ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 9 & 10, 2005

News & Views

Can Human Plasma Redox State Be Used to Monitor the Progression of Rheumatoid Arthritis?

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RHEUMATOID ARTHRITIS

MUSCULOSKELETAL CONDITIONS are prevalent, and their impact is pervasive. They are the most common cause of severe long-term pain and physical disability, affecting hundreds of millions of people around the world. At any given time, 30% of American adults are affected by joint pain, swelling, or limitation of movement (2). Rheumatoid arthritis (RA), one of the most disabling musculoskeletal diseases, affects ~0.5–1% of adults in Western nations (14). It is a chronic inflammatory systemic disease with onset in young or middle-aged adults. It is characterized by destructive and proliferative changes in the synovial membranes, periarticular structures, skeletal muscles, and perineural sheaths. Although the exact etiology of RA remains unknown, it is assumed that either a foreign agent or some alteration in the control of cellular responses, possibly genetically mediated, is involved in the chronic persistent synovial inflammation (5). On roentgenography, osteoporosis, destruction of cartilage, and narrowing of the joint intervals are characteristic. As the disease progresses, the articular cortex becomes thinned and almost indistinct. Eventually, joints are destroyed, ankylosed, and deformed. For RA patients, total joint arthroplasty provides reliable relief from pain, as well as functional improvement (Fig. 1).

The increasing incidence of RA will result in a dramatic increase in the burdens it places on individuals and on society.

TREATMENT FOR RA

Treatment of patients with RA involves a multidisciplinary approach with patient education, physical therapy, and medication. Because persistent synovitis leads to joint destruction that results in long-term morbidity and increased mortality, the aim of drug treatment should be the prevention of synovitis. Although traditional treatments for RA, such as nonsteroidal antiinflammatory drugs and corticosteroids, have some benefits for temporary symptomatic relief, dramatic improvements in disease control have been noted with the use of disease-modifying antirheumatic drugs (DMARDs), such as methotrexate (MTX). Inadequate responders to MTX are often given MTX in combination with other agents, particularly hydroxychloroquine, sulfasalazine, leflunomide, and cyclosporine (17). Recently, biological DMARD treatments for RA have been developed to inhibit the activity of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), which mediates the destructive synovitis that is the hallmark of RA. These agents have been successful in treating patients with RA who fail to respond adequately to traditional DMARD treatments (9). A long-term study has even demonstrated cartilage regeneration (15). Two biological DMARDs designed to inhibit TNF-α bioactivity have been approved by the United States Food and Drug Administration for the treatment of RA: etanercept and infliximab. These agents are both indicated for the treatment of moderate to severe DMARD-refractory RA (8). In Japan, infliximab was approved for the treatment of RA in 2004. Rapid clinical response to infliximab treatment was shown by the early decline in the number of cases of swollen and tender joints. Furthermore, these improvements were immediate and sustained (16, 20).

PLASMA REDOX STATE AND RA

The formation, behavior, and scavenging of reactive oxygen species (ROS) in biological systems have received much attention (3). It has been suggested that the prooxidant/antioxidant imbalance in RA may be due to either an acceleration of some cellular reactions or an insufficiency of the antioxidant defense system (18). Recently, reduced blood concentrations of antioxidants were reported in RA patients (12). Although these data suggest that the antioxidant defense system is compromised in RA patients, the plasma redox state in RA patients has not been clarified. We first measured blood

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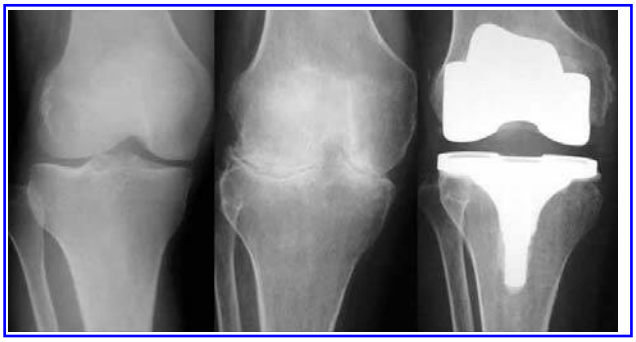

FIG. 1. Radiographs of a 45-year-old woman with RA. The subject complained of right knee joint pain. At entry into the study, there were no abnormalities detected on a radiograph (**left**). Although she received DMARD therapy for 3 years, her knee pain did not improve and joint destruction progressed (**middle**). At 5 years, she received a total knee replacement (**right**).

