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Oxidative stress parameters in different systemic rheumatic diseases

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Abstract

The involvement of oxidative stress in the pathogenesis of rheumatic disorders, such as systemic sclerosis (SSc) and chronic polyarthritides, has been suggested yet not thoroughly verified experimentally. We analysed 4 plasmatic parameters of oxidative stress in patients with SSc ($n = 17$), psoriatic arthritis (PsA) ($n = 10$) and rheumatoid arthritis (RA) ($n = 9$) compared with healthy subjects ($n = 22$). The biomarkers were: total antioxidant capacity (TAC) measured by ferric reducing antioxidant power (FRAP) method, hydroperoxides determined by ferrous ion oxidation in presence of xylenol orange (FOX) method and sulfhydryl and carbonyl groups assessed by spectrophotometric assays. The results showed significantly increased hydroperoxides in SSc, PsA and RA (3.97 ± 2.25, 4.87 ± 2.18 and $5.13 \pm 2.36\,\mu mol\,L^{-1}$, respectively) compared with the control group ($2.31 \pm 1.40\,\mu mol\,L^{-1}$; $P < 0.05$). Sulfhydryls were significantly lower in SSc ($0.466 \pm 0.081\,mmol\,L^{-1}$), PsA ($0.477 \pm 0.059\,mmol\,L^{-1}$) and RA ($0.439 \pm 0.065\,mmol\,L^{-1}$) compared with the control group ($0.547 \pm 0.066\,mmol\,L^{-1}$; $P < 0.05$). TAC in all three diseases showed no difference in comparison with controls. Carbonyls were significantly higher in RA than in the control group (32.1 ± 42 vs $2.21 \pm 1.0\,nmol\,(mg\ protein)^{-1}$; $P < 0.05$). The obtained data indicate augmented free radical-mediated injury in these rheumatic diseases and suggest a role for the use of antioxidants in prevention and treatment of these pathologies.

Introduction

The involvement of oxidative (oxidant) stress has been proposed in the destruction of cartilage and connective tissue occurring in rheumatic disorders, such as systemic sclerosis and chronic polyarthritis. Systemic sclerosis is a generalized multisystemic disorder characterized by microvascular damage eventually leading to tissue fibrosis accompanied by a range of severe complications. Its aetiology and pathogenesis has not been fully understood, yet generally it is believed that the causes are rather complex. The vascular involvement has been the pivotal aspect under investigation (Kahaleh 2004). There are many factors contributing to the inflammation and derangement of the vascular endothelium, mainly by alteration of immune processes or changes in the coagulative/fibrinolytic system (Simonini et al 1999). Oxidative stress has been proposed as one of the possible factors (Murrell 1993). The way the reactive oxygen and nitrogen species (RONS) can contribute to the pathogenesis of vascular disease is very extensive. They may damage endothelial cell function directly by chemical modification of macromolecules via peroxidation of lipids and oxidation of proteins or by activating various pro-inflammatory cytokines, which further initiate cascades of processes leading to activation of immune cells (Herrick & Matucci Cerinic 2001). Furthermore, RONS may well directly alter DNA and proteins from apoptotic cells and thus contribute to the development of autoimmune responses (Ahsan et al 2003).

Raynaud's phenomenon, present in most patients, is very often the first symptom to occur, usually preceding the development of systemic sclerosis by months or even years. Raynaud's phenomenon provokes frequent episodes of hypoxia–reperfusion, thus producing RONS responsible for endothelial injury. However, Raynaud's

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phenomenon and related events are not the only possible source of free radicals in the microvasculature. Other factors include activated polymorphonuclear cells, which can produce high amounts of RONS along with proteases (Lau et al 1992a, b), and also inducible nitric oxide (NO) synthase, which can generate pathological excess of NO (Matucci Cerinic & Kahaleh 2002). These early events are often followed by small vessel structural changes and ischaemia. The birth of a vicious circle of RONS generation along with related inflammatory processes leads to further endothelial damage, obliteration of microvasculature and fibrosis (Murrell 1993; Kahaleh 2004). Yet since only a small percentage of patients with primary Raynaud's phenomenon eventually develop systemic sclerosis, there must be, besides increased RONS formation, apparently some concurrent factors present. It is probably an abnormal susceptibility to oxidative damage mostly due to a deteriorated antioxidant defence system.

