Effective tumour necrosis factor-blocking therapy reduces reactive oxygen metabolite level in rheumatoid arthritis

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Abstract

Objective: To assess circulating levels of derived reactive oxygen metabolites (ROMs) in patients with active rheumatoid arthritis (RA), before and during antitumour necrosis factor (TNF-α) therapy.

Methods: Patients with active RA and failed previous treatment with disease-modifying antirheumatic drugs received subcutaneous anti-TNF-α for 52 weeks. Circulating hydrogen peroxide was quantified as a marker of oxidative stress at baseline and at 24 and 52 weeks.

Results: The study included 40 patients. Circulating dROM levels were significantly reduced compared with baseline after 24 and 52 weeks of anti-TNF-α treatment (33.2 ± 10.0 mgH₂O₂/dl, 29.5 ± 7.0 mgH₂O₂/dl and 29.3 ± 9.0 mgH₂O₂/dl, respectively). There was a significant direct correlation between disease activity score and ROM levels.

Conclusion: TNF-α inhibition can control disease activity and reduce circulating levels of reactive oxygen species in patients with RA.

Keywords
Anti-TNF, disease activity, oxidative stress, reactive oxygen metabolites, rheumatoid arthritis, ROS

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by hyperplasia of synovial tissues and structural joint damage, with chronic low-grade systemic inflammation; a combination of genetic...
susceptibility and environmental factors are critical in RA pathogenesis.\(^1\)

Reactive oxygen species (ROS) are products of aerobic metabolism that cause DNA mutation, lipid peroxidation and protein oxidation, and activate and perpetuate the autoimmune process.\(^2\) ROS are produced in many normal and abnormal conditions in humans including atheroma, asthma, Alzheimer’s disease, ageing and cancer.\(^3\) In many diseases of the joints, proinflammatory factors (cytokines and prostaglandins) and ROS are released at sites of inflammation, with tumour necrosis factor (TNF)-\(\alpha\) overproduction thought to be the main contributor to increased ROS release in patients with RA.\(^4\) In addition, high ROS levels have been shown to be related to RA disease activity.\(^5\)

The aim of this study was to assess circulating levels of reactive oxygen metabolites (ROMs) in patients with active RA, before and during anti-TNF-\(\alpha\) therapy.

**Patients and methods**

**Study population**

The study recruited patients with RA attending the outpatient clinic at the Rheumatology Unit, University of Bari, Bari, Italy between October 2013 and June 2014. Patients were required to meet American College of Rheumatology and European League Against Rheumatism 2010 classification criteria,\(^6\) and to have had previous failed treatment with disease-modifying antirheumatic drugs (DMARDs). Patients were evaluated at baseline and after 24 and 52 weeks’ subcutaneous anti-TNF-\(\alpha\) administration (adalimumab, etanercept or golimumab), at standard dose and administration regimens. Data included demographic and clinical characteristics, and Disease Activity Score (28)–C-Reactive Protein (DAS28–CRP)\(^7\) and Health Assessment Questionnaire (HAQ) findings.\(^8\) Circulating hydrogen peroxide was quantified as a marker of oxidative stress, using a Diacron automated method (d-ROM test),\(^9\) where \(<27\text{ mg H}_2\text{O}_2/dl\) indicates low oxidative stress, \(27–32\text{ mg H}_2\text{O}_2/dl\) indicates moderate oxidative stress, and \(>32\text{ mg H}_2\text{O}_2/dl\) indicates high oxidative stress.\(^10,11\)

**Statistical analyses**

Data were presented as mean±SD. Continuous variables were evaluated using one-way analysis of variance followed by paired \(t\)-test, and categorical data were compared using Fisher’s exact probability test or \(\chi^2\)-test, as appropriate. Pearson’s correlation coefficient was used to evaluate the relationship between DAS28–CRP score and dROM levels. Statistical analyses were performed using InStat\(^\text{®}\) version 3 (GraphPad Software, San Diego, CA, USA). \(P\)-values <0.05 were considered statistically significant.

**Results**

The study included 40 patients with RA (four male/36 female; mean age 53±13 years; age range 18–78 years). Patients’ demographic and clinical data are shown in Table 1.

Circulating dROM levels were significantly reduced compared with baseline (33.2±10.0 mgH\(_2\)O\(_2\)/dl) after 24 and 52 weeks’ anti-TNF-\(\alpha\) treatment (24 weeks, 29.5±7.0 mgH\(_2\)O\(_2\)/dl; 52 weeks, 29.3±9.0 mgH\(_2\)O\(_2\)/dl; \(P=0.01\) for each comparison). At baseline, 22 (55%) patients had high oxidative stress (\(>32\text{ mgH}_2\text{O}_2/dl\)). After 24 and 52 weeks’ anti-TNF-\(\alpha\) treatment, 50% (20/40) and 62.5% (25/40) of patients, respectively, achieved low disease activity (DAS28–CRP <3.2); all of these patients had low oxidative stress (\(<27\text{ mgH}_2\text{O}_2/dl\)).

