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Anti-tumour necrosis factor alpha therapy normalises fibrinolysis impairment in patients with active rheumatoid arthritis

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ABSTRACT

Objectives. *Rheumatoid arthritis (RA) is associated with increased cardiovascular risk and involvement of inflammation, coagulation and fibrinolysis. Treatment with infliximab, a tumour necrosis factor-α (TNF-α) blocking chimeric monoclonal antibody, induces a long-term reduction of inflammation and coagulation, but its effect on fibrinolysis is still unknown. We carried out an observational study investigating plasma biomarkers of inflammation and fibrinolysis in RA patients before and after 14 weeks of infliximab treatment given according to the therapeutic guidelines for RA.*

Methods. *We studied 20 selected patients with active RA and without any other atherosclerosis risk factor as well as 40 healthy controls. Patients, treated with a stable dose of methotrexate, received infliximab (3 mg/kg) at week 0, 2, 6 and 14. At week 0 and 14, we assessed clinical, inflammatory and fibrinolyitic parameters.*

Results. *At baseline, plasminogen activator inhibitor (PAI-1) antigen, PAI-1 activity and tissue-type plasminogen activator (t-PA) antigen were significantly higher in RA patients than in controls (p=0.01, p=0.001 and p=0.0001 respectively). After 14 weeks of infliximab treatment, the levels of PAI-1 antigen, PAI-1 activity and t-PA antigen significantly decreased till normalization (p=0.0001). Plasma levels of C reactive protein (CRP) and interleukin-6 (IL-6) were directly correlated with levels of PAI-1 antigen (p=0.011 and p=0.0001), PAI-1 activity (p=0.013 and p=0.027) and t-PA antigen (p=0.017 and p=0.040).*

Conclusions. *This study provides evidence that TNF-α blockade by infliximab not only decreases inflammation, but also reduces the inhibition of fibrinolysis. Such a combined effect may be pivotal in reducing the whole thrombotic risk in these patients.*

Introduction

It is increasingly recognised that patients with rheumatoid arthritis (RA) have a greater risk of premature death than the general population, mainly due to cardiovascular disease (CVD) (1). According to published studies (2, 3), RA is an independent risk factor for the development of CVD, and this can be explained at least in part by a direct impact of extensive chronic inflammation. In fact, it has been shown that the activation of the inflammatory cytokine network induces several pro-thrombotic conditions like insulin resistance, dyslipidemia, endothelial dysfunction and alteration of coagulation and fibrinolysis (4). In addition, the activation of the extrinsic coagulation system and the impairment of the fibrinolytic pathway may contribute to amplify and perpetuate the inflammatory response. The local imbalance between fibrin formation and removal results in the accumulation of fibrin deposits in the RA synovium (5). Fibrin degradation could be further impaired by anti-citrullinated protein antibodies (ACPA) which bind to citrullinated fibrin (6). The systemic imbalance between coagulation and fibrinolytic pathways, which has been reported in RA patients, may sustain a pro-thrombotic state (7-9).

The pro-inflammatory cytokine, tumour necrosis factor-α (TNF-α) is a pivotal mediator of the inflammatory cascade in RA, and it is also involved in modulating the expression of all of the major components of the fibrinolytic system (10). In RA patients, the treatment with the anti-TNF-α chimeric monoclonal antibody infliximab showed an improvement of clinical and laboratory parameters as well as a reduction in the activation of coagulation and endothelial dysfunction (11, 12). In particular, Agirbasli and colleagues (11) highlighted the potential short-term effect, after a single infliximab infusion, on fibrinolytic parameters in eight RA patients, however the fibrinolytic changes in the relatively long-term period and their correlation with cytokine levels in RA remains to be defined.

Thus, in the present study we investigated, in a cohort of patients with active RA and without any other atherosclerosis risk factor, the effects of infliximab on circulating fibrinolytic biomarkers and their correlation with clinical and inflammatory parameters as TNF-α, interleukin-6 (IL-6) and C-reactive protein (CRP) levels.

