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Compromised GCF total antioxidant capacity in periodontitis: cause or effect?

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Abstract

Background: Oxidative stress is implicated in the pathogenesis of periodontitis. The total antioxidant capacity (TAOC) of gingival crevicular fluid volume (GCF) and plasma appears compromised in periodontitis, but it is unclear whether this predisposes to, or results from the inflammatory process.

Aim: To investigate longitudinal changes in GCF and plasma TAOC following reductions in periodontal inflammation with successful non-surgical therapy.

Materials and Methods: Two longitudinal studies were run in series on non-smokers with chronic periodontitis (CP). Study-1 ($n = 17$) assessed index sites with mild disease; Study-2 ($n = 18$) investigated deep sites. GCF sampling and clinical measures were performed at baseline and 3 months post-therapy. Plasma and GCF TAOC was determined by enhanced chemiluminescence and 32 age/sex-matched periodontally healthy controls were used.

Results: Therapy improved clinical outcomes consistent with the literature. There were no differences in plasma TAOC between periodontitis patients ($507 \pm 92\,\mu$MTeq) and controls ($520 \pm 100\,\mu$MTeq; $p = 0.57$) at baseline, but GCF TAOC was lower ($p < 0.0001$) in CP patients ($680 \pm 371\,\mu$MTeq) than controls ($1129 \pm 722\,\mu$MTeq). Successful periodontal therapy did not alter plasma TAOC ($p = 0.56$), but GCF TAOC increased (by $449 \pm 722\,\mu$MTeq, $p < 0.001$) to control subject levels ($p = 0.47$)

Conclusions: Local total antioxidant capacity in CP appears to reflect increased oxygen radical activity during periodontal inflammation and can be restored to control subject levels by successful non-surgical therapy.

Key words: chronic periodontitis; enhanced chemiluminescence; gingival crevicular fluid; non-surgical therapy; plasma; total antioxidant capacity

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Chronic periodontitis (CP) is initiated by the sub-gingival biofilm (Madianos et al. 2005) but the progression of destructive disease appears to be dependent upon an abnormal host response to those organisms (Page & Kornman 1997). The periodontitis phenotype is characterized by hyperinflammation involving excess oxygen radical release by neutrophilic polymorphonuclear leucocytes (Gustafsson & Åsman 1996) and excess release of proteolytic enzymes such as neutrophil elastase (Figueredo et al. 1999). Evidence is emerging to implicate oxidative stress in the pathogenesis of periodontitis (reviewed by Chapple & Matthews 2007). Oxidative stress is a state of altered physiological equilibrium within a cell or tissue/organ, defined as ''a condition arising when there is a serious imbalance between the levels of free radicals in a cell and its antioxidant defences in favour of the former'' (Halliwell & Gutteridge 1989). It is estimated that 1–3 billion reactive species are generated/cell/day, and given this, the importance of the body's antioxidant defence systems to the maintenance of health becomes clear (Ames et al. 1993).

Oxidative stress is implicated in the pathogenesis of several chronic inflammatory diseases associated with periodontitis, such as type-2 diabetes (Evans et al. 2002), rheumatoid arthritis

Conflict of interest and source of funding statement

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(Hadjigogos 2003), vascular disease including stroke (Faraci 2005) and chronic inflammatory lung disease (Rahman et al. 1996). Excess reactive oxygen species (ROS) production by hyper-reactive peripheral blood neutrophils is one potential source of oxidative stress in periodontitis (Gustafsson & Åsman 1996, Sheikhi et al. 2000, Sheikhi et al. 2001), but how such activity translates to neutrophil behaviour within the periodontal tissues remains unclear.

Whatever the complexities involved in excess ROS generation, the study of the body's antioxidant systems is crucial to understanding the mechanisms involved in tissue damage. A number of recent studies have demonstrated increased oxidative stress in periodontitis patients. Sculley & Langley-Evans (2003), in a cohort study of 129 subjects, demonstrated increased levels of saliva protein carbonyls (a stable index of oxidative damage to proteins) in periodontitis patients relative to controls. Increased concentrations of 8-hydroxydeoxyguanosine (8-OHdG), a relatively stable biomarker of DNA damage, were found in the saliva of periodontitis patients, levels decreasing with successful periodontal therapy (Takane et al. 2002, Sugano et al. 2003). More recently, Panjamurthy et al. (2005) demonstrated raised levels of thiobarbituric acid reactive substances (TBARS) in tissue homogenates from gingival biopsies of periodontitis patients. As TBARS are now regarded as a poorly specific marker of lipid peroxidation (Halliwell & Whiteman 2004), it is important to note that malondialdehyde (MDA), a more specific biomarker of lipid peroxidation, was also found to be raised in gingival crevicular fluid (GCF) and saliva of patients compared with controls (Tsai et al. 2005). It seems, therefore, that oxidative stress is a strong feature of periodontitis and the study of antioxidant defence systems therefore becomes important in elucidating mechanisms of tissue damage and potentially new therapeutic strategies.

The scavenging or ''chain breaking'' antioxidants confer substantial protection on vital cell structures, because of their cellular and extracellular ubiquity and rapid rates of sacrificial oxidation (Halliwell & Gutteridge 1990). However, these species act in concert through redox-cycling reactions, regenerating each other from their respective radical species (Chapple 1996). An example is GSH, which will regenerate α-tocopherol and vitamin C from their radicals, preventing further lipid peroxidation and cell membrane damage (Sies & Murphy 1991). For this reason assays of global antioxidant defence were developed in the 1980s and 1990s, which enabled scientists to investigate the capacity of biological systems to withstand oxidative stress. Assays of total antioxidant capacity (TAOC) provide an overview of the biological interactions between individual antioxidant species and how efficiently these translate into host cell protection during periods of oxidative stress (Maxwell et al. 2006). One such system, based upon enhanced chemiluminescence (ECL), was developed in our laboratories and those of our collaborators and is sufficiently sensitive to measure the TAOC of GCF (Chapple et al. 1997).