There was a significant positive correlation between circulating dROM levels and DAS28–CRP \((r=0.22, P<0.01; \text{Figure 1})\).

**Discussion**

Our study confirmed the correlation between circulating ROS (evaluated via
dROM) levels and disease activity in patients with RA. The mechanisms responsible for the onset of RA remain unclear. Smoking has been implicated as one of the most important extrinsic risk factors for RA development and severity, and evidence suggests interrelations between smoking, oxidative stress, inflammation, autoantibody formation and epigenetic changes in RA.

Reactive oxygen species play an important role in progressive joint destruction (both upstream and downstream of nuclear factor κB and TNF-α pathways) that is central to the inflammatory response. Increased oxidative stress is considered the key determinant of RA comorbidities (mainly accelerated atherosclerosis) and the increased incidence of cardiovascular disease and mortality observed in people with RA. The immune response, via cytokines and chemokines that attract monocytes, characterizes the pathology from formation and stabilization to progression and rupture of the atherosclerotic plaque.

The therapeutic goal of controlling systemic inflammation in RA results not only in the remission of musculoskeletal symptoms but also in improvements to general health. Biological immunosuppressive therapies targeting proinflammatory cytokines have demonstrated ability to control disease activity and halt progressive joint destruction. TNF-α inhibitors with antioxidative activity may have multiple target effects that could exhibit excellent anti-inflammatory activities, although metabolic and cardiovascular effects remain unclear.

In the early 2000s, trials of anti-TNF-α drugs indicated problems with cardiovascular safety, including the progression of existing heart failure, as well as modification of lipids to atherogenic status with anti-interleukin 6 treatment. On the other hand, data from national registries of patients with RA appear to demonstrate a reduction in cardiovascular events in those patients responding to biological treatment. High levels of ROS are associated with obesity, cardiovascular diseases and atherosclerosis.

Few studies have investigated the effects of anti-TNF-α therapy on oxidative stress. In a finding similar to others, we observed that TNF-α antagonism reduces oxidative stress in responding patients. This reduction in circulating ROS levels during anti-TNF-α treatment may explain the ability of these drugs to reduce cardiovascular morbidity and mortality in patients who achieve good disease control. The relatively small number of patients in this study and the use of three different subcutaneously administered TNF-α inhibitors (with

| Parameter                     | N (%) |
|-------------------------------|-------|
| Sex, male/female              | 4/36  |
| Age, years                    | 53 ± 13 |
| Tobacco use                   | 8 (20.0) |
| Disease duration, months      | 6.8 ± 3.7 |
| RF positive                   | 31 (77.5) |
| Anti-CCP positive             | 29 (72.5) |
| ESR, mm/h                     | 57 ± 27 |
| CRP, mg/l                     | 6.4 ± 3.7 |
| DAS28                          | 6.4 ± 0.9 |
| HAQ                           | 1.8 ± 0.7 |
| Previous treatment            |       |
| CCS                           | 3 (7.5) |
| CCS + MTX                     | 27 (67.5) |
| CCS + LFM                     | 4 (10.0) |
| CCS + SSZ                     | 6 (15.0) |
| Anti-TNF-α treatment          |       |
| Etanercept                    | 15 (37.5) |
| Adalimumab                    | 17 (42.5) |
| Golimumab                     | 8 (20.0) |

RF, rheumatoid factor; CCP, cyclic citrullinated peptide; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, Disease Activity Score (28); HAQ, Health Assessment Questionnaire; CCS, corticosteroids; MTX, methotrexate; LFM, leflunomide; SSZ, sulphasalazine.
dissimilar doses and dosing intervals) are relevant study limitations; further studies with a larger series may confirm our preliminary findings.

In conclusion, TNF-α inhibition could control disease activity and reduce circulating ROS levels in patients with RA. This may explain the systemic effects of anti-TNF-α agents and justify early treatment to prevent cardiovascular morbidity. The observed correlation between the DAS28–CRP and the ROS level suggests that measurement of oxidative stress could serve as a biomarker of inflammation and disease severity.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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Figure 1. Pearson’s correlation coefficient analysis of the relationship between circulating levels of derived reactive oxygen metabolites (dROM; mg H2O2/dL) and Disease Activity Score (28)–C-Reactive Protein (DAS28–CRP) in patients with active rheumatoid arthritis undergoing anti-tumour necrosis factor (TNF)-α treatment (n = 40).
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