The involvement of oxidative stress in the pathogenesis of chronic polyarthritis, such as rheumatoid arthritis and psoriatic arthritis, has also been proposed (Halliwell 1995). Epidemiological studies have shown that rheumatoid arthritis occurs in previously healthy subjects with low levels of circulating antioxidants and, once established, is characterized by reactive oxygen products within affected joints. Besides, the local production of hydroxyl radicals seems to modify the structure of human IgG, thereby increasing the production of rheumatoid factors, the biological marker of rheumatoid arthritis. Along with ischaemia–reperfusion injury during joint movement, the affection of inflamed synovium by RONS is attributed mainly to activated neutrophils. These are, like in systemic sclerosis, kept at inflammatory sites by repeated formation of various chemotactic molecules, including cytokines, adhesion molecules, etc. Consequently, generated RONS cannot be effectively scavenged due to alterations in physiological antioxidant defences (Halliwell 1995; Taysi et al 2002).

The extent of free-radical-mediated injury is reflected in increased levels of different products of oxidative reactions. Since RONS can attack all biological macromolecules, there has been a wide range of assays determining various oxidative stress parameters in-vivo. The most sensitive targets for attacks of RONS are unsaturated fatty acids of cellular lipid membranes. This process yields many products, including hydroperoxides, conjugated dienes, aldehydes, F_2-isoprostanes, etc. Free-radical-mediated oxidation of proteins may similarly lead to many end-products.

The role of free radicals has been extensively studied in the pathogenesis of some diseases but less is known about their role in rheumatic diseases. The objective of this study was to assess four different parameters reflecting oxidative stress status changes in plasma and compare them with healthy controls, and thus help to clarify the hypothesis of oxidative stress involvement in the pathogenesis of systemic sclerosis, rheumatoid arthritis and psoriatic arthritis.

Materials and Methods

Patients

Thirty-six patients with rheumatic disorders were studied, from which 17 suffered from systemic sclerosis, 9 from rheumatoid arthritis and 10 from psoriatic arthritis. All patients were recruited from the Department of Rheumatology, University of Rome "La Sapienza", in agreement with the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm). Systemic sclerosis and rheumatoid arthritis patients were diagnosed and classified according to the criteria of the American College of Rheumatology (Anon 1980; Arnett et al 1988); psoriatic arthritis was diagnosed according to the criteria proposed by Moll & Wright (1973). Systemic sclerosis patients were divided into limited cutaneous (10 cases) and diffuse (7 cases) subsets (LeRoy et al 1988). Patients were receiving various medications, most commonly corticosteroids, vasodilators and non-steroidal anti-inflammatory drugs. Where suitable, individual patients received also ciclosporin, azathioprine, methotrexate or cyclophosphamide. Eight patients with lower disease activity took no medication at all. In both inflammatory arthritides the most commonly taken drugs were methotrexate and low-dose corticosteroids; a few patients also took infliximab, leflunomide or hydroxychloroquine. A summary of the patients' main characteristics is shown in Table 1. The control group consisted of 22 healthy subjects with no acute or chronic inflammatory disease and on no medication, excluding also any antioxidant supplementation (9 males, 13 females; age 25–72, mean 40).