Most of the relatively few studies investigating TAOC in periodontitis have analysed saliva, because of the larger volumes collectable (Moore et al. 1994, Meucci et al. 1998, Zappacosta et al. 1999, Diab-Ladki et al. 2003, Sculley & Langley-Evans 2003, Buduneli et al. 2006). However, we have previously demonstrated a very different AO profile for GCF than for saliva or plasma compartments (Brock et al. 2004), with GSH predominating in GCF (Chapple et al. 2002) and uric acid predominating in saliva (Moore et al. 1994) and in serum (Maxwell et al. 2006). We also demonstrated in a case–control study (Brock et al. 2004) that GCF TAOC was significantly higher in health than paired serum or plasma samples, whereas GCF TAOC levels were significantly reduced in periodontitis and approached the levels found within saliva and serum/plasma. Whether the total antioxidant compromise in GCF in periodontitis patients was constitutional and predisposed to oxidative stress, or resulted from the inflammatory lesion itself remains unclear. What is clear is that in periodontitis, the preventative antioxidant enzyme systems appear to be upregulated (Panjamurthy et al. 2005, Baltacioğlu et al. 2006) in an apparently protective response to oxidative stress. However, these levels decrease as pocket depth increases (Ellis et al. 1998) and the efficient radical scavengers appear reduced in periodontitis (Chapple et al. 2002, Brock et al. 2004, Pavlica et al. 2004), potentially by sacrificial oxidation.

The aim of the reported longitudinal intervention studies, therefore, was to determine the effect of successful non-surgical periodontal therapy upon plasma and GCF TAOC in non-smokers with chronic periodontitis.

Material and Methods

Study design

Two longitudinal intervention studies were run in series, the only intervention being conventional non-surgical periodontal therapy. The examiner (G.B.) and therapist (N.L.) were the same in both studies. While the baseline data from Study-1 ($n = 17$) formed the basis of a previously reported cross-sectional, case–control analysis (Brock et al. 2004), the data from Study 2 ($n = 18$) have not been previously reported, nor have the longitudinal data from Study 1.

The initial study (Study-1) was based on examination and analysis of samples from index sites (see Clinical Samples), with the patient used as the unit of analysis. It emerged that the mean probing pocket depths were 3.1 ± 0.5 mm at baseline, indicating that the index sites sampled were relatively shallow. On completion of Study 1, and in order to encompass a greater spread of disease severity, a second study (Study 2) was run where sites for clinical assessment and sampling were selected on the basis that they had the deepest pockets. Again, the patient remained the unit of analysis and different control subjects were recruited.

Subjects were recruited to both studies according to the same criteria and from patients referred to the periodontal department of Birmingham's Dental Hospital. CP was defined in both studies as previously described (Brock et al. 2004). Patients had to possess at least two non-adjacent sites on non-incisor/first molar teeth with probing pocket depths (PPDs) of $\geqslant 5$ mm, that bled upon probing (from the pocket base) and which demonstrated radiographic bone loss of $\geqslant 30\%$. Age- and sex-matched control subjects were recruited from staff of the dental hospital and controls took part in the baseline examination only. Control subjects had no evidence of interproximal attachment loss, no PPDs $\geqslant 3$ mm at any sites on any teeth and whole-mouth bleeding scores (from the base of the sulcus) of $< 10\%$.

All subjects were systemically healthy and exclusion criteria included a course of non-steroidal anti-inflammatory drugs or antimicrobial drugs within a 3-month period before the study commencing, pregnancy, use of mouthwashes or vitamin supplements within the previous 3 months. All volunteers had a negative history of current or previous smoking or of recreational drug use and no special dietary requirements.

Ethical approval was granted separately for each study by South Birmingham Local Research Ethics Committee (Study 1-LREC 0405, Study 2-LREC 5643). Informed consent to participate was initially obtained, followed by the completion of a medical questionnaire. Only those volunteers who fulfilled all inclusion and exclusion criteria were formally enroled into the study and studies were run to international good clinical practice standards.

After enrolment, all volunteers were re-appointed for collection of baseline GCF and venous blood samples, which were taken after an overnight fast and before recording clinical measures. Patients received oral hygiene instruction and conventional non-surgical therapy, which was performed under local analgesia on a quadrant by quadrant basis within 2 months. Patients were recalled 3 months post-therapy to re-sample GCF and venous blood and for clinical measures. 3 months were decided upon to allow for initial healing and reduce the risk of re-infection/disease re-activation.

Clinical samples

GCF samples were collected according to standard protocols (Chapple et al. 2002, Brock et al. 2004) from the inter-proximal surfaces of index teeth and subjects were asked to refrain from brushing within 1 h of sampling. In Study 1, a molar, pre-molar and incisor tooth was selected from the right maxillary quadrant in left-handed individuals and from the contra-lateral maxilla in right-handed individuals. In this study, six samples were collected per subject (mesio-buccal and disto-palatal of the three index teeth). Clinical measures (PPD, per cent sites with bleeding and plaque) were recorded immediately afterwards. This was also the protocol in Study 2, but clinical measures were recorded 1 week before GCF sampling of molar, pre-molar or incisor teeth with the deepest pockets. In Study 2, only mesio-buccal sites were sampled and four sites per subject were evaluated.

