Protein Oxidation Markers in the Serum and Synovial Fluid of Psoriatic Arthritis Patients

Omidreza Firuzi,^{1,2} Antonio Spadaro,³ Chiara Spadaro,¹ Valeria Ricciari,³
Rita Petrucci,⁴ Giancarlo Marrosu,⁴ and Luciano Saso^{1*}

¹Dipartimento di Fisiologia Umana e Farmacologia "Vittorio Erspamer," Sapienza Università di Roma, Rome Italy

²Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

³Dipartimento di Clinica e Terapia Medica Applicata, Divisione di Reumatologia, Sapienza Università di Roma, Rome, Italy

⁴Dipartimento di Ingegneria Chimica, dei Materiali, delle Materie Prime e Metallurgia, Sapienza Università di Roma, Rome, Italy

The role of oxidative stress has been studied in rheumatoid arthritis (RA) and other inflammatory joint diseases to some extent, but its importance in psoriatic arthritis (PsA) has rarely been investigated. The aim of this study was to analyze the levels of protein oxidation markers, sulfhydryl (SH) and carbonyl (CO) groups, in the synovial fluid (SF) and serum of PsA patients and compare them with the findings in RA and osteoarthritis (OA) patients. A total of 49 subjects with a knee-joint effusion including 16 PsA, 18 RA, and 15 OA patients were studied. In all patients, the levels of SH groups measured in the serum and SF inversely correlated with the number of white blood cells (WBC) ($P < 0.05$) and the percentage of polymorphonuclear leukocytes (PMN) ($P < 0.01$) in SF. Serum

SH levels inversely correlated with serum erythrocyte sedimentation rate (ESR) ($P < 0.02$) and C-reactive protein (CRP) ($P < 0.05$) values. The SH levels in SF were significantly lower in patients affected by PsA and RA compared to OA cases ($P < 0.02$). The serum SH levels in PsA were lower than OA ($P < 0.001$) and higher than RA patients ($P < 0.05$). The serum and synovial levels of CO groups in PsA, RA, and OA patients were similar. Our study provides novel evidence on the involvement of protein oxidation in PsA and confirms the important role of oxidative stress in the pathogenesis of RA. These data suggest that antioxidant agents can potentially be a useful addition to the conventional therapy in the management of these diseases. *J. Clin. Lab. Anal.* 22: 210–215, 2008. © 2008 Wiley-Liss, Inc.

Key words: carbonyl; sulfhydryl; synovial fluid; psoriatic arthritis; oxidative stress

INTRODUCTION

Oxidative stress, a pathological condition caused by an alteration of the equilibrium between free radical production and the normal physiological defense, causes the oxidation of lipids, proteins, and nucleic acids (1) and leads to significant changes of their biological activity. It is believed that oxidative stress plays an important role in the pathogenesis of several diseases including rheumatoid arthritis (RA) (2–4).

While, hydroperoxides (5), malondialdehyde (4,6,7), 4-hydroxy-2-nonenal (8), and F(2)-isoprostanes (9) are

Abbreviations: CO, carbonyl; CRP, C-reactive protein; DNPH, 2,4-dinitrophenyl hydrazine; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); ESR, erythrocyte sedimentation rate; OA, osteoarthritis; PMN, polymorphonuclear leukocytes; PsA, psoriatic arthritis; RA, rheumatoid arthritis; ROS, reactive oxygen species; SF, synovial fluid; SH, sulfhydryl; WBC, white blood cell.

*Correspondence to: Luciano Saso, Dipartimento di Fisiologia Umana e Farmacologia "Vittorio Erspamer", Sapienza Università di Roma, P.le Aldo Moro 5, 00185 Rome, Italy.

E-mail: luciano.saso@uniroma1.it

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the main lipid peroxidation parameters, some of the most important protein oxidation markers include; 1) 3-nitrotyrosine, produced by the reaction of peroxynitrite radical (10) with tyrosine residues; 2) carbonyl (CO) groups, formed by the oxidation of proline, histidine, lysine, and arginine residues (3,11,12); and 3) sulfhydryl (SH) groups, which can be oxidized; their level correlates inversely with oxidative stress (13).

