the damaging effects of periodontal

disease progression. Other studies have shown that patients with periodontal disease demonstrate signi-ficantly elevated activities of myeloperoxidase, glutathione peroxidase, glutathione S-transferase, thiobarbituric acid reactive substances and oxidized glutathione levels in gingival tissues compared with control subjects [20].

Myeloperoxidase is found in the lysosomal granules of myeloid cells, particularly macrophages and neutrophils, responsible for generating potent bacteriocidal activity; by the hydrolysis of hydrogen peroxide produced in the metabolic burst in the presence of halide ions. This enzyme, found in leukocytes is linked to inflammation and cardio-vascular disease. An elevated blood level of the enzyme predicts the early risk of myocardial infarction. Glutathione peroxidase is a detoxifying enzyme that eliminates hydrogen peroxide and organic peroxides. Glutathione is an essential cofactor for the enzyme and its reaction involves the oxidation of glutathione (GSH) to glutathione disulphide (GSSG). The GSSG is then reduced to GSH by glutathione reductase. Lactoferrin is an iron-binding glycoprotein with a wide spectrum of biological activities. It may contribute to protection against pathogens and their metabolites by enhancing phagocytosis, cell adherence, and controlling release of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor (TNF)-α. Plasma markers of oxidative stress and enzymatic / non-enzymatic antioxidants have been investigated in patients with Alzheimer's disease, demonstrating an antioxidant deficit in comparison with age matched healthy controls [21].

Increased oxidative stress as a result of excessive production of reactive oxygen species has been attributed to lipid, protein and DNA components of tissue damage. Mitochondrial DNA (mtDNA) is a reliable marker of oxidative DNA damage. The mtDNA deletions in gingival tissues of patients with periodontal disease were investigated with a view to clarifying a correlation between the deletion and periodontal disease parameters, in the context of the age of the patient [22]. Gingival tissue and blood samples were collected from 30 patients with periodontal disease and 30 healthy controls. Clinical periodontal disease parameters were recorded. Using the polymerase chain reaction method (PCR), both 7.4- and 5-kbp mtDNA deletions were investigated in tissue and blood samples. There were no 7.4-kbp mtDNA deletions in either the blood samples or gingival tissues obtained from healthy or periodontally diseased subjects. However, the 5-kbp mtDNA deletion was detected in 24 of the 30 subjects (80%) with chronic periodontal disease and not detected in healthy subjects. There was a significant correlation between all clinical periodontal parameters and the occurrence of the 5-kbp mtDNA deletion; there was a further correlation between the age of the patient and the 5-kbp mtDNA deletion. It was concluded that a 5-kbp mtDNA deletion was evidence of premature oxidative damage in patients with periodontal disease, due to overproduction of ROS by activated PMNs. Levels of the marker of oxidant-induced DNA damage, 8-hydroxy-2-deoxyguanosine were significantly elevated in periodontits patients with decreased plasma total antioxidant capacity, when compared with controls [23].

The association between Down's syndrome and susceptibility to early onset rapidly progressive periodontal disease is well established in the literature and provides a good model for studying immune mediated responses relevant to periodontal disease progression. There is less information available on the influence of reactive oxygen species on periodontal disease progression in Down's syndrome patients. A recent study was carried out to investigate the generation of reactive oxygen species in gingival fibroblasts (DS-GF) isolated from patients with Down's syndrome [24]. The aim of the investigation was to use electron spin resonance spin trapping with 5,5-dimethyl-1-pyrolline-N-oxide (DMPO) and to establish whether ROS generation plays a role in the pathogenesis of periodontal disease progression in Down's syndrome patients. There was evidence of DMPO-OH spin adduct formation indicative of hydroxyl radical (HO) generation from DS-GF and non-DS-GF. Generation of HO by DS-GF was significantly decreased in the presence of the hydrogen peroxide scavenger catalase or the iron chelator desferal. This could result from over expressed CuZn-superoxide dismutase in Down's syndrome leading to the catalysis of hydrogen peroxide formation from oxygen. This increases the availability of hydrogen peroxide as substrate for the iron dependent generation of HO via the Fenton reaction. Generation of HO from DS-GF may be involved in the pathogenesis of periodontal disease in Down's syndrome patients.

A significant decrease in glutathione concentration was reported in Down's syndrome patients when compared with controls in an investigation of glutathione metabolism in this population [25]. Evidence of oxidative stress has been reported in Down's syndrome individuals (trisomy 21) with significantly elevated levels of ubiquinone-10, the oxidized component of coenzyme Q-10 and decreased ratio of the reduced form ubiquinol-10: total conenzyme Q10; which was normalized by supplementation with ubiquinol-10, to correct the pro-oxidant status of trisomy 21 [26]. Preliminary studies in pro-oxidant cultures of oral periosteal fibroblasts and well characterized osteoblasts demonstrated that coenzyme Q10, phytoestrogens and the antioxidant Pycnogenol derived from French maritime pine bark, alleviated oxidative stress induced by nicotine in this model [27], indicating a possible role for these antioxidants in the adjunctive therapy of inflammatory diseases characterized by a pro-oxidant profile.

The implications of oxidative stress induced damage, periodontal disease progression and impaired oxidant /antioxidant status are well documented in the literature. There are clear indications of a hyper-inflammatory status which can influence or fuel the progression of co-existing diseases with similar inflammatory pathogeneses. Existing documented evidence is suggestive of scope for adjunctive therapeutic targets for antioxidants in the management of periodontal and associated diseases.

ANTIOXIDANT STATUS IN PERIODONTAL TISSUES

The pathogenesis of periodontal diseases associated with reactive oxygen species highlights the importance of antioxidant status as a growing focus of interest. Immuno-

histochemical parameters provide important information on the inflammatory status linked to tissue morphology and vascular proliferation. During gingivitis there is a large inflammatory infiltrate of lymphocytes, plasma cells and neutrophils in the lamina propria and the squamous epithelium is vascular endothelial growth factor (VEGF) positive. Investigation of involved tissue demonstrated a concurrent dramatic reduction in tissue levels of Vitamin E, while CoQ10 levels remained similar [28]. Continuous oxidative stress associated with long-term chronic inflammation characteristic of periodontal disease could affect the antioxidant status of the affected tissues. Nuclear factor-kappaB (NF-kappa B) plays a key role in the induction of genes that respond to inflammation and maintain physiological homeostasis. The actions of NF-kappaB are significantly inhibited by the dithiocarbamate antioxidants. In this context, the effects of pyrrolidine dithiocarbamate (PDTC) was investigated in a rat model of periodontitis, induced by placing 2/0 braided silk around a lower first molar [29]. At day eight, the mucogingival tissue surrounding the molar was removed for biochemical and histological analysis, demonstrating increased neutrophil infiltration associated with significant expression of TNF-α, inducible nitric oxide synthase (iNOS) and nitrotyrosine formation. Ligation induced significant alveolar bone destruction. It is relevant that intra-peritoneal injection of PDTC exerted an anti-inflammatory effect and was able to overcome tissue damage induced by experimental periodontitis by reducing the above parameters of inflammation. This has potential implications for adjunctive therapeutic targeting in the management of periodontal disease.