All GCF and blood samples were collected the morning following an overnight fast and subjects were asked to refrain from drinking (except water) or chewing gum during that period. Compliance with abstention from these activities was checked before sampling. One subject was lost to Study 1 for consuming a cup of tea within 2 h of their appointment. Sites were isolated with cotton rolls and gently air-dried before sampling. Samples were collected over 30 s by insertion of Periopaper™ strips until light resistance was felt and volumes read on a pre-calibrated Periotron 8000™ (Oraflow, Plainview, NY, USA) as previously described (Chapple et al. 1999). Samples were eluted for 30 min. at room temperature into a phosphate-buffered saline (PBS)/bovine serum albumin (BSA) (50 mg/l BSA) buffer at a ratio of six strips to 600 μl (Study 1) or four strips to 400 μl (Study 2) and pooled into cryogenic vials before immersion in liquid nitrogen. GCF samples were stored under liquid nitrogen as previously reported (Chapple et al. 1997, 2002, Brock et al. 2004). Pooled samples were analysed on a subject rather than a site level, given that the subject formed the basis for statistical analyses.

Venous blood samples for plasma were collected from the ante-cubital fossa into Vacutainer™ lithium heparin tubes, and allowed to stand for 30 min. before centrifugation at $1000 \times g$ for. (4°C). Samples were aliquoted into cryogenic vials and stored under liquid nitrogen (Study 1) or at -80°C (Study 2). Previous studies demonstrated that plasma was stable for TAOC measurements under either conditions (Chapple et al. 1997).

Clinical measurements

All clinical measures for both studies were performed by the same individual (GB). PPDs were recorded in duplicate using a Hu-Friedy (UNC-PCP15) probe and where measures differed by >1 mm a third recording was made. The mean pocket depth per site was calculated from the closest two of triplicate probing measures. Marginal bleeding on probing was recorded as percentage positive sites of whole-mouth measures and plaque presence was also assessed dichotomously in the same manner after disclosing. Clinical data were double entered into a spreadsheet (Microsoft excel, Windows XP) and means of subject means were calculated as the ''per patient'' value for subsequent statistical analysis. GCF volumes were calculated on a per-site basis and volumes derived from a Periotron 8000™ calibrated with 1:5 diluted serum (in PBS) as previously reported (Chapple et al. 1999). GCF volumes were added for each subject to obtain a total volume collected over 30 s from which antioxidants had been sampled.

TAOC assay

The ECL assay of TAOC has been previously reported and widely validated by our group (Whitehead et al. 1992, Chapple et al. 1997, Maxwell et al. 2006). Briefly, it relies upon the total and reversible inhibition of hydrogen peroxide-mediated luminol oxidation, by antioxidant scavengers present within the test solution. The reaction is catalysed by horse-radish peroxidase and utilizes an enhancer (ρ-iodophenol-sodium salt) to prolong and intensify the resulting luminescent signal. Light emission is measured in arbitrary units and time (min.) and the assay is calibrated on the day of sample analysis using a water-soluble vitamin-E analogue (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid-Trolox™). Standard curves are constructed using a series of 20–80 μM Trolox standards, run (in triplicate) before, and on completion of assays of clinical samples from patients and their matched controls (at the same time). Samples were defrosted and assayed immediately and plasma was diluted before assay (1:10) with PBS containing 50 mg/l BSA. Twenty microlitres volumes are used routinely for plasma assays and also for Trolox standards. Hundred microlitre volumes of GCF eluates were used without dilution and analysed in triplicate in combination with matched volumes of Trolox standards for assay calibration. Plasma TAOC was expressed as (μM) Trolox equivalents (μMTeq) and GCF TAOC was expressed both as μmTeq/30 s GCF sample and also as μMTeq, to account for the influence of GCF volume upon derived TAOC values.

Data handling and statistical analysis

The primary outcome measures were change (Δ) in GCF TOAC (μMTeq)

and Δ plasma TAOC (μMTeq). Secondary outcome measures were Δ GCF TAOC/30-s sample (μMTeq/30 s) and Δ GCF volume. A post hoc power calculation on the primary outcome measure (Δ GCF TAOC) from Study 1 ($n = 17$) demonstrated a significant difference of 1.6-fold detected at the $p < 0.05$ level, with a power of 0.79. Based on this finding, 18 patients were recruited for Study 2. Post hoc analysis demonstrated that this study had a power of 0.93 to detect differences in GCF TAOC at the $p < 0.05$ level.

Data were recorded manually into individual case record folders (CRFs) before double entry into a Microsoft Excel (windows XP) spreadsheet. Between-group data (periodontitis *versus* control & Study 1 *versus* 2) were analysed by one-way analysis of variance, and changes in outcome measures (pre- *versus* post-therapy) were assessed by a paired *t*-test. A significance level of $p < 0.05$ was used for assigning statistical significance.

Results

Demographics

In both studies, two patients did not meet the inclusion criteria and were therefore not enroled. A further patient in Study 1 was eliminated because they consumed a drink of tea within 2 h of GCF sampling. Seventeen patients exited Study 1, with a mean age of 41.11 ± 14 years (range 32–60 years); seven were male and nine female. In Study 2, 18 subjects completed the study, with a mean age of 47.11 ± 6.4 years (36–61 years); four were male and 14 female. Data were therefore available for 35 periodontitis patients with mild to moderate disease and 32 age- and sex-matched controls.