Materials and methods

Patients

The study involved 20 RA patients (15 women and five men) attending the Department of Rheumatology at Istituto Gaetano Pini, Milano, Italy, who fulfilled the revised American College of Rheumatology (formerly the American Rheumatism Association) criteria for the classification of RA (13). Their median age was 54.8 years (range 24-63 years) and the median disease duration was 6.1 years (range 5 months to 27 years). All patients had active disease with a 28-joint disease activity score (DAS-28) of at least 3.2 and were treated with a stable dose of methotrexate (10-15mg/week) and prednisone (5mg a day). None of our patients was assuming non-steroidal antiinflammatory drugs (NSAIDs). These patients, previously studied for coagulation activation (12), have been re-evaluated for atherosclerosis risk factors: they had normal blood pressure, lipidemia, glycemia, body mass index and absence of cigarette smoking.

At week 0, 2, 6 and 14, they were treated with infliximab at the standard intravenous dose of 3 mg/kg, according to the therapeutic international guidelines for RA (14). During routine clinical assessment with DAS-28 and laboratory evaluation, patients gave their consent to use their blood for measuring fibrinolytic parameters which were assessed at week 0, before infliximab treatment, and at week 14. The study was approved by the local review board and informed consent was obtained from all patients.

Controls

The control group consisted of 40 age- and sex-matched healthy subjects. They were blood donors with no personal or family history of thrombosis and without traditional cardiovascular risk factors.

Blood sampling

Morning fasting blood samples were collected in vacutainer tubes (Beckton & Dickinson, Rutherford, NJ) by means of the clean puncture of an antecubital vein with minimal stasis, using sodium citrate 3.8% as anticoagulant. The blood collection was performed before infusion of infliximab (baseline) and before the forth infusion at week 14, to evaluate its effect. The samples were centrifuged at 2000 x g, at 4°C, divided into aliquots, frozen and stored at -80°C until testing.

Fibrinolysis parameters

Plasminogen activator inhibitor type 1 (PAI-1) antigen was measured by a commercial enzyme-linked immunosorbent assay – ELISA (Innotest PAI-1, Byk Gulden, Konstanz, Germany). Intra- and inter-assay coefficients of variation (CVs) were 8% and 13%, respectively.

PAI-1 activity was measured using a commercial bioimmunoassay (Chromolize PAI-1; Biopool, Umea, Sweden) with intra- and inter-assay CVs of 2.4% and 4.5%.

Tissue plasminogen activator (t-PA) antigen was measured by a commercial enzyme-linked immunoassay method (Imunolyse tPA; Biopool, Umea, Sweden) according to the manufacturer's instructions. Intra- and inter-assay CVs were 6.5% and 8%, respectively.

Inflammatory parameters

TNF-α plasma levels were measured using a direct solid-phase immunoassay (Enzyme Amplified Sensitivity Immunoassay; EASIA, Biosource, Flerus, Belgium); with intra- and inter-assay CVs of 8% and 10%.

IL-6 levels were measured in plasma by an ELISA (R&D Systems, Minneapolis, MN). Intra- and inter-assay CVs were 4.2% and 6.4%.

C-reactive protein (CRP) concentration was measured using a sandwich ELISA (Zymutest CRP, Hyphen BioMed, Neuville-sur-Oise, France). Intra- and inter-assay CVs were lower than 11%.

Statistical analysis

The descriptive statistics are reported as median and interquartile range (25^{th} and 75^{th} percentiles). Statistical analysis was performed by non-parametric tests for independent samples when differences between groups were evaluated (Mann-Whitney) and for paired samples when the effect of treatment was assessed (Wilcoxon). Significance level was set at $p<0.05$. Data were analysed using the SPSS PC statistical package, version 17.00 (SPSS Inc., Chicago, IL).