Changes of these markers have been reported in both serum and synovial fluid (SF) of patients affected by RA:

- Serum: Malondialdehyde (14), superoxide dismutase (7), 3-nitrotyrosine (10) were reported to be increased in patients with RA compared to healthy subjects. Changes in concentrations of CO and SH groups in the serum of RA patients were also reported (3,13,15).
- SF: Increased levels of lipid peroxidation (16), nitric oxide (17,18), 4-hydroxy-2-nonenal (8), and 3-nitrotyrosine (10) were reported in SF of RA patients. An increase of the CO levels in SF of RA patients has also been reported (3,19).

Although protein oxidation phenomena were studied in detail in RA, only a few authors (9,20) have measured the markers of oxidative stress in psoriatic arthritis (PsA). PsA is a disease classified in spondyloarthritides according to European Spondyloarthropathy Study Group criteria (21) with special clinical features. In fact, the clinical picture of PsA shares features of spondyloarthropathies and RA, but asymmetrical oligoarticular disease predominantly of the lower limbs, distal interphalangeal joint (DIP) involvement, enthesitis, dactylitis, and typical radiological features help to distinguish it from RA (22).

The aim of this study was to analyze the levels of CO and SH groups, two important markers of protein oxidation, in SF and serum of PsA patients in comparison to subjects affected by RA and osteoarthritis (OA).

MATERIALS AND METHODS

Patients

A total of 49 patients with a knee-joint effusion including 16 PsA patients, classified according to the modified Moll and Wright criteria (23), 18 RA patients, classified according to ACR criteria (24), and 15 OA patients were studied. All patients were being treated with at least one nonsteroidal antiinflammatory drug (NSAID). None of the patients had received intra-articular steroid injections at least 3 months before the study.

All patients underwent a careful evaluation of clinical and laboratory features. SF, obtained by therapeutic

TABLE 1. Main clinical and laboratory features of patients with psoriatic arthritis (PsA), rheumatoid arthritis (RA), and osteoarthritis (OA)

	PsA	RA	OA
Patients (number)	16	18	15
Age (years, mean/range)	48.4/31–68	52.9/30–71	59.3/ 45–78
Males/females (number)	11/5	5/13	5/10
Disease duration (months, mean/range)	78.3/3–336	110/6–360	83.7/ 7–420
DMARD, % (n)	56.2 (9)	27.8 (5)	0 (0)
Corticosteroid, % (n)	37.5 (6)	50.0 (9)	0 (0)
Serum RF positive, % (n)	6.2 (1)	77.8 (14)	0 (0)
Serum ESR (mm/L hr)*	39 (20–57)	36 (23–49)	13 (8–17)
Serum CRP (mg dL ⁻¹)*	1.4 (0.6–2.1)	2.2 (0.9–3.5)	0.3 (0.3–0.3)
SF low viscosity, % (n)	81.2 (13)	72.2 (13)	26.7 (4)
SF mucin clot, % (n)			
Good	18.7 (3)	27.8 (5)	73.3 (11)
Fair	68.7 (11)	66.7 (12)	26.7 (4)
Poor	12.5 (2)	5.5 (1)	–
SF WBC (per µl)*	9280 (7009–11551)	7365 (4976–9753)	695 (79–1311)
SF PMN %*	74 (68–80)	68 (59–77)	21 (7–35)

*Mean (lower–upper 95% confidence interval).

DMARD, disease-modifying antirheumatic drug.

arthrocentesis, and paired serum samples were stored at –20°C until determination of SH and CO groups. Routine laboratory tests included analysis of WBC and PMN in SF, and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF) in serum. Clinical information, including corticosteroid and disease-modifying antirheumatic drug (DMARD) treatments, and laboratory features of the patients are shown in Table 1.

Reagents

Bovine serum albumin (BSA) (cat. No. A-3294), Bradford's reagent (cat. No. B-6916), 2,4-dinitrophenylhydrazine (DNPH, cat. No. 42210), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, cat. No. D-8130), ethylene diamine tetraacetic acid (EDTA, cat. No. 25.404-5), glutathione (cat. No. G-4251), guanidine hydrochloride (cat. No. 50940), sodium phosphate dibasic (cat. No. S-7907), sodium phosphate monobasic (cat. No. S-9638), trichloroacetic acid solution 6.1 N (cat. No. T-0699), trifluoroacetic acid 99% (cat. No. 30.203-1), and tris[hydroxymethyl]aminomethane (trizma base) (cat. No. T-6066) were all obtained from Sigma-Aldrich Co. (St. Louis, MO; www.sigma-aldrich.com). Ethanol 99.8% (cat. No. 414608), ethyl acetate (cat. No. 448256), hydrochloric acid 37% (cat. No. 403871), and methanol (high-performance liquid chromatography [HPLC]-grade, cat. No. 412383) were purchased from Carlo

Erba (Milan, Italy; www.carloerbareagenti.com). Potassium dihydrogen phosphate (cat. No. 4873) was obtained from Merck (Darmstadt, Germany; www.merck.de). Deionized water was prepared with MilliQ4 (Millipore, Molsheim, France).