Cross-sectional analysis at baseline

Whole mouth mean probing pocket depths (PPDs) were higher in Study 2 than Study 1 (Table 1) at baseline ($p < 0.002$), and post-therapy ($p < 0.0001$). There were more sites exhibiting bleeding on probing (% BOP) in Study 2 patients than Study 1 patients, both pre- and post-therapy ($p < 0.0001$). GCF volumes were also higher in Study 2 than Study 1 at baseline ($p = 0.008$), whereas no differences were detected post-therapy ($p = 0.44$). There were no differences in plasma or GCF TAOC (μMTeq) between Study 1 or Study 2 pre- or post-therapy ($p = 0.14$) and these data were therefore pooled for graphical representation and for comparison with control groups. Baseline GCF TAOC/30 s samples were higher in Study 2 than Study 1 ($p < 0.001$), possibly due to the higher GCF volumes collected in study-2, but no differences were recorded post-therapy ($p = 0.09$) when GCF volumes were very similar between studies.

Pooled data from studies 1 and 2 demonstrated no differences in plasma TAOC between patients (507 ± 92 μMTeq) and controls (520 ± 100 μMTeq; $p = 0.57$) at baseline or indeed post-therapy (patients 515 μMTeq; $p = 0.85$), despite a marginally significant difference between Study 1 patients and controls (controls higher than patients, $p = 0.05$) at baseline. However, GCF TAOC (μMTeq) was significantly lower in patients ($n = 35$, 680 ± 371 μMTeq) relative to controls ($n = 32$, 1129 ± 722 μMTeq) at baseline ($p < 0.0001$). The GCF TAOC/30 s sample was no different between patients (2.1 ± 1.2 μMTeq) and controls (2.02 ± 1.0 μMTeq; $p = 0.7$).

Longitudinal changes in clinical outcomes

Reductions in PPD following non-surgical therapy were significant ($p < 0.00001$) in both studies, but the magnitude of change (Δ PPD) did not differ between studies 1 and 2 (1.1 ± 0.4 mm Study 1, 1.0 ± 0.5 mm Study 2; $p = 0.6$). Statistically significant reductions in per cent sites BOP were higher in Study 2 (51 ± 15%, $p < 0.00001$) than Study 1 (18 ± 6%, $p < 0.00001$: difference between studies = $p < 0.00001$). GCF volume changes followed a trend similar to per cent sites BOP with significant reductions in both studies (Study 1 Δ = 0.12 ± 0.13 μl, $p < 0.000001$; Study 2 Δ = 0.24 ± 0.11 μl, $p < 0.00001$). The reductions in GCF volume in Study 2 were greater than Study 1 ($p = 0.03$). When GCF volume data were pooled, volumes from patients (0.36 ± 0.2 μl) were higher than controls (0.17 ± 0.06 μl; $p < 0.00001$) pre-treatment, but post-therapy GCF volumes were the same as control patient levels (0.15 ± 0.2 μl; $p = 0.09$). In summary, clinical improvements in PPD and GCF volume with conventional non-surgical periodontal therapy were consistent with those reported in the wider literature.

Table 1. Clinical and biochemical data for Study 1 ($n = 17$) and Study 2 ($n = 18$). Means ± SD (range)

	Study 1 ($n = 17$)			Study 2 ($n = 18$)		
	pre-therapy	post-therapy	controls	pre-therapy	post-therapy	controls
Probing pocket depths (mm)	3.1 ± 0.5 (2.2–4.1)	2.1 ± 0.2 (1.7–2.4)		3.6 ± 0.5 (3–4.8)	2.6 ± 0.2 (2.3–3.1)	
% sites bleeding on probing	22.5 ± 7.3 (9–37)	4.2 ± 3.8 (0–16)		65 ± 13 (39–91)	14 ± 8 (5–40)	
Plasma [TAOC] (μMTeq)	483 ± 111 (362–765)	489 ± 119 (341–728)	545 ± 102(393–705)	529 ± 66 (424–653)	539 ± 132 (360–811)	497 ± 96 (380–738)
GCF volume (μl)	0.27 ± 0.12 (0.12–0.59)	0.19 ± 0.14 (0.07–0.65)	0.14 ± 0.03(0.10–0.19)	0.44 ± 0.22 (0.23–1.1)	0.23 ± 0.09 (0.08–0.38)	0.20 ± 0.07 (0.13–0.34)
GCF [TAOC] (μMTeq)	632 ± 343 (274–1443)	1015 ± 549 (430–2970)	1287 ± 696(692–3706)	730 ± 103 (207–1613)	1249 ± 872 (356–3750)	1220 ± 708 (387–2413)
GCF TAOC/30 s sample	1.4 ± 0.6 (0.7–3.0)	1.7 ± 0.7 (0.8–3.3)	1.75 ± 0.8(1.1–4.6)	2.8 ± 1.3 (0.9–6.7)	2.3 ± 1.4 (0.8–5.3)	2.3 ± 1.1 (0.7–4.6)