Table 1 Main demographic, clinical and laboratory parametres of the patients with systemic sclerosis (SSc), rheumatoid artritis (RA) and psoriatic arthritis (PsA)

Characteristic	Ssc (n=17)	RA (n=9)	PsA (n=10)
Age (years), mean (range)	60 (41–79)	58 (45–76)	57 (36–74)
Sex (female/male)	13/4	9/0	2/8
Disease duration (years), mean (range)	9.8 (1–39)	10.9 (2–38)	12.3 (1–28)
Erythrocyte sedimentation rate ($mm\,h^{-1}$), mean (range)	18.2 (4–62)	21 (10–35)	31.8 (5–86)
C-reactive protein ($mg\,L^{-1}$), mean (range)	*	12 (2–24)	30 (3–110)

*Only in 4 cases mildly (max 2-fold higher than physiological levels) increased.

Samples

Venous blood was collected into EDTA-coated tubes (for the measurement of carbonyl and sulfhydryl groups) or sodium citrate-coated tubes (for hydroperoxides (ROOHs) and total antioxidant capacity (TAC) measurements). Plasma was separated within 30 min by centrifugation at 1500 *g* for 10 min at 4°C. Fresh samples were analysed within 3 h of sample collection. The rest of the plasma samples were divided into portions and stored at −70°C within 1 h of sample collection for later measurements. ROOHs, TAC and sulfhydryl groups of plasma samples were measured in fresh samples. Carbonyl groups were assessed on the next day.

Total antioxidant activity of plasma

The FRAP assay (ferric reducing antioxidant power) was performed according to the method of Benzie & Strain (1996) with minor modifications. Briefly, to prepare the FRAP solution, 10 mL of acetate buffer 300 mM, adjusted to pH 3.6 with acetic acid, were mixed with 1 mL of ferric chloride hexahydrate 20 mM dissolved in distilled water and 1 mL of 2,4,6-tris(2-pyridyl)-s-triazine 10 mM dissolved in HCl 40 mM. Ten microlitres of plasma were added to 1.8 mL of a freshly prepared FRAP solution in borosilicate test tubes in quadruplicate and the absorbance was measured at 593 nm after 6 min of incubation at room temperature against a blank of acetate buffer. Each day of experiment, Trolox in two different concentrations (0.2 and 0.4 mM) was used to obtain a new calibration curve. TAC, expressed as Trolox equivalent concentration, was then calculated by dividing the absorbance change (A6–A0) by the slope of the calibration curve. A0 was considered the sum of the absorbance of plasma diluted 180 times and the absorbance of the FRAP solution.

Hydroperoxides

ROOHs were determined by the FOX assay (ferrous ion oxidation in presence of xylenol orange) (Nourooz-Zadeh 1999) with minor modifications. All glassware was cleaned with warm concentrated nitric acid before use. Briefly, FOX solution was a mixture of two solutions, A and B. Solution A was prepared by dissolving butylated hydroxytoluene in pure methanol at 4.4 mM concentration. Solution B consisted of xylenol orange 1 mM and ammonium ferrous sulfate 2.5 mM dissolved in sulfuric acid 250 mM. Working solution was prepared by mixing A and B solutions at a proportion of 9:1, respectively. Working solution was kept at 4°C for a maximum of two weeks. The molar extinction coefficient for each freshly prepared working solution was determined by a calibration curve with different concentrations of hydrogen peroxide in the range 0.1–2.5 μM. The concentration of hydrogen peroxide was measured spectrophotometrically ($\varepsilon_{240} = 43.6\,cm^{-1}\,M^{-1}$) (Nourooz-Zadeh et al 1994). For measuring ROOHs in plasma, 90 μL of plasma was mixed with 10 μL triphenylphosphine 20 mM in methanol (in quadruplicate) or with 10 μL of methanol (in quadruplicate) in 1.5 mL microcentrifuge vials. The vials were vortex-mixed for 10 min each and incubated at room temperature in the dark for 30 min before adding 900 μL of FOX solution. Then the samples were again incubated for 1 h at room temperature in the dark, being vortex-mixed every 10 min, and centrifuged at 16 000 *g* for 7 min. The absorbance of the supernatant was determined at 560 nm by spectrophotometer (Hewlett-Packard 8452AX). The absorbance of the samples treated with triphenylphosphine was subtracted from non-treated samples to calculate the concentration of ROOHs.