Increased levels of reactive oxygen species are associated with impaired antioxidant capacity. They contribute to denaturation of critical enzymes which renders them inactive. Blood is a good medium for testing antioxidant capacity. The antioxidant capacity of total blood was shown to be reduced in patients with periodontal disease and in aged subjects compared with controls [30]. This study highlights the importance of increased oxidative stress in periodontal patients. In severe cases, this has implications on systemic inflammatory loading and possible implications on progression of conditions such as metabolic syndrome, also mediated by an inflammatory pathogenesis. Previous work has reported reduced antioxidant capacity of saliva in patients with periodontal disease. Urate is the main salivary antioxidant with lesser amounts of albumin and ascorbate. The influence of diet on salivary antioxidant capacity is less clear than the effect of dietary constituents on plasma levels which show a clear link. The main antioxidants in plasma are ascorbate, albumin and urate. A clearer understanding of the link between nutritional status, antioxidant capacity and periodontal diseases would aid therapeutic targeting along these lines for adjunctive management of periodontal diseases [31]. In age matched controls with or without periodontal disease the activities of enzymatic and non-enzymatic antioxidants were investigated at interproximal gingival sites. The enzymatic antioxidants evaluated were catalase, glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase; the non-enzymatic antioxidants tested were total glutathione and reduced glutathione, oxidized glutathione (GSSG), thiobarbituric acid

reactive substances (TBARS), and myeloperoxidase (MPO) activity. The periodontally diseased sites showed significantly raised levels of MPO, GPx, GST, and also those of TBARS and GSSG in gingival tissue compared with controls [20]. These findings demonstrate an important correlation between biomarkers of oxidative stress and periodontal disease, in response to an inflammatory stimulus.

The antioxidant capacity of whole saliva was investigated in 30 healthy adults with implant supported overdentures [32]. The initiation and progression of peri-implantitis is associated with predominantly Gram-negative sub-gingival colonizers that are gram negative and anaerobic or micro-aerophilic. The resultant inflammatory changes as a result of direct effects of toxins and the host response are similar to those seen in chronic periodontal disease around natural teeth, associated with reactive oxygen species. Pocket probing depths, the total antioxidant capacity of saliva and levels of urate, ascorbate and myeloperoxidase were compared in patients with healthy peri-implant tissue and those demonstrating peri-implant disease. It is relevant that significantly lower levels of the above antioxidants were detected in peri-implant disease. ROS mediated oxidative stress could contribute to reduced antioxidant capacity at diseased sites. The pathogenesis of peri-implantitis is rather similar to that of periodontitis in this context and may warrant adjunctive supplementation with antioxidants in addition to traditional intervention for pocket debridement . A similar study compared inflammatory markers around natural teeth and implants. Levels of the inflammatory markers nitrite, an end product of nitric oxide (NO) oxidation and myeloperoxidase (MPO) were investigated in gingival crevicular fluid of natural teeth with a healthy or diseased periodontium and in peri-implant sulcular fluid [33]. There were raised levels of MPO from inflamed sites of implants and natural teeth when compared with non-inflamed sites. Peri-implant sulcular fluid also showed increased levels of nitrite at inflamed sites compared with healthy sites. Peri-implant sulcular fluid may have useful diagnostic implications in predicting biological changes occurring around endosseous implants.

The effects of lipid A-associated proteins from the periodontal pathogen *Porphyromonas gingivalis* (Pg) on the modulation of inducible nitric oxide synthase (iNOS) expression and production of nitric oxide was investigated in a murine macrophage cell line [34]. A standard procedure was used for extraction of the endotoxin. The accumulation of nitrite in culture supernatant was an index of nitric oxide production. Western blot was used to analyse iNOS and RT-PCR products were also assayed. This study showed that lipid A-associated proteins of *Pg* are effective in stimulating nitric oxide synthesis and in inducing NOS. Multiple signal transduction mechanisms involving nuclear factor-kappaB, microtubule polymerization and protein kinase are implicated. The ability of *Pg* to induce NO and NO synthase has important implications in the aetiopathogenesis of periodontal diseases and therapeutic targets for their control.

When human gingival fibroblasts were incubated with lipopolysaccharide (LPS) obtained from *Escherichia coli* (*E.coli*) and the periodontal pathogens *Aggregatibacter actinomycetemcomitans* (*Aa*) and *Porphyromonas gingivalis*

(*Pg*) in the presence or absence of the antioxidant N-acetyl-cysteine (NAC), it was demonstrated that NAC suppressed the pro-inflammatory profile induced by LPS; associated with increased production of the pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, raised levels of ROS, the formation and activation of matrix metallo- proteinase 2 (MMP2). These findings suggest the relevance of LPS and ROS in the modulation of inflammation in this model and the therapeutic potential of antioxidants. NAC was also shown to prevent LPS-induced activation of p38 MAPK and JNK indicating an application for its down-regulation of JNK and p38 MAPK and controlling oxidative stress induced periodontal damage as an adjunctive therapy to root surface debridement [35]. Similar effects have been demonstrated in response to omega-3 fatty acids [36], berry proanthocyanidins [37] and green tea polyphenolics [38].

TOTAL ANTIOXIDANT CAPACITY IN PERIO-DONTAL DISEASE AND HEALTH

In vivo antioxidant systems modulate reactive oxygen species and the net outcome would drive the progression of periodontal disease. The local antioxidant capacity of saliva, gingival crevicular fluid and peripheral antioxidant capacity of plasma and serum were determined in age and sex matched non-smokers with or without chronic periodontal disease [39], using an enhanced chemiluminescence method. Antioxidant levels in GCF were significantly lower in subjects with periodontal disease, compared with healthy controls. The mean values for peripheral and salivary total antioxidant capacity were lower for the test group and significantly so for plasma, but less significant than the values for GCF. These findings indicate that GCF total antioxidant capacity shows qualitative and quantitative differences from that of saliva, plasma and serum. They are likely to reflect the oxidative stress induced pathogenesis of periodontal disease; variation in antioxidant capacity amongst individuals could reflect susceptibility to the disease process and altered buffering capacity.