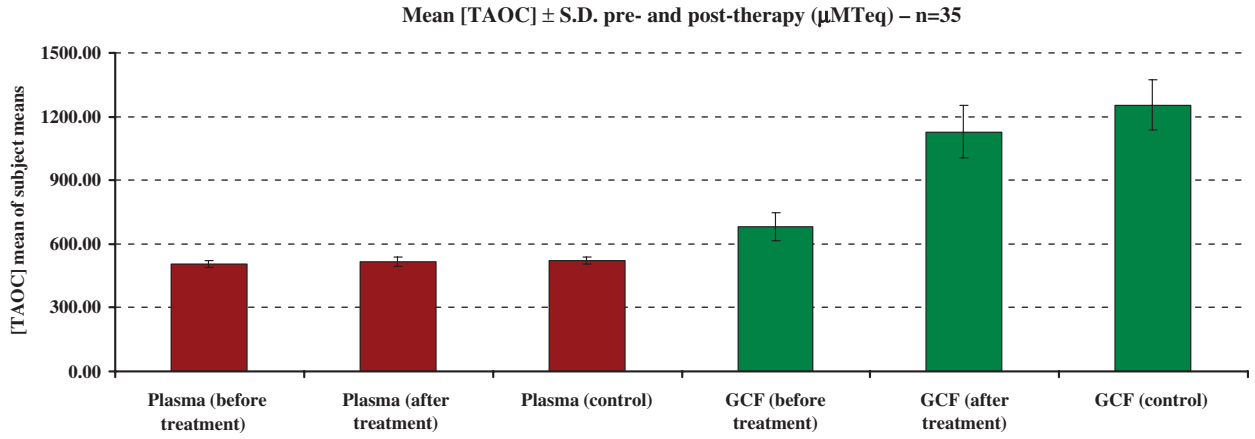

Fig. 1. Mean total antioxidant capacity (TAOC) ± standard deviation pre- and post-therapy (μMTeq) – $n = 35$.

Longitudinal changes in plasma and GCF total antioxidant capacity

Figure 1 illustrates GCF and plasma TAOC (μMTeq) pre- and post-therapy relative to periodontally healthy controls for all subjects. Plasma TAOC did not change with successful therapy (ΔTAOC $= 8 \pm 80\,\mu$MTeq, $p = 0.56$), whereas GCF TAOC increased significantly (by $449 \pm 722\,\mu$MTeq, $p < 0.001$) to reach control patient levels ($p = 0.47$). Interestingly, the differences between GCF and plasma TAOC (μMTeq) were highly significant in patients post-therapy and in controls ($p < 0.00001$), but less so for pre-therapy GCF TAOC relative to respective plasma levels ($p = 0.02$). GCF TAOC per 30-s sample did not alter with successful therapy ($p = 0.55$) from pre-therapy levels despite the significant decreases in GCF volume with treatment.

Influence of gender

Plasma TAOC was higher in males than females both pre- and post-therapy ($p < 0.0001$) as illustrated in Fig. 2, a finding consistent with the existing literature (Maxwell et al. 2006). Interestingly, these gender differences were not evident for GCF TAOC in patients pre-treatment ($p = 0.21$), post-treatment ($p = 0.4$) or in controls ($p = 0.4$).

Discussion

This is the first study to investigate how peripheral (plasma) and local (GCF) total antioxidant capacity is influenced by reductions in periodontal inflammation following successful non-surgical therapy. It is clear that GCF TAOC is compromised in periodontitis; however, the increases recorded in this study, following reductions in disease activity and inflammation, reached control subject levels, suggesting that the total antioxidant compromise may result from the inflammatory lesion, rather than predisposing to it. It is important, however, to interpret data on global antioxidant activity with care, because the balance of individual and often highly influential antioxidants may in fact not be altered by treatment. Some such species (e.g. reduced glutathione, GSH) can also be influential in the regulation of redox-sensitive transcription factors and subsequent inflammation (Chapple 1997, Chapple et al. 2002). Without data on dynamic changes in individual antioxidant concentrations following successful therapy, it would be dangerous to assume that there is no constitutional basis for deficiencies in individual antioxidant species. GSH deficiencies have been demonstrated in inflammatory lung diseases (Rahman & MacNee 1999), rheumatoid arthritis (Hassan et al. 2001) and other free-radical mediated pathologies such as diabetic neuropathy (Bravenboer et al. 1992) as well as in CP (Chapple et al. 2002), and their investigation in longitudinal intervention studies is warranted.

There is a potential dilemma when attempting to analyse the effects of oxidative stress on tissue damage, specifically in deciding whether to analyse individual antioxidant concentrations, or global antioxidant defence. While specific antioxidant species may be of importance to a given pathogenic process, their analysis in isolation from other antioxidant systems can provide misleading information. Assays of TAOC have the advantage that they analyse the combined effectiveness of contributing species, which may be greater than the sum of the effects of the individual antioxidants. They also account for the influence of antioxidants that are as yet undiscovered or difficult to assay, and the assays themselves are more efficient, cheaper and less time consuming than performing large numbers of individual assays (Maxwell et al. 2006). However, the disadvantage of using assays of TAOC is that they differ in their sensitivities to individual antioxidant species and therefore may not reflect the activity of an important antioxidant for a particular biological system or disease process, for example the ECL assay utilized in the current study is poorly sensitive to GSH (Maxwell et al. 2006).

The strongest predictor of serum TAOC in the ECL assay utilized in this study has been shown to be uric acid, followed by vitamins A, C and E (Maxwell et al. 2006), proteins having a low influence on derived TAOC. It is also important to recognize that different compartments of the body differ in their antioxidant profile and in the contribution of individual antioxidants to tissue homeostasis and stability. Brock et al. (2004) pointed out that the most influential antioxidants in GCF were not the same as for serum or saliva, with uric acid predominating in the latter (Moore et al. 1994, Halliwell 1996, Maxwell et al. 2006) and reduced glutathione predominating in the former (Chapple et al. 2002).