Sulfhydryl groups

Sulfhydryl groups/thiols in plasma were measured by a spectrophotometric method using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB or Ellman's reagent) (Hu 1994) with minor changes. Briefly, 60 μL of plasma were mixed with 200 μL of Tris-EDTA buffer (Tris base 0.25 M, EDTA 20 mM, pH 8.2) in 1.5-mL microcentrifuge tubes in quadruplicate. Subsequently, 15 μL of DTNB 10 mM in methanol were added to the samples, except one blank to which the same quantity of methanol was added. After 15 min of incubation at room temperature in the dark, 1 mL of methanol was added to all samples and they were centrifuged at 3000 *g* for 10 min after 5 min of incubation. The absorbance of the supernatant was then measured at 412 nm on a spectrophotometer and the concentration of sulfhydryl groups was calculated ($\varepsilon = 13600\,cm^{-1}\,M^{-1}$).

Carbonyl groups

Carbonyl groups in plasma were determined using the reagent 2,4-dinitrophenyl hydrazine (DNPH) as described previously (Levine et al 1990; Firuzi et al 2003) with minor changes. Briefly, in 1.5-mL microcentrifuge tubes 15 μL of plasma were mixed with 400 μL of DNPH 15 mM in HCl 2 M in quadruplicate. Four-hundred microlitres of HCl 2 M were added to 15 μL of plasma in duplicate as blanks. The samples were vortex-mixed for 10 min each and after 1 h of incubation at room temperature in the dark, 1 mL of trichloroacetic acid 14% (w/v) was added. After another 10 min of incubation all the samples were centrifuged at 11 000 *g* for 5 min. Then the supernatant was discarded and precipitates were washed 3 times with 1 mL of a mixture of ethanol–ethylacetate (1:1, v/v) and, after 10 min, centrifuged. Finally, the protein precipitates were dissolved in 900 μL of guanidine hydrochloride 6 M in KH_2PO_4 20 mM adjusted to pH 2.3 with diluted trifluoroacetic acid. Subsequently, the samples were placed in a water-bath at 37°C for 1 h and further incubated for 2 h in the dark at room temperature. The absorbance was measured at 360 nm with a spectrophotometer against guanidine solution as a blank. The concentration of carbonyls was calculated using the molar extinction coefficient (ε) $22000\,cm^{-1}\,M^{-1}$. The protein concentration in the samples was determined by the modified Bradford assay (Macart & Gerbaut 1982). Measurements were performed by a

microplate reader (Bio-Rad 3550) at 595 nm and calibration curves were prepared with bovine serum albumin as a standard.

Statistics

The obtained data were analysed using one-way analysis of variance with Holm-Sidak (all versus control) post-hoc test performed with SigmaStat version 3.00 for Windows (SPSS Inc., Chicago, IL). The same software was used for Pearson's correlation analyses. $P < 0.05$ was considered statistically significant. All quantitative data are expressed as the mean ± standard deviation.

Results

Total antioxidant capacity

Raw data and means are shown in Figure 1A (expressed as molar concentration equivalent of Trolox, a water-soluble analogue of α-tocopherol). The total antioxidant capacity (TAC) was either not significantly different from the control group in systemic sclerosis or any arthritis. The values in patients tended to be even higher though the elevation was not significant. Furthermore, TAC in systemic sclerosis was found to negatively correlate with the disease duration ($P < 0.05$) (data not shown). The mean TAC value was 0.395 ± 0.095 in systemic sclerosis, 0.329 ± 0.074 in rheumatoid arthritis, 0.399 ± 0.142 in psoriatic arthritis and 0.327 ± 0.068 mmol L^{-1} (equivalent of molar concentration of Trolox) in the control group, respectively.