The cell membrane-permeable radical scavenging agent Tempol (4-hydroxy-2,2,6,6-tetramethypiperidine-N-oxyl) exerts protective effects in diverse models of inflammation. Reactive oxygen species play a significant role in the induction of genes associated with inflammatory pathology. The effect of Tempol was investigated in a rat model of periodontitis induced by placing a braided silk ligature around a molar tooth [40]. At day 8 the mucogingival tissue and bone around the tooth was removed for evaluation of tissue permeability, PMN infiltration, histology, radiology, nitrotyrosine formation and poly-(ADP-ribose) polymerase (PARP) activation. There was a significant inflammatory response to placement of a silk ligature around the molar with a heavy infiltrate of PMNs, positive staining for nitrotyrosine formation and activation of PARP. There was extravasation of Evans blue in the mucogingival tissue as a result of inflammatory damage and evidence of alveolar erosion demonstrated by radiographic evaluation. Intraperitoneal injection of 10mg/kg Tempol for 8 days resulted in significant reduction in the inflammatory parameters described above. These antioxidant effects of Tempol indicate that adjunctive therapeutics with antioxidants to

overcome the effects of ROS has an application in the management of chronic periodontal disease.

ROLE OF NUTRITION IN TARGETING OXIDATIVE STRESS INDUCED BY INFLAMMATION

Inflammation results in the formation of acute phase proteins, inflammatory mediators and the need for defence against free radical damage and promotion of tissue repair. It alters protein, fat and carbohydrate metabolism due to oxidative mechanisms involved in the generation of reactive oxygen species [41]. Key antioxidants and nutrients would play an important role in overcoming a deficit incurred by excessive demand. Some nutritional components can be targeted for their anti-inflammatory effects in controlling the inflammatory process. New knowledge arising from molecular biology and nutritional genomics is likely to provide important correlations between the adjunctive role of diet and the progression of periodontal disease. Experimental studies have demonstrated that proanthocyanidins extracted from cranberry (Vaccinium macrocarpon) have an inherent anti-adhesive mechanism for prevention of bacterial attachment to tissues *in vitro* [37]. The applications for cranberry juice as a prophylactic agent against recurrent urinary tract infections are well documented in the literature. These anti-adhesive actions of cranberry - proanthocyanidins are also a useful therapeutic target against other diseases with an inflammatory pathogenesis such as gastritis associated with *Helicobacter pylori* and periodontal diseases. It was demonstrated that patients with periodontal disease had significantly lower plasma and salivary melatonin levels than healthy control patients [42] when 46 periodontal patients were compared with 26 age matched controls. Lymphocyte subpopulations of CD3, CD4, CD8, C19 and natural killer cells were analyzed using flow cytometry. Patients with more severe periodontal disease and higher counts of CD4 and total lymphocyte numbers showed higher levels of melatonin. Melatonin could act in a protective capacity in overcoming inflammatory periodontal disease.

Omega-3 fatty acid (omega-3 FA) is an effective anti-inflammatory anti-oxidant found in fish oil. A recent investigation studied the influence of omega-3 FA found in fish oil on gingival tissues of rats infected with *P.gingivalis* [36]. Rats infected with two strains of *Pg* were fed either fish oil or corn oil during a 22 week period, ad libitum and sacrificed. The RNA of rat gingival tissue was analysed for proinflammatory mediators IL-1β, TNF-α, IL-6, T helper cytokines, interferon- (IFN) γ, IL-4, IL-10, antioxidant enzymes catalase, superoxide dismutase and genes responsible for the production of eicosanoid mediators (COX-2 and 5-lipoxygenase), by reverse transcription-polymerase chain reaction (RT-PCR), using primers specific for rats. An omega-3 FA diet significantly decreased expression of IL-1β, TNF-α and enhanced messenger RNA expression of IFN γ, catalase and superoxide dismutase compared to corn-oil fed rats. Alveolar bone resorption in the rats showed a significant positive correlation with IL-1β, IL-6 and COX-2 and negative correlations with catalase and superoxide dismutase. These findings demonstrate that diets enriched in omega-3 FA are effective in minimizing *Pg* induced inflammation and alveolar bone loss in the rat model. This may be extrapolated to humans and has potential appli-

cations in the adjunctive management of periodontal diseases.

The efficacy of green tea catechin against black pigmented Gram–negative anaerobic rods was investigated in the context of periodontal disease control [38]. In order to measure the minimum inhibitory concentration of green tea catechin against gram negative rods, hydroxylpropyl cellulose strips impregnated with green tea catechin were inserted in periodontal pockets as a slow-release device. They were applied weekly for 8 weeks. Green tea catechin was shown to have a significant bactericidal effect against *Prophyromonas gingivalis* and *Prevotella spp in vitro* with an MIC of 1mg/ml. There was significant reduction in probing depths and the proportion of black pigmented rods at week 8 in the catechin group with mechanical debridement in comparison with placebo sites of debrided groups. Peptidase activities in gingival crevicular fluid were maintained at lower levels at test sites compared with placebo sites which maintained 70% of baseline values. Green tea catechin used as a slow release local delivery system in periodontal pockets in combination with their mechanical debridement has been shown to be effective in improving periodontal disease control in this study.

A HYPER-INFLAMMATORY STATE RELEVANT TO CO-EXISTING SYSTEMIC PATHOLOGIES AND THERAPEUTIC POTENTIAL

Periodontal Diseases and Systemic Diseases

With the recent accumulation of data suggestive of several links between oral and systemic health the divide between these entities is rapidly closing, with several common pathogenic mechanisms and risk factors associated with disease progression. This is particularly true in entities such as cardiovascular disease, diabetes mellitus and osteoporosis. Significant scientific advancement in this field demonstrates links in the aetiopathogenesis of dental and systemic diseases. These links provide therapeutic targets for amelioration of periodontal and systemic diseases; it is possible that in very severe cases of periodontal disease, removal of this inflammatory focus could result in improved systemic health [43]. The oxidative stress inducing effects of smoking, compound the effects of free radical damage in periodontal and systemic diseases.