Our data show that GCF TAOC (μMTeq) is significantly reduced in periodontitis patients relative to unaf-

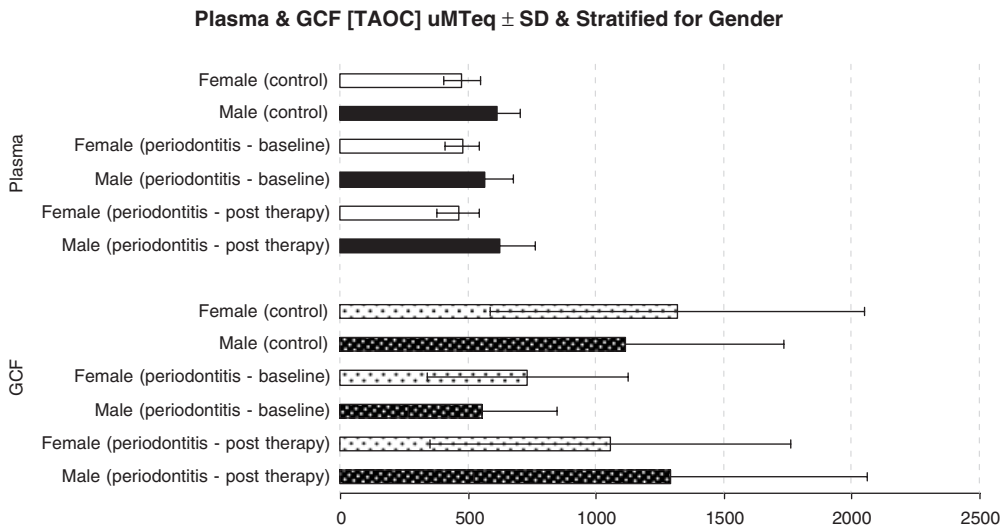

Fig. 2. Plasma and gingival crevicular fluid total antioxidant capacity (TAOC) uMTeq ± standard deviation and stratified for gender.

fected controls, but plasma levels do not differ. The resolution of inflammation with successful non-surgical periodontal treatment resulted in an increase in GCF TAOC reaching the levels found in control subjects. This suggests that the oxidative stress that characterizes periodontitis (Takane et al. 2002, Sculley & Langley-Evans 2003, Sugano et al. 2003, Panjamurthy et al. 2005) causes a reduction in the radical scavenging antioxidant species, most influential on the ECL TAOC assay, and that removal of the oxidative stress restores a normal TAOC. However, this does not exclude the possibility that other key antioxidant species may be constitutionally lowered in periodontal tissues and, as such, their deficiency may predispose to oxidative stress and may be unaffected by periodontal therapy. However, such antioxidants are clearly not major components of GCF TAOC as measured using the ECL assay. Moreover, these data do not eliminate the possibility that increasing tissue concentrations of certain antioxidants of strategic importance to the periodontium may offer protection against the oxidative stress that characterizes periodontal hyper-inflammation and that appears to underpin aspects of tissue destruction.

Our baseline plasma data are in contrast to that of Baltacioğlu et al. (2006), who investigated the TOAC of serum and GCF in a cohort of Turkish pre- and post-menopausal women with and without periodontitis. They found significantly lower serum and GCF TAOC in periodontitis patients, irrespective of menopause. There are several explanations for the differences. Firstly, Baltacioğlu et al. used a novel automated TAOC assay for which they did not describe the assay's sensitivity to individual antioxidant species. It is likely that the contribution of individual antioxidants to the overall balance of TAOC differed from that of the ECL assay reported in the current study. Second, their patients were all female and gender differences in serum TAOC are significant (Brock et al. 2004, Maxwell et al. 2006) because of the higher concentrations of urate in male sera; this was confirmed again by the gender data in this current study. Third, the serum TAOC ($720 \pm 210\ \mu$MTeq) of the periodontally healthy pre-menopausal patients in Baltacioğlu et al's study were higher than previously reported (Whitehead et al. 1992, Maxwell et al. 2006). Given the differing lifestyles of the Turkish women, with traditional diets high in fish oils, the non-periodontitis controls in their study may have had unusually high serum TAOC compared with other European groups.

Smokers were specifically excluded from our current studies, because of the lack of available data on the effects of smoking on GCF TAOC. While the negative impacts of smoking upon peripheral and respiratory antioxidant status (Rahman et al. 1996) and lung glutathione levels (Rahman & MacNee 1999) are widely recognized, data on periodontitis are limited and conflicting. Zappacosta et al. (1999) demonstrated that the smoking of a single cigarette was capable of inducing a significant reduction in salivary glutathione concentration and a dose-dependent reduction of periodontal ligament GSH was also reported in smokers (Chang et al. 2003). Levels of the free radical-induced DNA oxidation product 8-hydroxydeoxyguanosine excreted in the urine are also known to be related to smoking and gender (Loft et al. 1992). Buduneli et al. (2006) recently reported no differences in the TAOC of saliva in smokers and non-smokers, while Nagler et al. (2000) have demonstrated that the in vitro exposure of saliva to smoke reduces salivary GSH levels. Saliva is not the ideal medium for examining the TAOC of periodontitis subjects, given its demonstrably different antioxidant profile from GCF (Brock et al. 2004); however, there are no data concerning the impact of smoking on GCF TAOC in the literature and such studies are required.

Only four studies, to our knowledge, have investigated TAOC in serum/plasma from periodontitis patients and controls and one of those was in dogs (Pavlica et al. 2004). All demonstrated significantly lower TAOC in serum and plasma samples from periodontitis subjects (Chapple et al. 2002, Brock et al. 2004, Pavlica et al. 2004, Baltacioğlu et al. 2006). However, all studies involved relatively small numbers of patients in each group and may have been underpowered. Brock et al. (2004) found that the reduced serum TAOC concentration in periodontitis did not quite reach statistical significance, whereas differences in plasma levels did, possibly reflecting differences in the handling of serum and plasma before assay (serum is prepared

at higher centrifugal forces and is more prone to oxidation), effects of clotting factor removal or presence of anticoagulant. Interestingly, the TAOC of plasma was significantly lower than that of serum in this study. Panjamurthy et al. (2005) found lower plasma vitamin C, vitamin E and GSH in periodontitis patients even after adjusting for protein levels, whereas AO-enzyme levels were raised, the authors attributing this to a protective response to oxidative stress.