ROS levels (see Appendix, note 1) of healthy controls and RA patients (see Appendix, note 2). Plasma ROS levels were significantly higher in subjects with RA [healthy controls: 254 ± 36 Carr units (CARR U); RA subjects: 432 ± 92 CARR U; data are expressed as the means ± SD; $*p < 0.01$ versus control] (Fig. 2). Although the overproduction of ROS by RA neutrophils has been shown (6), the clinical relevance has not been apparent. This is the first report demonstrating an enhanced plasma redox state in subjects with RA. Several different pathways can lead to the increased formation of ROS in inflamed joints (11). This enhanced level of oxidants plays a significant role in tissue damage and the inflammation-perpetuating process in the rheumatoid synovium.

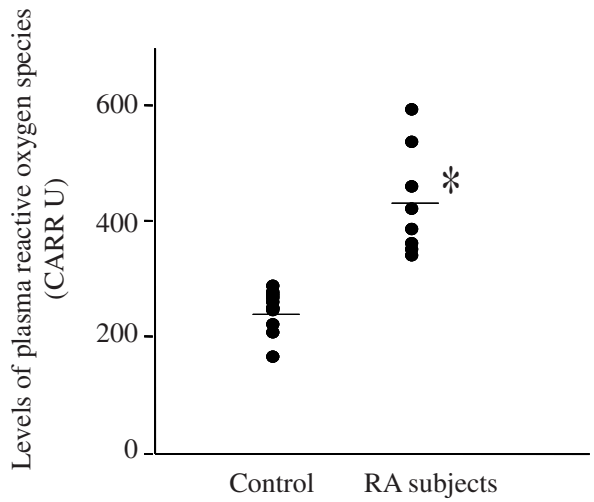

FIG. 2. Plasma ROS levels of healthy age- and sex-matched controls and subjects with RA before infliximab treatment. All subjects with RA were diagnosed according to the revised ACR criteria. Levels of plasma ROS were measured using d-ROMs assay kits. Bars represent the means. $*p < 0.01$.

For some time, C-reactive protein (CRP) and the rheumatoid factor have been used to determine the pathological activity of RA. However, the relationship between these two parameters and the susceptibility to cartilage degradation, which is the most important determinant of the prognosis in RA, is notclear. In this context, Tanaka *et al.* and Yamazaki *et al.* reported that ROS caused the degradation of articular cartilage *in vitro* (19, 23). Accordingly, it is possible that blood levels of ROS may become a marker of disease activity in RA patients.

INFLIXIMAB REDUCED ROS LEVELS IN RA PATIENTS

We hypothesized that the plasma redox state is a marker for disease activity in RA. If so, there would be alterations in plasma ROS levels in parallel with disease activity. In the present study, we measured the plasma ROS levels of subjects with RA who were treated with infliximab (see Appendix, note 3). To examine the efficacy of infliximab, we determined the American College of Rheumatology (ACR) response (see Appendix, note 4), which is the standard parameter used to assess the treatment. The ACR20 response was significant for infliximab at each evaluation throughout the study, *i.e.,* 88, 88, and 75%, at 2, 6, and 10 weeks, respectively. We also examined the alteration of plasma ROS levels and found a dramatic decrease in almost all cases treated with infliximab (Fig. 3). Only one subject out of eight did not respond to infliximab; her plasma ROS levels did not decrease and, instead, slightly increased during the infliximab treatment (Fig. 3). In this one case, after 10 weeks of treatment with infliximab, different DMARDs were challenged. According to the ACR20 response criteria at 10 weeks, the other seven subjects with RA were determined to be responders to infliximab. We next examined the alterations of plasma ROS levels in these responders. Significant decreases in plasma ROS levels were clear (Fig. 4). Therefore, treatment with infliximab resulted in not only the improvement of RA symptoms, but also a decreased plasma redox state. However, not all subjects with RA respond to this TNF-α blocker, as in the case of one member of the current study group. In some cases, side effects, such as tuberculous and fungal infections, were reported with infliximab treatment. In these cases, an important practical question is whether it is worthwhile to prescribe the other TNF-α blocker (21) or whether this is simply a waste of time and money, in terms of a cost–benefit analysis (10, 13). It is important that a precise and sensitive marker to define responders to TNF-α blocker treatment be found (22).

In conclusion, the plasma redox state may be a new marker for monitoring the progression of RA, although long-term and randomized studies will be necessary to determine the precise role of ROS.