The levels of free radicals and antioxidants were evaluated in order to find a correlation between cigarette smoking and periodontal damage [44]. The study group consisted of thirty five healthy subjects ranging from 25-56y with chronic moderate inflammatory periodontal disease (attachment loss of 3-4mm). All subjects were matched with regard to plaque index, gingival index and attachment loss. Gingival tissue obtained at the time of surgery and blood samples were analysed for lipid peroxide, superoxide dismutase, catalase, glutathione and total thiol. The lipid peroxide level in tissue and blood was lowest in non-smokers and highest in smokers who smoked more than 50 cigarettes /day. There were similar trends for catalase, while thiol levels had similar implications in those who smoked less than 20 cigarettes / day. The superoxide dismutase levels were higher in tissue and blood in non-smokers than in

heavy smokers. The glutathione level was consistently lower in smokers than in controls in tissue samples. In the context of pulmonary function [45] and diabetic nephropathy [46] oxidative stress induced by smoking has been identified as an independent risk factor for their progression. These studies indicate that a pro-oxidative status enhanced by smoking could influence the progression of periodontal and systemic diseases.

The aetiopathogenesis of periodontal disease, associated with bacterial plaque and a host modulated inflammatory response resulting in oxidative stress shares a common mechanism for inflammatory loading with several systemic conditions. This common sequence of inflammopathology pre-empts likely links with systemic conditions such as stroke, type-2 diabetes and atheromatous heart disease. In an analysis of 11,480 with multiple logistic regression for dual case definitions of mild and severe disease in adults over 20y, serum concentrations of vitamin C, bilirubin and the total antioxidant capacity were inversely associated with periodontitis [47]. The association was stronger in cases of severe disease. Vitamin C and total antioxidant capacity were protective in never-smokers and increased serum antioxidant concentrations were shown to be associated with reduced risk of periodontitis. A case of severe deficiency of ascorbic acid (vitamin C) has been reported in a 2-year old Caucasian girl [48] following a diet consisting of organic milk, barley and corn syrup lacking in fruits and vegetables, which are sources of ascorbic acid, a diet based on belief. Ascorbic acid is essential for collagen formation, iron absorption and reduction in free radicals; a deficiency in dietary ascorbic acid results in scurvy, not commonly reported today. The 2-year old presented with pale bloated skin, oedematous, violaceous gingivae, loosening of a few of her teeth, multiple ecchymoses of the skin and perifollicular haemorrhage. Following 3 days of treatment with oral ascorbic acid and significant improvement in diet, there was dramatic improvement. This report highlights the importance of a dietary history in aiding the diagnosis of mucosal and dermatological conditions.

HYPER-INFLAMMATORY STATUS IN DIABETES MELLITUS (DM) AND PERIODONTAL DISEASES WARRANTS ANTIOXIDANT THERAPEUTIC TARGETS

Dysregulation of innate immunity associated with the multifactorial complications of diabetes mellitus contributes to a hyper-inflammatory status [49]. High glucose levels lead to the formation of diacylglycerol via the polyol pathway resulting in the activation of electrons and superoxide formation. This chain of events leads to the formation of advanced glycation end products as a consequence of non-enzymatic glycosylation of proteins. Complications of uncontrolled DM such as periodontal disease, athero-sclerosis, nephropathy, retinopathy and impaired healing are characterized by altered cell signalling and activation of the MAP kinase pathway or transcription factors such as NF-kappaB. This is associated with increased expression of inflammatory cytokines such as TNF-α, IL-1β and IL-6 [50]. The cause and effect relationship demonstrated in the inflammatory pathogenesis of diabetic complications has implications for therapeutic targets of anti-inflammatory and

antioxidant agents also relevant to periodontal disease with which it often co-exists [51,52].

Glucose tolerance and obesity have been shown to be linked to periodontal disease. Levels of the adiposite derived cytokine adiponectin decrease in obese and Type 2 diabetic patients. There is a distinct inverse relationship between adiponectin levels and diabetes risk [53]. An investigation was carried out to study the effect of adiponectin on the regulation of osteoclast formation in response to a stimulus from the lipopolysaccharide of gram negative bacteria in a highly responsive clone of osteoblasts [54]. Adiponectin was a potent inhibitor of osteoclast formation, stimulated by toll-like receptor 4 ligand and receptor activator of NF-kappaB (RANK). Due to the importance of the latter as a transcription factor for osteoclasts, its transcription activity was assayed, using a luciferase assay. Adiponectin was also able to inhibit TLR4-mediated expression of the gene for inducible nitric oxide synthase and formation of nitric oxide in the cells. These findings suggest the likely role of adiponectin in controlling LPS/RANKL mediated osteoclast formation in periodontal disease, acting as a negative regulator. Adipose tissue plays a central role in lipid and glucose metabolism, producing a range of hormones and cytokines relevant to diabetes and cardiovascular disease [55]. These interactions are relevant to the progression of periodontal diseases in uncontrolled diabetic patients.

Monocytes and macrophages may play an important role in the prevalence of severe periodontal disease seen in uncontrolled diabetic patients. Monocytes isolated from poorly controlled diabetics produce larger amounts of inflammatory cytokines than healthy individuals and this has been demonstrated in gingival crevicular fluid. In this context, when histiocytes were cultured in medium containing either normal (5mM) or high (25mM) concentrations of glucose and subjected to lipopolysaccharides for 24h, pre-exposure to higher concentrations of glucose resulted in significantly higher levels of cytokine secretion and inducible levels of cellular nitric oxide in response to increased levels of glucose [56]. Enhanced secretion of cytokines was associated with increased mRNA expression. Cytokine release enhanced by high concentrations of glucose was inhibited by simvastatin but not fenofibrate or pioglitazone. It is relevant that statins inhibited cytokine release in hyperglycaemic cultures triggered by LPS. This study identifies a novel mechanism whereby the inflammatory response of macrophages is enhanced by hyper-glycaemia and there may be an application for statins in the management of periodontal diseases with or without co-existing uncontrolled diabetes mellitus.

Subjects on the statins, simvastatin and atorvastatin show significantly fewer periodontal lesions than non-medicated controls [57]. Their pleiotropic anti-inflammatory effects are likely to have a beneficial effect on periodontal tissue. Ligature-induced periodontal bone loss in rats was reversed by 46% in response to simvastatin which stimulated osteo-blastic function [58]. Similarly when simvastatin in a carrier of polylactic / polyglycolic acid co-polymer was implanted in extraction sockets of male Wistar rats, a greater rate of bone formation and improved bone quality were observed when compared with controls [59]. This has implications in

the preservation of residual alveolar ridge following extractions. These further reinforce the link between disease presentation and therapeutic targeting amongst co-existing diseases with inflammation lead pathology.