Overall, the balance of evidence supports a mild antioxidant compromise in the plasma of periodontitis patients, which may or may not reach statistical significance, depending upon the patient group (gender and habitat) and method of TAOC determination. Such minor changes in plasma/serum TAOC are likely to lack relevance or clinical significance, given their low concentrations relative to those of GCF in health, at least in non-smokers. However, data are needed on serum/plasma TAOC in smokers with periodontitis as well as on GCF TAOC.

Studies that have investigated GCF TAOC (Brock et al. 2004, Pavlica et al. 2004, Baltacioğlu et al. 2006) have been consistent in their findings, which, in agreement with the current study, demonstrate significantly reduced GCF TAOC in periodontitis patients. The current study has demonstrated that global antioxidant defences measured using an ECL assay appear to increase to control subject levels when the inflammation within the tissues is reduced by successful conventional periodontal therapy. The data suggest that the antioxidant compromise may result from the periodontal inflammation. However, such an increase in oxidative stress may prolong the inflammatory response by activating redox-sensitive transcription factors like NFκB and AP-1, thereby providing a rationale for antioxidant therapy in the prevention of disease progression.

In conclusion, the data presented in the current studies indicate that the reduced TAOC in periodontitis is likely to result from the inflammatory lesion, rather than predisposing to it. However, these data do not exclude the possibility that constitutional deficiencies in individual antioxidant species, known to influence pro-inflammation via redox-regulated gene transcription factors, may underpin tissue damage in periodontitis, or that boosting tissue levels of specific antioxidants may afford protection against the tissue damage mediated by oxidative stress.

References

Ames, B. N., Shigenaga, M. K. & Hagan, T. M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Science USA* **90**, 7915–7922.

Baltacioğlu, E., Akalin, F. A., Alver, A., Balaban, F., Ünsal, M. & Karabulut, E. (2006) Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. *Journal of Clinical Periodontology* **33**, 385–392.

Bravenboer, B., Kapelle, A. C., Hamers, F. P., Van Buren, T., Erkelens, D. W. & Gispen, W. H. (1992) Potential use of glutathione for the prevention and treatment of diabetic neuropathy in the streptozosin-induced diabetic rat. *Diabetalogica* **35**, 813–817.

Brock, G. R., Matthews, J. B., Butterworth, C. J. & Chapple, I. L. C. (2004) Local and systemic antioxidant capacity in periodontitis health. *Journal of Clinical Periodontology* **31**, 515–521.

Buduneli, N., Kardeşler, H., Işik, H., Steadman Willis III, C., Kinane, D. F. & Scott, D. A. (2006) Effects of smoking and gingival inflammation on salivary antioxidant capacity. *Journal of Clinical Periodontology* **33**, 159–164.

Chang, Y. C., Hsieh, Y. S., Lii, C. K., Huang, F. M. & Chou, M. Y. (2003) Induction of c-fos expression by nicotine in human periodontal ligament fibroblasts is related to cellular thiol levels. *Journal of Periodontal Research* **38**, 44–50.

Chapple, I. L. C. (1996) Role of free radicals and antioxidants in the pathogenesis of the inflammatory periodontal diseases. *Journal of Clinical Pathology: Molecular Pathology* **49**, M247–M255.

Chapple, I. L. C. (1997) Reactive oxygen species and antioxidants in inflammatory diseases, *Journal of Clinical Periodontology*, **24**, 287–296.

Chapple, I. L. C., Brock, G., Eftimiadi, C. & Matthews, J. B. (2002) Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. *Molecular Pathology* **55**, 367–373.

Chapple, I. L. C., Landini, G., Griffiths, G. S., Patel, N. C. & Ward, R. S. N. (1999) Calibration of the Periotron 8000® and 6000® by polynomial regression. *Journal of Periodontal Research* **34**, 79–86.

Chapple, I. L. C., Mason, G. M., Matthews, J. B., Thorpe, G. H. G., Maxwell, S. R. J. & Whitehead, T. (1997) Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Annals of Clinical Biochemistry* **34**, 412–421.

Chapple, I. L. C. & Matthews, J. B. (2007) The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology 2000* **43**, 161–233.

Diab-Ladki, R., Pellat, B. & Chahine, R. (2003) Decrease in the total antioxidant activity of saliva in patients with periodontal disease. *Clinical Oral Investigations* **7**, 103–107.

Ellis, S. D., Tucci, M. A., Serio, F. G. & Johnson, R.B (1998) Factors for progression of periodontal diseases. *Journal of Oral Pathology and Medicine* **27**, 101–105.

Evans, J. L., Goldfine, I. D., Maddux, B. A. & Grodsky, G. M. (2002) Oxidative stress and stress-activated signalling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine Reviews* **23**, 599–622.

Faraci, F. M. (2005) Oxidative stress: the curse that underlies cerebral vascular dysfunction? *Stroke* **36**, 186–188.

Figueredo, C. M., Gustafsson, A., Asman, B. & Bergstrom, K. (1999) Increased release of elastase from in vitro activated peripheral neutrophils in patients with adult periodontitis. *Journal of Clinical Periodontology* **26**, 206–211.

Gustafsson, A. & Åsman, B. (1996) Increased release of free oxygen radicals from peripheral neutrophils in adult periodontitis after Fcγ-receptor stimulation. *Journal of Clinical Periodontology* **23**, 38–44.

Hadjigogos, K. (2003) The role of free radicals in the pathogenesis of rheumatoid arthritis. *Panminerva Medica* **45**, 7–13.