Hydroperoxides

The plasma levels of hydroperoxides (ROOHs) in all three groups are shown in Figure 1B. The mean values indicate that in all three diseases the ROOHs were increased compared with the control group ($P < 0.05$). Both inflammatory arthritides showed ROOHs even higher than systemic sclerosis. The mean level of hydroperoxides was 3.97 ± 2.24 in systemic sclerosis, 5.13 ± 2.36 in rheumatoid arthritis, 4.87 ± 2.18 in psoriatic arthritis and 2.31 ± 1.41 μmol L^{-1} in healthy controls, respectively.

Sulfhydryl groups

Plasma sulfhydryls were decreased, as compared with healthy controls, in all three groups of patients ($P < 0.05$). The scatter of sulfhydryl values, as well as the position of the mean, in all four groups are shown in Figure 2A. The lowest level was found in rheumatoid arthritis. In addition, in systemic sclerosis a significant negative correlation was found between elevated plasma hydroperoxides and decreased sulfhydryl groups ($P < 0.01$; Figure 3). In psoriatic arthritis patients sulfhydryls were negatively correlated with patients' ages ($P = 0.04$) and there was also a correlation with C-reactive protein levels ($P = 0.064$) (data not shown). The mean level of sulfhydryls was 0.466 ± 0.081 in systemic sclerosis, 0.439 ± 0.069 in rheumatoid arthritis, 0.467 ± 0.057 in psoriatic arthritis and 0.547 ± 0.066 mmol L^{-1} in the control group, respectively.

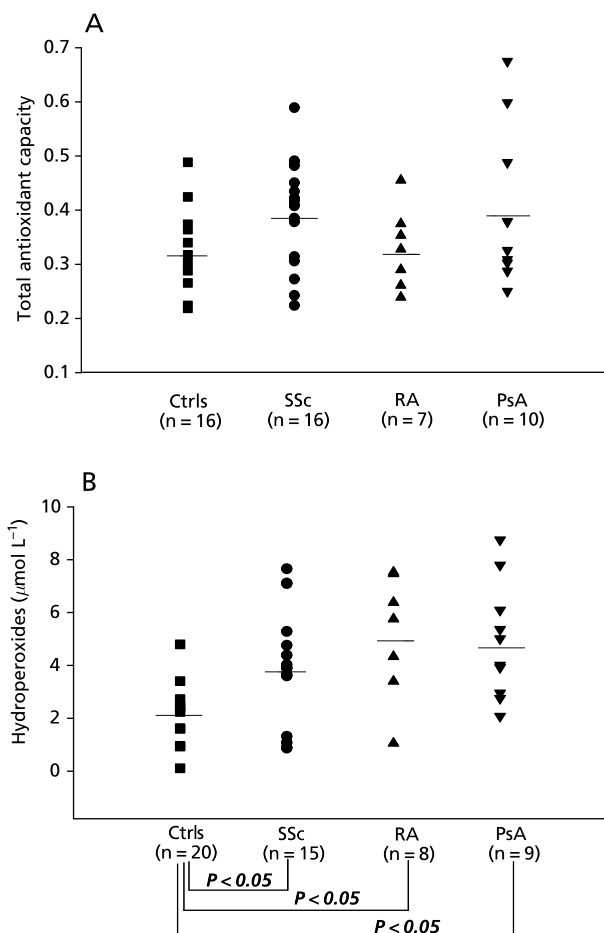

Figure 1 Total antioxidant capacity (A) and plasmatic hydroperoxides (B) in control group (Ctrls), systemic sclerosis (SSc), rheumatoid arthritis (RA) and psoriatic arthritis (PsA). The mean values for each group are shown as a horizontal line. Total antioxidant capacity is expressed as mmol L^{-1}, concentration equivalent to Trolox.