OXIDATIVE STRESS, ATHEROSCLEROTIC PLA-QUE AND ROS: COMMONALITY WITH DIABETES MELLITUS AND PERIODONTAL DISEASE

Functional impairment of the vascular endothelium is an early indicator of atherosclerotic events. The vessel wall adapts to various changes occurring within the vasculature and functions as a receptor- effector structure. This main-tains homeostasis during multiple physical and chemical stimuli and their counteraction by producing a variety of agonists and antagonists which modify the impulses and maintain a balance. When adverse stimuli are no longer contained by homeostatic mechanisms the endothelium is invaded by lipids, monocytes and T lymphocytes. Oxidised LDL promotes monocyte dynamics for trans-endothelial movement in the context of atherogenesis [60]. An oxidative stress induced inflammatory response results in the initiation of atheromatous plaque formation which manifests as fatty streaks in the vessel wall. Progressive development of fatty streaks sets off a cascade of events leading to exposure of atheromatous plaque resulting in rupture, thrombogenesis and vascular occlusion [61]. As a result of aerobic metabolism, biologically active reactive oxygen species (ROS) are formed. Prevailing physiological homeostatic measures would usually overcome a hyper-inflammatory oxidative response with anti-oxidants in order to prevent oxidative damage. In pathological states the relative excess of ROS results in oxidative stress induced cellular and tissue damage. This cycle of events results in further release of oxidative stress inducing agents resulting in the maintenance of a cascade of oxidative stress induced damage which triggers the release of further risk factors and other events.

These features of oxidative stress also operate in Type 1 and Type 2 diabetes mellitus, which are both very significant independent risk factors for coronary heart disease, stroke and peripheral arterial disease. Glycosylation of proteins and phospholipids occurs as a result of hyperglycaemia and increased oxidative stress. Some of the early non-enzymatic glycosylation products which are reversible, subsequently re-arrange themselves to form more stable compounds which progressively undergo a series of molecular re-configu-rations resulting in the formation of advanced glycosylation end products (AGEs). Once formed, the process is irre-versible and AGEs are relatively stable compounds which generate ROS. This process contributes to vascular damage, atherogenesis and thromboembolic events [62]. The correlation between cardiovascular disease and glucose metabolism is well documented. They share an oxidative stress induced pathogenesis which precedes either of these conditions with common genetic and environmental antecedents. Large vessel atherosclerosis can precede the development of diabetes and vice versa. Antioxidants can be used as selective targets in overcoming these effects. In a diabetogenic cell culture model of well characterized osteoblasts, it was demonstrated that the oxidative effects of AGE and nicotine were overcome by glutathione and insulin

like growth factor [63] demonstrating relevant therapeutic targets in diabetic smokers with periodontal disease.

There is growing acceptance in the literature of the effects of polyphenols, including those found in cranberries in reducing the risk of cardiovascular disease [64], dental caries and periodontal disease [65]; being effective in reducing hyper-inflammatory responses associated with biofilm formation, adherence and co-aggregation of periodontal pathogens leading to proteolytic activity and destruction of extra-cellular matrix. The American cranberry (Vaccinium macrocarpon) is a significantly good source of phenolic phytochemicals. This includes phenolic acids such as benzoic, hydroxyl cinnamic and ellagic acids; and the flavinoids such as anthocyanins, flavonols and flavan-3-ols. The bioactive mechanisms of polyphenols, including those found in cranberries involve reducing the risk of cardio-vascular disease by increasing the resistance to LDL oxidation, platelet aggregation, reducing blood pressure and other actions associated with controlling inflammation and thrombosis. These applications are also relevant to the hyperinflammatory status of aggressive periodontal diseases.

Resveratrol (3,4',5-trihydroxystilbene) is a plant derived anti-inflammatory, antioxidant effective against platelet aggregation and atherogenesis. These actions extend to anti-ageing effects which are likely to have implications on age-related human diseases. It has been isolated from grapes, berries, peanuts and also found in red wine. It also has oestrogen like growth promoting effects, immunomodulatory and chemopreventive actions. Several *in vitro* and *in vivo* investigations have been carried out to identify its biological properties and actions. Resveratrol was shown to overcome repetitive oxidative stress induced by hydrogen peroxide in lung fibroblasts by preventing a rise in ROS and inhibiting p38 MAPK activation [66]. Similar findings have been reported in a cell culture model applicable to oxidative stress in smokers with periodontal disease [67]. Smoking and periodontal inflammation induced by gram negative pathogens such as *Porphyromonas gingivalis* (*Pg*) are risk factors for alveolar bone destruction. The combined effects of a cigarette smoke constituent the aryl hydrocarbon benzo pyrene and *Pg* lipopolysaccharide were investigated in a model comprising rat bone marrow cell osteogenesis. Bone formation was inhibited by these agents and reversed by the aryl hydrocarbon receptor antagonist resveratrol, suggestive of its therapeutic potential in progressive periodontal disease in smokers.

ANTIOXIDANT INTERVENTION IN RHEUMATOID ARTHRITIS

Reactive oxygen species are implicated in the aetiology of autoimmune diseases such as rheumatoid arthritis (RA). A possible link between the aetiopathogeneses of periodontal and arthritic diseases has been reported [68-72] and therapeutic potential reviewed [73]. It would be logical to assume that antioxidants have beneficial effects on the clinical manifestations of RA as bourne out by supporting evidence. But the correct balance for their optimal clinical efficacy has not been determined. A recent pilot study was carried out to investigate the potential benefit of antioxidant intervention in a group of eight non-smoking female patients

positive for rheumatoid factor and RA [74]. They received stable non-steroidal anti-inflammatory drug treatment or 20g antioxidant-enriched spread daily for 3months. The pilot group receiving the antioxidant consumed it for 10 weeks, followed by a wash out period of 4 weeks. The number of swollen and painful joints and general health measured using the disease activity score were significantly improved and the antioxidant supplement was considered beneficial. An increase in the disease activity score after the wash out period confirmed a cause and effect benefit of antioxidant usage. While these conclusions need to be validated in a larger controlled study population, these preliminary results of the pilot study indicate the clinical relevance of therapeutic intervention with an antioxidant in patients with rheumatoid arthritis. The stilbene resveratrol has been shown to prevent chondrocyte apoptosis by preventing depolari-zation of the mitochondrial membrane; and it is also protective against catabolic effects induced by IL-1β and PGE_2 by inhibiting its synthesis [75]. These effects provide a potential therapeutic target against oxidative injury and apoptosis seen in progressive osteoarthritis. Resveratrol could have similar therapeutic benefits in the management of other ROS driven inflammatory diseases such as periodontal disease.