Halliwell, B. (1996) Uric acid: an example of antioxidant evaluation. In: Cadenas, E. & Packer, L. (eds). *Handbook of Antioxidants*, pp. 243–256. New York: Marcel Dekker Inc.

Halliwell, B. & Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine*, 2nd edition. Oxford, UK: Oxford University Press.

Halliwell, B. & Gutteridge, J. M. C. (1990) The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics* **280**, 1–8.

Halliwell, B. & Whiteman, M. (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology* **142**, 231–255.

Hassan, M. Q., Hadi, R. A., Al-Rawi, Z. S., Padron, V. A. & Stohs, S. J. (2001) The glutathione defence system in the pathogenesis of rheumatoid arthritis. *Journal of Applied Toxicology* **21**, 69–73.

Loft, S., Vistisen, K., Ewertz, M., Tjonneland, A., Overvad, K. & Poulsen, H. E. (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Cancinogenesis* **13**, 2241–2247.

Madianos, P. N., Bobetsis, Y. A. & Kinane, D. F. (2005) Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. *Journal of Clinical Periodontology* **32** (Suppl. 6), 57–71.

Maxwell, S. R. J., Dietrich, T. & Chapple, I. L. C. (2006) Prediction of serum total antioxidant activity from the concentration of individual serum antioxidants. *Clinica Chimica Acta* **372**, 188–194.

Meucci, E., Littaru, C., Deli, G., Luciani, G., Tazza, L. & Littaru, G. P. (1998) Antioxidant status and dialysis: plasma and saliva antioxidant activity in patients fluctuating urate levels. *Free Radical Ressearch* **29**, 367–376.

Moore, S., Calder, K. A. C., Miller, N. J. & Rice-Evans, A. (1994) Antioxidant activity of saliva and periodontal disease. *Free Radical Research* **21**, 417–425.

Nagler, R., Lischinsky, S., Diamond, E., Drigues, N., Klein, I. & Reznick, A. Z. (2000) Effect of cigarette smoke on salivary proteins and enzyme activities. *Archives of Biochemistry and Biophysics* **15**, 229–236.

Page, R. C. & Kornman, K. (1997) The pathogenesis of human periodontitis: an introduction. *Periodontology 2000* **14**, 9–11.

Panjamurthy, K., Manoharan, S. & Ramachandran, C. R. (2005) Lipid peroxidation and antioxidant status in patients with periodontitis. *Cellular and Molecular Biology Letters* **10**, 255–264.

Pavlica, Z., Petelin, M., Nemec, A., Erzen, D. & Skaleric, U. (2004) Measurement of total antioxidant capacity in gingival crevicular fluid and serum in dogs with periodontal disease. *American Journal of Veterinary Research* **65**, 1584–1588.

Rahman, I., Morrison, D., Donaldson, K. & MacNee, W. (1996) Systemic oxidative stress in asthma. COPD and smokers. *American Journal of Respiratory and Critical Care Medicine* **154**, 1055–1060.

Rahman, I. & MacNee, W. (1999) Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. *American Journal of Physiology (Lung Cellular and Molecular Physiology)* **277**, L1067–L1088.

Sculley, D. V. & Langley-Evans, S. C. (2003) Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clinical Science* **105**, 167–172.

Sheikhi, M., Bouhafs, R. K. L., Hammarstrom, K-J. & Jarstrand, C. (2001) Lipid peroxidation caused by oxygen radicals from *Fusobacterium*-stimulated neutrophls as a possible model for the emergence of periodontitis. *Oral Diseases* **7**, 41–46.

Sheikhi, M., Gustafsson, A. & Jarstrand, C. (2000) Cytokine, elastase and oxygen radical release by *Fusobacterium nucleatum*-activated leukocytes: a possible pathogenic factor in periodontitis. *Journal of Clinical Periodontology* **27**, 758–762.

Sies, H. & Murphy, M. E. (1991) Role of tocopherols in the protection of biological systems against oxidative damage. *Journal of Photochemistry and Photobiology.B, Biology* **8**, 211–224.

Sugano, N., Yokoyama, K., Oshikawa, M., Kumagai, K., Takane, M. & Ito, K. (2003) Detection of streptococcus anginosus and 8-hydroxydeoxyguanosine in saliva. *Journal of Oral Science* **45**, 181–184.

Takane, M., Sugano, N., Iwasaki, H., Iwano, Y. & Shimizu, N. (2002) New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *Journal of Periodontology* **73**, 551–554.

Tsai, C. C., Chen, H. S., Chen, S. L., Ho, Y. P., Ho, K. Y., Wu, Y. M. & Hung, C. C. (2005) Lipid peroxidation: a possible role in the induction and progression of chronic periodontitis. *Journal of Periodontal Research* **40**, 378–384.

Whitehead, T. P., Thorpe, G. H. G. & Maxwell, S. R. J. (1992) An enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Analytica Chimica Acta* **266**, 265–277.

Zappacosta, B., Persichilli, S., DeSole, P., Mordente, A. & Giardina, B. (1999) Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. *Archives of Oral Biology* **4**, 485–488.

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Clinical Relevance

Scientific rationale for study: Oxidative stress and antioxidant depletion are important components of the pathogenic processes of periodontitis. It is unclear whether reported depletions in TAOC of periodontitis patients predispose to, or result from such oxidative stress. Understanding this relationship requires intervention studies rather than case–control or association studies.

Principal findings: Plasma TAOC was unaffected by successful non-surgical periodontal therapy, but GCF TAOC increased in patients post-therapy, to levels found in control subjects.

Practical implications: The data suggest that total antioxidant depletion results from oxidative stress within the inflammatory lesion and therapeutic strategies may need to focus on disease prevention rather than adjunctive therapy.