Carbonyl groups

The amount of carbonyl residues in systemic sclerosis did not differ from the control group; neither did it in psoriatic arthritis (Figure 2B). In rheumatoid arthritis patients, however, two different groups were formed, one (24%, n = 3) having a level 70- to 90-fold higher than the rest. Carbonyl levels were thus on average more than 10-fold higher (32.08 ± 41.95 vs 2.21 ± 1.03 nmol (mg of protein)$^{-1}$) compared with the control group ($P < 0.001$). No difference in other markers or available clinical characteristics was found in the respective three patient groups, though. Mean values were 2.22 ± 0.84 in systemic sclerosis, 32.1 ± 41.94 in rheumatoid arthritis, 2.67 ± 2.05 in psoriatic arthritis and 2.21 ± 1.03 nmol (mg protein)$^{-1}$ in healthy controls.

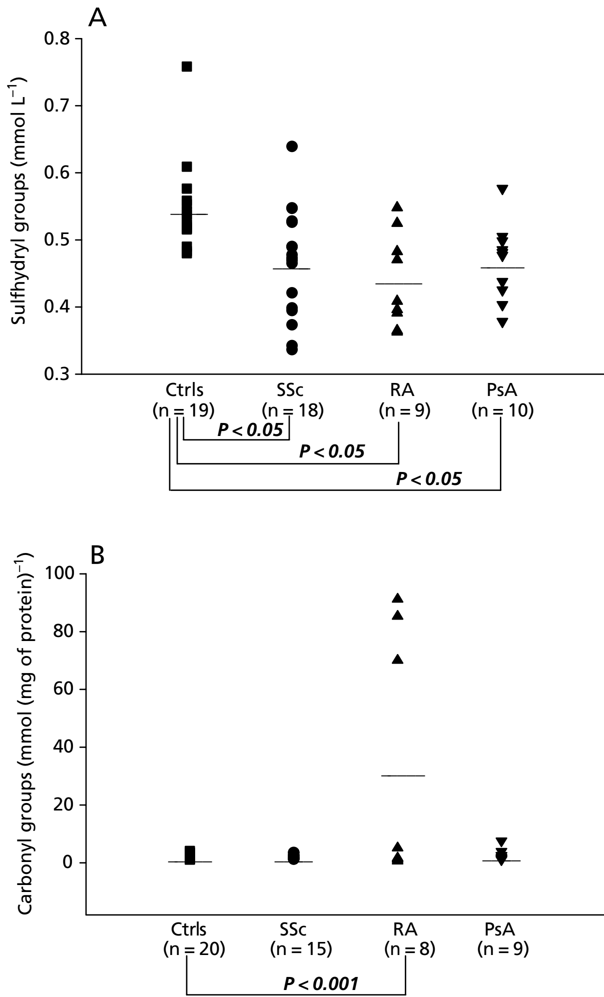

Figure 2 Sulfhydryl (A) and carbonyl (B) groups in control group (Ctrls), systemic sclerosis (SSc), rheumatoid arthritis (RA) and psoriatic arthritis (PsA). The mean values are shown as a horizontal line.

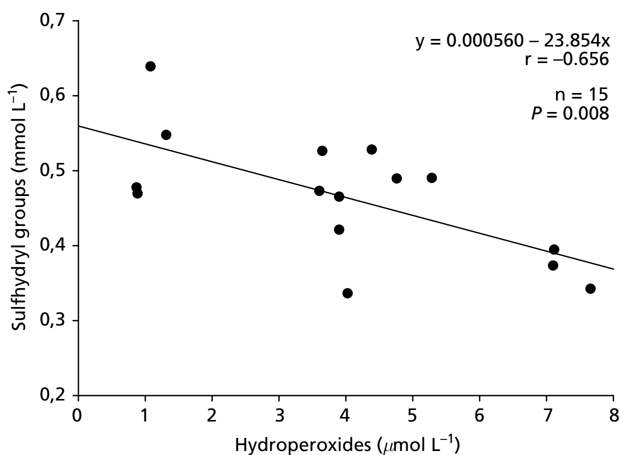

Figure 3 Linear regression between plasmatic hydroperoxides and sulfhydryl groups in systemic sclerosis.