Results

As shown in Fig. 1, at baseline, PAI-1 antigen and active PAI-1 were significantly higher in RA patients, median 22.28 ng/ml (interquartile range 10.18–61.95 ng/ml) and 8.77 ng/ml (7.12-12.04 ng/ml), than in healthy controls 11.80 ng/ml (5.90-21.75 ng/ml) and 4.00 ng/ml (1.50-7.05 ng/ml) (p=0.01 and p=0.001 respectively); t-PA was also significantly higher in patients, 46.75 ng/ml (13.33-71.05 ng/ml), than in controls, 7.55 ng/ml (4.60-12.02 ng/ml) (p=0.0001).

After 14 weeks of infliximab treatment, the levels of PAI-1 antigen and active PAI-1 significantly decreased to 9.49 ng/ml (4.30-15.65 ng/ml) and 4.93 ng/ml (0.57-8.88 ng/ml) (p=0.0001 for both) (Fig. 2); t-PA was also significantly lower than baseline after 14 weeks, 8.16 ng/ml (4.58-31.63 ng/ml) (p=0.0001). The median changes between baseline and week 14 were: PAI-1 antigen -9.52 ng/ml (-3.64-34.83 ng/ml), PAI activity -4.88 ng/ml (-2.16-21.57 ng/ml) and t-PA -9.00 ng/ml (-4.45-16.32 ng/ml). At week 14, the three parameters reached the normal range without any significant difference with normal controls.

Plasma levels of CRP were directly correlated with levels of PAI-1 antigen (r=0.569, p=0.0001), PAI-1 activity (r=0.410, p=0.013) and t-PA antigen (r=0.394, p=0.017). Also for plasma levels of IL-6, a direct correlation was found with levels of PAI-1 antigen (r=0.421, p=0.011), PAI-1 activity (r=0.368, p=0.027) and t-PA antigen (r=0.343, p=0.040). None of the fibrinolytic parameters correlated with TNF-α plasma levels. Finally, DAS-28 score was directly correlated with PAI-1 antigen (r=0.414, p=0.01), PAI-1 activity (r=0.343, p=0.047) and t-PA antigen (r=0.370, p=0.031).

Discussion

The results of our study in a group of patients with active RA and without any other atherosclerosis risk factor show that fibrinolysis is inhibited,

mainly due to an increase in plasma levels of PAI-1 both as activity and antigen. Infliximab treatment reduces both inflammation and the inhibition of fibrinolysis.

The fibrinolytic system is finely regulated by means of plasminogen activators such as t-PA, and natural inhibitors such as PAI-1. Among the plasminogen activators, t-PA is thought to play a relevant role in initiating fibrinolysis and thrombolysis. The high baseline circulating levels of t-PA antigen in our patients are not contradictory, because the t-PA immunoassay measures, to a large extent, circulating complexes between t-PA and PAI-1. Consequently, increased concentrations of t-PA antigen give indirect information on PAI-1 expression and indicate a reduced rather than increased fibrinolysis (15). The small number of our patients could be counterbalanced by the absence of traditional risk factors for atherosclerosis potentially affecting fibrinolysis.

Activation of coagulation and fibrin deposition are a consequence of inflammation and the impairment of fibrinolytic system has been previously reported in RA patients (7), and found to be associated with disease activity and rheumatoid vasculitis. PAI-1 is the most important endogenous suppressor of fibrinolysis, powerfully upregulated by TNF-α (10), and elevated plasma levels of PAI-1 have been linked to an increase in the risk for vascular complications in several diseases as well as in RA (10, 16).