PERIODONTAL DISEASE, FOETAL DEVELOPMENT AND MORTALITY

Adverse foetal developmental outcome including preterm labour and foetal mortality may be influenced by maternal bacterial infections. There are several complications associated with periodontal disease, including foetal death. An investigation was carried out to study the response of pregnant ewes to intra-amniotic injection of LPS derived from *Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn)*, *Eschericia Coli* (*E Coli*), or saline (controls) at 118 days of pregnancy. The outcome studied included rate of foetal death, features of inflammation and lung maturation in survivors [76]. The foetuses were delivered abdominally at 125 days (term 150 days). When compared with *E. coli*-LPS, those from periodontal pathogens induced a high level of mortality of 50% and 75% in response to *Aa* and *Pg*- LPS respectively. There was evidence of inflammation in amniotic fluid and cord blood and enhanced lung maturation at birth of fetuses that survived exposure to LPS. There are implications on foetal mortality and other complications of pregnancy from inflammatory pathology at sites distant from the uterus, such as periodontal diseases if the inflammatory loading is substantial.

Increased risk of prematurity and low birthweight asso-ciated with maternal periodontal infection is well docu-mented in certain populations. Developmental impairment of the foetus in response to oxidative stress mediated by inflammatory pathways responsible for prematurity and low birth weight has also been reported. An investigation was carried out in a mouse model, to ascertain whether maternal infection with *Campylobacter rectus (C rectus)* impairs survival and neurodevelopment in addition to causing restricted growth of the foetus [77]. Challenge with *C rectus* on gestation day 7.5 resulted in abnormal placental archi-tecture, inflammation and a significant increase in foetal

brain mRNA expression of TNF-α and interferon (IFN)- γ. Mortality was increased by 3.9-fold in response to *C rectus* challenge. Brain tissue of the 9 day old neonate demonstrated ultrastructural changes that were similar to those seen in white matter of the brain in humans following maternal infection. These findings could be extrapolated for comparison with exposure to oral infections including periodontal disease which could potentially influence foetal loss and perinatal neurological development depending on the status of inflammatory loading.

Chorioamniotic pathogenesis is significantly influenced by oxidative stress. A recent investigation in a murine model demonstrated that exposure to LPS on day 18 of gestation resulted in an increase in the abortion rate and foetal demise; this was accompanied by an elevated inflammatory response associated with raised levels of cytokines, chemokines, inducible nitric oxide synthase expression and leukocytic infiltration of the placenta [78]. There appeared to be increased metabolism of phospholipids resulting in increased expression of cytosolic and secretory phospholipase A2. There was also increased secretion of prostaglandin-2, leukotriene B4, expression of cycloxygenase-2 and synthesis of the oxidative stress marker malondialdehyde in the placenta. Conversely pre-treatment with N-acetyl-cysteine nullified these effects of LPS in the placenta. This study demonstrates that antioxidant-based therapies could block adverse foetal outcome, caused by LPS-induced inflammatory changes at the foeto-maternal interface resulting from maternal bacterial infections. This is applicable to periodontal diseases which are associated with LPS-induced inflammatory pathology.

Bacterial lipopolysaccharide is associated with foetal death and growth retardation. LPS-induced inflammation and resultant oxidative stress are associated with these effects. In this context, the effects of the antioxidant N-acetylcysteine (NAC) a glutathione precursor on LPS-induced intra-uterine foetal death and intra-uterine growth retardation were investigated in pregnant mice [79]. All pregnant mice were injected with 75µg/kg LPS on gestational day 15-17 and NAC was administered either as pre-treatment prior to LPS administration or post-treatment after LPS administration. Results indicated that pre-treatment with NAC significantly alleviated LPS-induced foetal mortality and prevented retardation in growth and development. There were corresponding reductions in levels of TNF-α in maternal serum and amniotic fluid; and lipid peroxidation in maternal and foetal livers. In contrast, post-LPS delivery of NAC was not protective and aggravated LPS-induced preterm labour indicating dichotomous effects of NAC. Judicious adjunctive treatment with antioxidants timed correctly could avert foetal distress in response to maternal LPS loading as encountered in severe and florid manifestations of periodontal diseases.

Foetal inflammation is the strongest predictor of brain lesions. Both antenatal and neonatal exposure to LPS could sensitise the foetal brain to subsequent hypoxic / ischaemic events in adulthood. Intracerebral / intravenous LPS injections evoke a marked cytokine response in the brain and significant white matter lesions. More distant routes of administration such as intracervical, intrauterine or maternal LPS from bacterial infections result in a reduction of markers for myelin in the absence of obvious macroscopic lesions. These aspects of LPS induced damage to the foetal brain have been reviewed [80].

Recent studies have shown that administration of LPS to pregnant rats increases the expression of IL-1β, TNF-α and IL-6 in the foetal brain. The role of erythropoietin (EPO) documented for its effects on repair and maintenance of the nervous system was investigated for its effects on injury of white matter in Sprague-Dawley rats [81]. An LPS suspension of 500 µg/kg was administered to pregnant rats at 18 and 19 days of gestation, while the control group was treated with pyrogen-free saline. They received 5,000 U/kg erythropoietin. Seven day old Sprague-Dawley rat pups were divided into 4 groups consisting of control, LPS-treated group, pre-natal maternal EPO-treated group and post-natal EPO-treated group. Pro-inflammatory cytokine levels were determined by the ELISA method. Levels of IL-1β, TNF-α, and IL-6 were significantly elevated in the LPS treated group compared with controls. Pre-natal maternal EPO treatment significantly reduced levels of TNF-α and IL-6 in the newborn rat brain in response to LPS injection. Post-natal EPO treatment resulted in significant reduction of only IL-6. Using immunohistochemistry techniques, this study demonstrated that staining for myelin basic protein as a marker of myelination was significantly weaker in the LPS treated group when compared with the pre-natal maternal EPO treated group, which was stronger than the post-natal EPO treated group, due to the myelin sparing effect of EPO by reducing the expression of inflammatory cytokines. This is the first study to demonstrate the protective effect of EPO on LPS-induced white matter injury in the developing brain. This agent is more likely to be beneficial in treating LPS-induced brain injury in the perinatal period than in premature newborns as it is often used.

These studies indicate that early intervention in reducing oxidative stress associated with maternal LPS loading could reduce the sequelae of inflammatory pathogenesis leading to adverse foetal outcome. This has implications on effective management of the nidus of inflammation and suggestive of a role for strategic use of adjunctive antioxidants, applicable to periodontal diseases in pregnancy.