Determination of SH Groups in SF and Serum

SH groups in serum and SF were measured by a spectrophotometric method using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB or Ellman's reagent) (25,26). Briefly, SF or serum samples were mixed with Tris-EDTA buffer (pH 8.2) in microcentrifuge tubes in quadruplicate. Subsequently, 10 mM DTNB in methanol was added to the samples. After incubation at room temperature in the dark, methanol was added to all samples and, after 5 min, they were centrifuged at 3,000 *g*. The absorbance was subsequently measured at 412 nm and the concentration of SH groups was calculated using the molar extinction coefficient (ϵ) of 13,600 $\text{cm}^{-1} \text{M}^{-1}$ (26).

Determination of CO Groups in SF and Serum

CO groups in SF and serum were determined using the reagent DNPH as described by Levine et al. (11) with some modifications (26). Briefly, after incubation of DNPH with samples, trichloroacetic acid (TCA) 10% (w/v) was added and samples were centrifuged at 11,000 *g*. The precipitates were washed with a mixture of ethanol/ethyl acetate, 1:1 (v/v) three times and then dissolved in guanidine hydrochloride 6 M (pH 2.3). The absorbance was then measured at 360 nm and the concentration of CO groups was calculated using the molar extinction coefficient (ϵ) of 22,000 $\text{cm}^{-1} \text{M}^{-1}$ (11). Protein concentration of the samples was determined by

the Bradford's (27) assay as modified by other authors (28) using a microplate reader (Bio-Rad 3550; Bio-Rad, Hercules, CA) at 595 nm and BSA as standard.

Statistical Analysis

Statistical analysis was performed using the program SigmaStat version 3.0 for Windows (SPSS Inc. Chicago, IL). The results are presented as mean and lower—upper 95% confidence intervals (CI). The statistical significance between groups was determined using paired or independent samples *t*-test when appropriate. Categorical variables were analyzed by χ^2 test or Fisher's exact test. The statistical significance of correlations was determined by Pearson's correlation coefficient. *P* values less than 0.05 were considered statistically significant.

RESULTS

SH Groups Measured in SF and Serum

In all patients the levels of SH groups measured in SF and serum inversely correlated with white blood cell (WBC) (Fig. 1) and polymorphonuclear leukocyte (PMN) cell count of SF (Fig. 2). Serum ESR and CRP values correlated only with serum SH values (Fig. 3) and not with synovial SH values (data not shown). SH values of SF did not correlate with SH levels in serum or with CO levels in SF (data not shown).

The concentration of SH groups in SF (nmol/mg proteins, mean/lower-upper 95% CI) was significantly lower in patients affected by PsA (2.430/1.794–3.067) and RA (2.545/1.728–3.361) compared to patients affected by OA (4.168/3.008–5.328) (Fig. 4).

The serum SH levels in PsA patients (2.499/2.022–2.976) was lower than in OA patients (3.993/

Fig. 1. Linear regression of SF (—) and serum (- - -) sulfhydryl groups (nmol/mg proteins) vs. SF WBC count (cells/mm³) in all patients including PsA, RA, and OA.

3.271–4.715) and higher than in RA patients (1.853/1.399–2.307) (Fig. 4). We did not find any significant difference in SH levels between paired SF and serum samples (data not shown).

CO Groups Measured in SF and Serum

In all patients the levels of CO groups measured in SF and serum correlated with each other (data not shown, $r = 0.518$; $P < 0.001$). We did not find any correlation between CO groups and parameters of local inflammation in the SF (WBC, PMN cells) or systemic inflammation (ESR, CRP). The levels of CO groups (nmol/mg proteins, mean/lower-upper 95% CI) in PsA (SF = 0.804/2.267–3.341; serum = 3.213/2.522–3.903), RA (SF = 3.517/2.674–4.360; serum = 2.863/

Fig. 2. Linear regression of SF (—) and serum (- - -) sulfhydryl groups (nmol/mg proteins) vs. SF PMN count (%) in all patients including PsA, RA, and OA.