ACKNOWLEDGMENTS

This work was supported by a research grant from the Sports Medicine Facilitating Program in Kinki University and

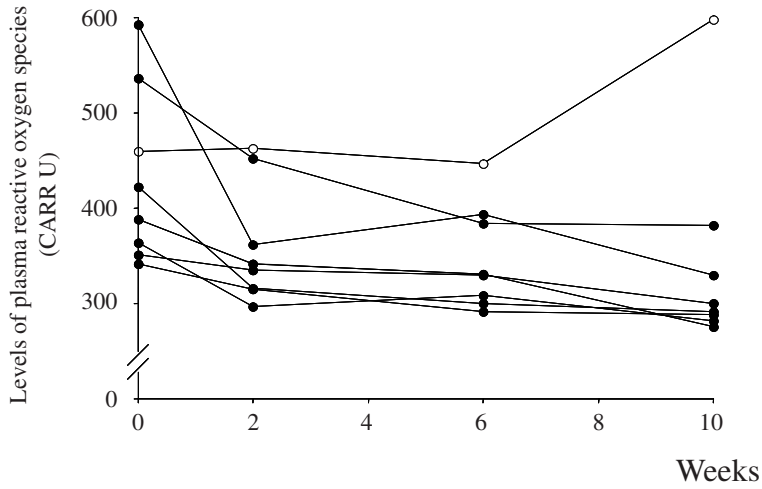

FIG. 3. Alteration in the plasma ROS levels in subjects with RA treated with infliximab. Levels of plasma ROS were measured using the d-ROMs assay kit. Almost all patients receiving infliximab showed an early decline in the number of swollen and tender joints (responder, ●). However, one patient did not respond to infliximab and, after 10 weeks, different DMARDs were challenged (○).

the Scientific Research Fund of the Ministry of Education, Science and Culture of Japan.

ABBREVIATIONS

ACR, American College of Rheumatology; CARR U, Carr unit; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; MTX, methotrexate; RA, rheumatoid arthritis; ROS, reactive oxygen species; SJC, swollen joint count; TJC, tender joint count; TNF-α, tumor necrosis factor-α.

APPENDIX

1. Levels of plasma ROS were measured using the d-ROMs assay kit (Diacron, Grosseto, Italy). The d-ROMs test is a spectrophotometric test that permits the assessment, in a biological sample, of the concentration of hydroperoxides (4, 11). In brief, a drop of blood obtained from the subject is mixed with a chromogenic substrate. Depending on the concentration of hydroperoxides in the drop of blood, a colored derivative (pink to red) develops. This colored complex is detectable and quantifiable using an integrated analytical system consisting of a dedicated photometer with incorporated centrifuge (FRAS3, Diacron). The concentration of hydroperoxides, which directly correlates with detected color intensity, is expressed as arbitrary units (CARR U).
2. The study was conducted in the Department of Orthopaedic Surgery of the Kinki University School of Medicine, Osaka, Japan. The study group consisted of eight female RA subjects (mean age: 41.7 ± 7.3 years) who were treated with infliximab. Patients were eligible for this study if they had a diagnosis of RA according to the revised 1987 American College of Rheumatology (ACR) criteria (1). Fourteen healthy female individuals (mean age: 38.3 ± 9.5 years) formed the control group.
3. Infliximab was intravenously administered, as instructed by the manufacturer, as a 3 mg/kg infusion, at baseline (0 weeks) and at 2 and 6 weeks. To determine plasma redox state, blood was collected at baseline and at 2, 6, and 10 weeks.
4. Clinical and laboratory efficacy assessments were conducted on subjects with RA at baseline and at 2-week intervals for 10 weeks. The ACR core criteria were monitored at each study visit: tender joint count (TJC) and swollen joint count (SJC); the assessment of pain, disease activity, and disability by the subject; the global assessment of disease activity by the doctor; and the erythrocyte sedimentation rate and CRP levels (7). The primary measure of efficacy was the number of subjects meeting ACR20 response criteria at 10 weeks. Subjects were classified as responders if they fulfilled the ACR20 core criteria for a clinical response to treatment (that is, >20% improvement in TJC and SJC, plus >20% improvement in three or more of the following five criteria: the assessment of pain, disease activity, or disability by the subject; the global assessment of disease activity by the doctor; and plasma CRP levels).

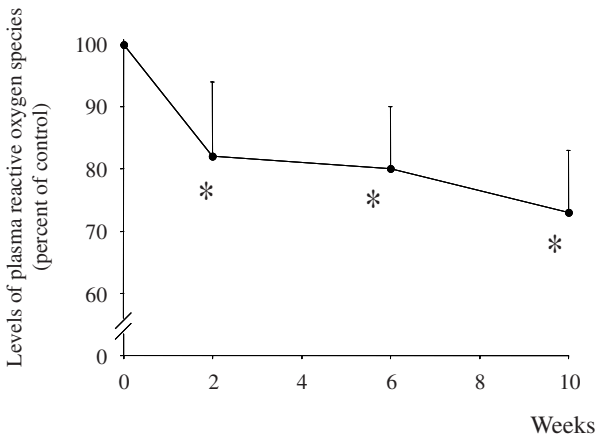

FIG. 4. Alteration in the plasma ROS levels in responders. Data are expressed as the percentage of baseline control, *i.e.,* ROS levels before treatment in each patient. Circles and bars represent the means and the standard deviations, respectively. *$p < 0.05$.

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Received for publication December 15, 2004; accepted March 1, 2005.