OXIDATIVE STRESS AND NEURODEGENERATIVE CHANGES ASSOCIATED WITH AGEING AND DEMENTIA LINKED TO PERIODONTAL DISEASE

There is documentation of the link between tooth loss and dementia [82]. Numerous studies have linked mental deterioration with subsequent worsening of oral health. However oral disease as a risk factor for dementia has received increased prominence. A decreased number of teeth increases the risk of a higher prevalence and incidence of dementia. Edentulism or a few remaining teeth could be a predictor of dementia in later life [83]. Peripheral infection and inflammation can affect the status of the central nervous system. Chronic periodontal disease is associated with elevated serum inflammatory markers such as C-reactive proteins, linked to Alzheimer's disease. The pathogenesis of chronic periodontal disease and the role of inflammation in Alzheimer's disease has been reviewed [84,85]. Being a

modifiable risk factor, management of periodontal disease in curbing the effects of a hyper-inflammatory state, could potentially improve the status of Alzheimer's disease, depending on the extent and severity of the inflammatory pathology.

The superimposition of neurodegenerative diseases such as Alzheimer's or Parkinson's disease on an ageing brain could exacerbate deficiencies in motor, cognitive and behavioural coordination characteristically seen in senescence. Delaying age related mental decline is an essential component of health care in order to minimize health costs. Therapeutic targets for reversing age-related neuronal deficits and their behavioural manifestations would aid healthy ageing. Diets rich in polyphenolic antioxidants and anti-inflammatory agents such as berry fruits, could reduce the risk of neurodegenerative changes [86]. A beneficial effect is seen as a result of their anti-inflammatory and anti-oxidant effects, in addition to altering signal transduction pathways, calcium buffering capacity and neuroprotective stress shock proteins. These actions contribute towards protection against age related changes in cognitive and motor function. The benefits of these interventions and putative molecular mechanisms involved have been investigated and discussed in rodent models. Astrocytes have been shown to be actively engaged in synaptic transmission and neuro-vascular coupling in the central nervous system. Severe oxidative insult results in the formation of mitochondrial ROS (mROS) formation resulting in impaired mitochondrial movement, apoptosis or cell death. Mitochondrial targeted antioxidants such as Mitoquinone (MitoQ), melatonin and others can overcome generation of mROS associated with severe oxidative stress [87]. An adaptive, pre-conditioning firewall-like effect attempts to prevent propagation of mROS. Mitochondria- targeted therapeutic strategies may have applications in the treatment of neoplasias such as astrocytomas, gliomas, neurodegenerative changes associated with astrocytes, mitochondrial diseases and ageing, including other inflammatory diseases.

PERIODONTAL DISEASE AND CANCER

The association between periodontal disease, tooth loss, cardiovascular disease and systemic diseases has been reported in the recent literature; in view of the significant global prevalence of chronic inflammatory periodontal disease amongst adults. This is inclined to be greater in an ageing population, partly due to cumulative effects. Global estimates for the prevalence of severe periodontal disease are in the range of 10-15% Several population studies have reported the prevalence of tooth loss and risk of oral, upper gastrointestinal, lung and pancreatic cancer. These findings have been reviewed recently with a summary of the biological mechanisms involved [88].

Studies indicate that the inflammatory loading resulting from periodontal diseases manifesting as tooth loss may have associations with developing cancer. However smoking could confound these results. A study was conducted to determine cancer risk in male health professionals aged 40-75y [89]. There was a significant association between cancer of the lung, kidney, pancreas, haematological cancers and a history of periodontal disease. There were significant increases in total and haematological cancers in never-smokers, associated with periodontal disease. It is relevant that periodontal disease was associated with a small but significant overall cancer risk which persisted in non-smokers. Periodontal disease may be a useful marker of a susceptible immune system, or directly affect cancer risk as a result of inflammatory loading. Formulation of an effectively targeted package from agents that are active in this context is a therapeutic challenge.

The development of oxidative stress with malignant progression of a tumour has been reported, with evidence for the efficacy of antioxidants as anticancer agents. Previous work established the potency of dehydroascorbic acid in eliminating aggressive mouse sarcomas and carcinomas. It has been shown that dehydroascorbic acid reacts with homocysteine thiolactone found in cancer cells resulting in the formation of the toxic compound 3- mercapto-propionaldehyde which kills cancer cells [90]. The efficacy of dehydroascorbic acid may be increased by combining it with adjuncts such as methotrexate. Other effective agents reported in this context are green tea polyphenols, melatonin and vitamin D, also reported to be beneficial in periodontal disease outcome. A threshold serum level of Vitamin D (75-120 nmol/l) has been shown to improve bone mineral density, oral health, cancer prevention and incident hypertension amongst other effects [91]. A higher intake is required in order to achieve an average desired level of 75nm/l in at least 50% of the population. The implications of higher dosing needs to be investigated in future studies. Green tea polyphenols are able to induce apoptosis in various tumour cell systems. This apoptotic mechanism has been shown to be targeted at mitochondria and executed by caspase 3 [92]. This has been demonstrated by subjecting tumour cells which had either a caspase 3 deletion or expression of wild type caspase 3 to increasing concentrations of green tea polyphenols. There was gradual decline in mitochondrial function, but the caspase 3 null cells did not undergo apoptosis indicating mitochondrial targeting and mediation by caspase 3 for apoptosis.

Nitric oxide (NO) is a short lived biologically active molecule that is produced by a complex family of enzymes called nitric oxide synthase from the amino acid L-arginine. High reactivity of this free radical with transitional metals and thiol residues brings about structural changes in proteins, enzymes and DNA. Elevated levels can be cytotoxic; the levels isolated in many human cancers are likely to facilitate tumour growth and dissemination. The actions of NO and its manipulation for therapeutic advantage have been reviewed recently [93]. Drug resistant cell populations can emerge in response to a milieu of oxidative stress [94]. Cellular adaptation is likely to be multifactorial coordinating factors that induce hypoxia, nuclear factor kappaB (NF-kappaB) and their targets downstream that are linked to resistance mechanisms. This resistance can be overcome by treating the cells with NO mimetic agents to restore their sensitivity to cytotoxic agents both *in vivo* and *in vitro*. Preliminary clinical trials indicate the efficacy of this approach by chemosensitising resistant cells. Some of the mechanisms involved include vascular changes which improve perfusion, tumour oxygenation and antioxidant effects, down regulation of the glutathione detoxification /redox buffering system,

inhibition of key transcription factors and DNA repair systems.