Discussion

This study investigated and confirmed increased oxidative stress occurring in systemic sclerosis, rheumatoid arthritis and psoriatic arthritis. Under conditions of oxidative stress there is a host of various reactive species generated. These oxidants, mostly free radicals, are capable of attacking all biological macromolecules giving rise to different end-products. Therefore a battery of assays has been proposed for evaluation of oxidative stress in-vivo. The criteria for methods used in this study were for them to be fast and simple, thereby rendering them possible to be performed together soon after sample collection so as to prevent any further oxidative reactions. The assays we employed were FRAP (ferric reducing antioxidant power) (Benzie & Strain 1996), assessing total antioxidant capacity (TAC), FOX (ferrous ion oxidation in presence of xylenol orange) method (Nourooz-Zadeh et al 1994) measuring hydroperoxides (ROOHs), a marker of lipid peroxidation, and assays for determination of sulfhydryl (Hu 1994) and carbonyl groups (Levine et al 1990), both reflecting oxidation of proteins.

In systemic sclerosis, two of four investigated parameters were found to be significantly different from the control group – plasmatic ROOHs and sulfhydryl groups. Moreover, these markers clearly inversely correlate with each other, suggesting that free-radical-mediated injury occurs in systemic sclerosis. Elevated ROOHs also confirm previously reported increases in lipid peroxidation, although other studies evaluated different markers (Simonini et al 1999). The decrease in plasmatic thiols also indicates oxidation of proteins. Similar reduction of sulfhydryl groups, further associated with white blood cell activation, has been also been previously reported (Lau et al 1992a). However, this was not supported by the carbonyl-group-evaluating assay, where no significant difference was found. In contrast to our results, in the only previously made study evaluating carbonyl residues they were found higher than in control group (Borderie et al 2004).

Inconsistent with these findings that implicate an increase of oxidative stress in systemic sclerosis, TAC was not decreased as anticipated. Moreover, it was even higher than in healthy controls. This also goes against the assumption based on other authors' studies describing impairment in antioxidant defences in systemic sclerosis. Some of these studies reported decreased plasmatic levels of micronutrients participating in scavenging of free radicals, such as ascorbic acid, α-tocopherol, β-carotene or selenium (Lundberg et al 1992; Herrick et al 1994). Besides these studies investigating single blood levels of antioxidants, there are no previous reports on plasma antioxidant capacity evaluation by other authors. Even though the FRAP assay was originally designed for, and shown capable of, representing changes in antioxidant properties of biological fluids, it must be noted that the reaction conditions are far from physiological and in-vivo hierarchies and activity of individual antioxidants may not be directly related to (and thus represented by) the reducing

potential of such a complex sample as plasma. Therefore the test measuring reducing potential may lack enough sensitivity and fail to reveal minor changes in antioxidant defences. Another hypothetical explanation of the lack of significant changes in TAC may lie in some as yet unknown feedback mechanism being a response to increased oxidative stress (Prior & Cao 1999). Likewise, adaptation to increased oxidative stress has been so far reported in the case of antioxidant enzymes, and considering endogenous molecules, such as ceruloplasmin or glutathione, they could be overproduced to manage the rise of RONS. Even though none of these molecules could per se contribute to FRAP (or other TAC-measuring) assay, their antioxidant/chelating properties may in part replace other low molecular weight antioxidants (ascorbic acid, α-tocopherol, etc.) in binding free radicals and cause them to remain in their reduced forms, thus keeping the overall TAC higher. Indeed, accepting such an assumption, we could explain the inverse correlation between TAC and disease duration we found in systemic sclerosis (data not shown). Nonetheless, previously stated findings suggest that oxidative stress, according to three of the four markers evaluated, is more pronounced in the later stages of the disease. It includes oxidation of proteins (signified by reduced sulfhydryl groups), deterioration of antioxidant defences (decreased TAC) and also lipid peroxidation (elevated ROOHs). In a study evaluating lipid peroxidation, higher oxidative damage in the early stages of the disease was reported (Simonini et al 1999). This contrast may be attributed to different ways of assessing the progress of systemic sclerosis. It may be also due to the different accumulative properties of the markers under examination as well as to different molecular mechanisms leading to their formation. There was no difference in any of the four oxidative stress parameters found between limited and diffuse subsets of systemic sclerosis.