Fig. 4. Concentration (mean/95% CI) of sulfhydryl groups (nmol/mg proteins) in serum (S) and SF of patients affected by PsA, RA, and OA.

Fig. 5. Concentration (mean/95% CI) of CO groups (nmol/mg proteins) in serum (S) and SF of patients affected by PsA, RA, and OA.

Fig. 3. Linear regression of serum ESR (—) and CRP (- - -) values vs. serum sulfhydryl groups (nmol/mg proteins) in all patients including PsA, RA, and OA.

1.872–3.853), and OA (SF = 2.875/2.197–3.553; serum = 2.469/1.844–3.094) patients were similar (Fig. 5).

DISCUSSION

In this study, SH and CO groups, two important protein biomarkers widely used for the evaluation of oxidative stress status in biological fluids, were measured in the SF and serum samples of patients affected by PsA. We compared the PsA patients with subjects affected by RA, an inflammatory disease, in which protein oxidation phenomena play an important pathogenic role. Furthermore, we compared PsA cases with patients affected by OA, commonly used as control by other authors (9,18,29), because of the evidence that oxidative stress does not seem to have a relevant role in this disease (30). The importance of oxidative stress in the joint compartment is confirmed by our finding that levels of SH groups in SF are inversely correlated with the number of WBC and percentage of PMN cells, which are both parameters of local inflammation in SF. These findings and the lack of correlation between SF and serum levels of SH groups suggest that SH levels in SF represent an expression of local inflammation. Besides, serum SH levels showed a negative correlation with ESR and CRP, which may reflect a systemic inflammatory response.

The pathogenic role of oxidative stress in PsA is suggested by other authors, who found that the serum levels of CO groups (12) and F(2)-isoprostanes (9) were changed in comparison with healthy subjects. This hypothesis was further supported by our recent data that showed elevated levels of plasmatic hydroperoxides and reduced levels of plasmatic SH in PsA compared to healthy subjects (20). In the present study, we used OA patients as control and found that serum SH groups were reduced in PsA. We also found a reduction of SH groups in RA, which agrees with the results reported by other authors (13,15). There are even fewer studies on the oxidative parameters in SF of PsA. In one study, SF of patients with various arthritic disorders, including four patients with PsA, were studied, and increased levels of F(2)-isoprostanes (8-iso-PGF_{2α} and 15-keto-dihydro-PGF_{2α}), were reported (9). To our knowledge, there are not any studies regarding the effects of oxidative stress on proteins in PsA SF. Our study showed that synovial SH groups was significantly lower in patients affected by PsA and RA compared to control. This oxidative condition may lead to the inactivation of protective proteins such as α₂-macroglobulin and α₁-antitrypsin, and to worsening of inflammation and tissue damage. We did not find any difference between synovial and serum levels of SH groups either in PsA or in RA. These results agree with

those of Koster et al. (13), who reported that serum and SF from RA patients contained similar levels of SH groups.

We found no significant difference in the synovial or serum CO levels among PsA, RA, and OA patients. In a previous study, the CO content in the SF of RA patients was elevated over OA patients but this difference did not reach significance ($P < 0.1$), although the control group included only four OA patients (19). According to another report, the level of free radical-induced damage to SF proteins (determined as protein CO derivative) was approximately two-fold higher in RA compared to OA patients, but there was no significant difference in the serum levels between RA patients and controls (3). These discrepancies could be due to the fact that CO groups are less sensitive than SH groups in discriminating between inflammatory and noninflammatory arthropathies. The analysis of various parameters of oxidative stress indicates that the SH groups are the most sensitive in discriminating between RA and healthy subjects (15). In fact CO groups poorly reflect local (WBC, PMN cells) and systemic (ESR, CRP) inflammatory response, as shown by the lack of correlation between CO groups and these parameters in our study. Furthermore, different treatments among inflammatory and noninflammatory arthropathies, including DMARDs, corticosteroids, and NSAIDs may have a variety of effects on inflammatory and immune mediated responses, as well as on the generation of free radicals.

In conclusion, our study provides novel evidence on the involvement of protein oxidation in PsA and confirms the important role of oxidative stress in the pathogenesis of RA. These data suggest that antioxidant agents may be used, in association with conventional therapy, for the treatment of these arthritides.

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