The stilbene compound resveratrol blocks the stages involved in carcinogenesis during tumour initiation and progression. The anti-cancer effects may be linked to its dual pro-oxidant effects depending on its concentration leading to oxidative breakage of DNA in the cells in the presence of metal ions such as copper. This mechanism is thought to be significant in the context of anti-cancer properties of plant polyphenols. The anti-oxidant and pro-oxidant properties of resveratrol and its therapeutic implications have been reviewed recently [95]. It has been shown to exhibit pro-oxidant properties depending on the presence of transitional metal ions such as copper, cell type and the concentration of phytoalexin. This action could contribute to oxidative breakage of cellular DNA and may be utilised in anticancer and chemopreventive applications. The antioxidant and pro-oxidant targets enhance the therapeutic potential of resveratrol, also applicable to periodontal diseases, particularly in smokers [67]. This oestrogenic plant stilbene compound resveratrol has been studied intensively with regard to its anti-inflammatory and anti-cancer properties. Similar potential clinical applications of structurally similar stilbene compounds piceatannol, pinosylvin, rhapontigenin and pterostilbene based on current pharmaceutical research have been reported [96]. Therapeutic applications for natural antioxidants in the management of dermatoses has been reviewed [97]. Dermal cellular DNA, cell membrane lipids and proteins are constantly exposed to oxidative stress as a result of environmental stressors. Topical application of antioxidant formulations could overcome some of these damaging effects. Due to their anti-inflammatory properties, some of the natural antioxidants such as Coffea Arabica, Pinus pinaster (Pycnogenol) could be targeted to treat oxidative damage associated with photoageing and skin cancer.

The role of the oral cavity as a source of oxidative stress is a relevant one, particularly in the context of periodontal diseases. Similarly a significant amount of lipid peroxidation has been demonstrated in breast cancer patients with raised levels of thiobarbituric acid reactive substances (TBARS). This was accompanied by an increase in enzymic and non-enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione S-transferase (GST) in samples from breast cancer patients compared to controls, as a compensatory mechanism [98]. Raised levels of malondealdehyde a marker of oxidative stress have been detected in breast cancer patients, with reduced circulatory levels of the antioxidant vitamins A, E and also Selenium [99]. Improved total antioxidant capacity could help ameliorate the condition in these patients.

The antioxidant melatonin has applications in combating the sequelae of an over-exuberant inflammatory response in periodontal diseases and cancer [100]. Melatonin is formed in the pineal gland and in the gastro-intestinal tract. Being a non-toxic highly lipophilic indole it has effective penetration and activity in stimulating type 1 collagen and bone formation. Its actions encompass therapeutic benefit in a range of conditions including autoimmune disorders,

periodontal diseases and neoplasias where it may be used as an adjunct to conventional therapy. The polyphenolic anti-oxidants have several applications in chronic inflammatory diseases including periodontal diseases and colonic cancer. In a recent investigation utilizing anthocyanin extracts from Cabernet Sauvignon grapes containing delphinidin-, petunidin-, peonidin- and malvidin-3 glucosides it was demonstrated that when they were incubated in porcine gut of freshly slaughtered pigs known to have a similar gut microflora to that of humans, the compounds were converted to gallic acid, syringic acid and trihydroxybenzaldehyde; metabolites responsible for their protective actions against colonic cancer [101]. A recent study has demonstrated that retinol is protective against colorectal cancer [102]. A systemic inflammatory response reduced circulating amounts of lipid soluble antioxidant vitamins and increased lipid peroxidation at an advanced stage of the tumour. Oxidative stress associated with the systemic inflammatory responses and tumour progression has potential for adjunctive therapeutics with antioxidants.

SUMMARY AND CONCLUSIONS

A hyper-inflammatory state seen in severe uncontrolled periodontal diseases could contribute to damage to organs distant from the focus of inflammation resulting in a significant systemic impact. There is rapid advancement in knowledge linking local and systemic inflammation, particularly in the fields of periodontal disease, cardiovascular disease and diabetes and other chronic inflammatory diseases. There is some overlap and commonality in the risk markers for these entities corresponding with surrogate markers associated with clinical end points of treatment. The size of the inflammatory burden would have implications on the impact factor. A common pathogenesis demonstrating established mechanisms for a pro-oxidant state amongst these diseases points to potential therapeutic targets. A cause and effect relationship would depend on the size of the inflammatory burden, genetic susceptibility and other confounding environmental variables. A distinct consensus regarding oxidative stress induced injury associated with periodontal and co-existing diseases, points to the application of antioxidant targets for adjunctive therapeutic measures; to minimize side-effects of conventional therapy and curb the progression of diseases characterized by a hyper-inflammatory state.

ABBREVIATIONS

Aa	=	*Aggregatibacter actinomycetemcomitans*
ADRP	=	Adipose differentiation related protein
AGE	=	Advanced glycaemic end product
CAT	=	Catalase
CD-14	=	Cluster of differentiation (gene)-14
COX	=	Cyclooxygenase
C rectus	=	Campylobacter rectus
CRP	=	Complement reactive protein
DM	=	Diabetes mellitus

DMPO	=	5,5-Demethyl-1-pyrolline-N-oxide
DS-GF	=	Down's syndrome gingival fibroblasts
E coli	=	*Escherichia coli*
EPO	=	Erythropoietin
FA	=	Fatty acids
GCF	=	Gingival crevicular fluid
GPx	=	Glutathione peroxidase
GSH	=	Glutathione
GSSG	=	Glutathione disulphide
GST	=	Glutathione S-transferase
HGF	=	Human gingival fibroblasts
HUVECs	=	Human umbilical vein endothelial cells
ICAM	=	Intercellular adhesion molecule
IFN	=	Interferon
IL	=	Interleukin
i NOS	=	Inducible nitric oxide synthase
JNK	=	c-Jun amino terminal kinase
LDL	=	Low density lipoprotein
LPS	=	Lipopolysaccharide
MAPK	=	Mitogen activated protein kinase
MDA	=	Malondialdehyde
MitoQ	=	Mitoquinone
MMP	=	Matrix metalloproteinase
MPO	=	Myeloperoxidase
mt DNA	=	Mitochondrial DNA
NAC	=	N-acetylcysteine
NF-kappa B	=	Nuclear factor - kappa B
NO	=	Nitric oxide
PARP	=	Poly- (ADP0ribose) polymerase
PCR	=	Polymerase chain reaction method
PDTC	=	Pyrrolidine dithiocarbamate
Pg	=	Porphyromonas gingivalis
PMN	=	Polymorphonuclear leukocytes
RA	=	Rheumatoid arthritis
RANK	=	Receptor activator for nuclear factor kappa
RANKL	=	Receptor activator for nuclear factor kappa ligand
ROS	=	Reactive oxygen species
RT-PCR	=	Reverse transcriptase polymerase chain reaction
SOD	=	Superoxide dismutase
TBARS	=	Thiobarbituric acid reactive substance
TEMPOL	=	4-Hydroxy-2,2,6,6-tetramethypiperidine-N-oxyl
TLR	=	Toll like receptor
TNF	=	Tumour necrosis factor
VEGF	=	Vascular endothelial growth factor

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