In rheumatoid and psoriatic arthritis, all four assays showed similar results to those of systemic sclerosis. The present data are among the first to be reported on oxidative stress status in psoriatic arthritis. In both arthritides, ROOHs were higher than in the control group. This difference was even greater than in systemic sclerosis. An array of studies on oxidative stress status in rheumatoid arthritis has been performed and some of them describe augmented lipid peroxidation in rheumatoid arthritis as well (Gambhir et al 1997; Taysi et al 2002). Even though different markers were determined, these reports are in line with our findings. Plasma thiols were significantly lower in both diseases when compared with healthy controls; in rheumatoid arthritis they were the lowest of all the three diseases. This is in concordance with other authors' reports (Jaswal et al 2003). Further supporting the idea of increased oxidation of proteins in rheumatoid arthritis, we detected increased levels of carbonyl groups. Although higher amounts of carbonyl residues in rheumatoid arthritis have been previously reported by other authors (Mantle et al 1999; Renke et al 2000), the mean increase found in our study (13 fold) is remarkably high. Yet this statement needs to be regarded with particular caution, since high diversity was found among the patients, clearly separating them into two groups. No other difference (in other oxidative stress markers, biochemical data, type of medication, etc.) explaining this remarkable occurrence was observed however (see Results). It would be certainly interesting to further extend the group of patients and investigate this issue more in detail. Nevertheless, the finding of very low sulfhydryl groups and elevated carbonyl groups indicates a serious oxidative injury, particularly in proteins. However, no correlation between these two parameters was observed. Unlike in the other diseases, sulfhydryl groups in psoriatic arthritis are inversely correlated with the age of patients. There is also an attenuated inverse correlation showing sulfhydryl groups decreasing as C-reactive protein levels increase, which may suggest an increase in the oxidation of proteins along with the severity of inflammation. TAC was not found in any arthritis to be significantly different from the control group. One study evaluating oxidative stress in rheumatoid arthritis (De Leo et al 2002) described a decreased TRAP (total radical trapping antioxidant parameter) value compared with controls. To explain our opposing results we can adopt the hypothesis suggested above for systemic sclerosis, that increased oxidative stress in-vivo may be faced by some inducible compensatory mechanisms other than only free-radical-scavenging enzymes. The results of studies evaluating blood antioxidants levels in RA (Gambhir et al 1997) have also proposed such an explanation.

In conclusion, our data support the hypothesis that oxidative stress occurs in systemic sclerosis as well as in rheumatoid arthritis and psoriatic arthritis. This implies a possible benefit of antioxidant treatment. In scleroderma, however, only a few studies have been undertaken in this respect so far, and they yielded inconsistent outcomes (Denton et al 1999; Herrick et al 2000; Kalin et al 2002). The difficulty in assessing the benefit of antioxidant treatment lies probably in the subjectivity or insensitivity to change of most of the measures currently in use (Herrick & Matucci Cerinic 2001). Future trials focused on patients in earlier stages of systemic sclerosis, along with improved study design, could clarify the real clinical effect of antioxidant therapy. In the case of inflammatory arthritides, our data further support the opinion of clear benefit in adding antioxidants and micronutrients to conventional treatment, as has been confirmed by several studies in rheumatoid arthritis (Darlington & Stone 2001; Jaswal et al 2003); the same proposal can be offered also for psoriatic arthritis.

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