We provide evidence that the clinical beneficial effects, induced by intravenously administered anti-TNF-α after 14 weeks of therapy, are associated with a significant decrease in PAI-1 levels which correlate with IL-6 and acute-phase reactants such as CRP. This finding supports the view that normalisation of fibrinolysis is likely related to the reduction of inflammation in RA patients. The lack of correlation between PAI-1 and TNF-α plasma levels may be due simply to the fact that the ELISA for TNF-α antigen may detect not only active TNF-α but also biologically inactive TNF-α/anti-TNF-α complexes (17). However, it is well known that plasma levels of TNF-α measured by ELISA are not affected by anti-TNF-α therapy (17).

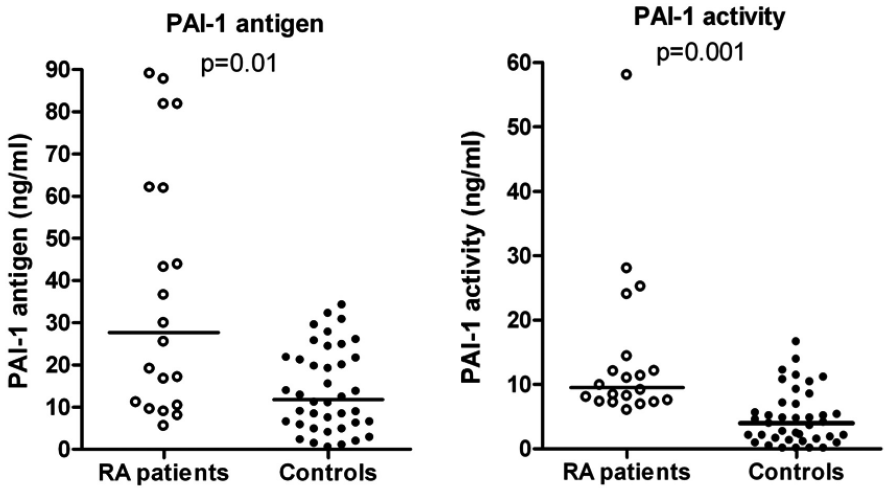

Fig. 1. Plasma levels of plasminogen activator inhibitor type 1 (PAI-1) antigen and PAI-1 activity in 40 healthy controls and 20 rheumatoid arthritis patients. Horizontal lines represent medians.

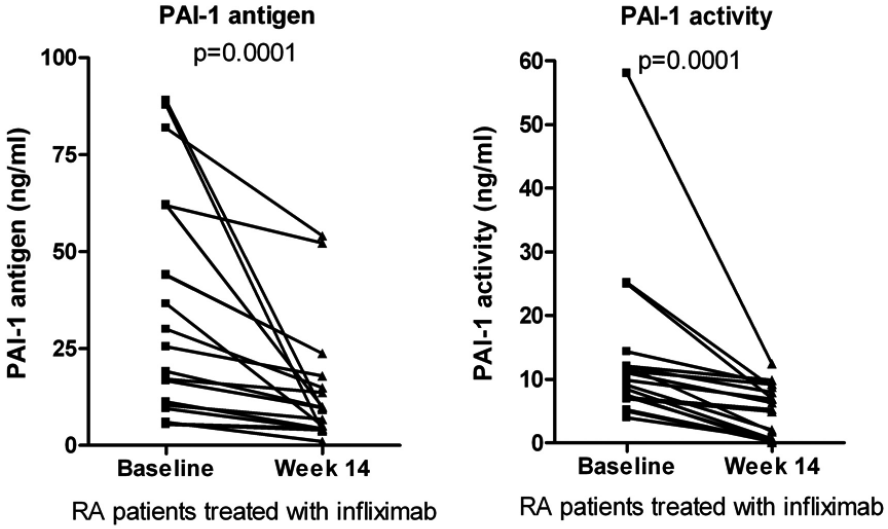

Fig. 2. Effects of infliximab treatment on plasma levels of plasminogen activator inhibitor type 1 (PAI-1) antigen and PAI-1 activity in 20 rheumatoid arthritis (RA) patients before (baseline) and after 14 weeks of treatment.

Overall, the reduction of fibrinolysis inhibition together with the reduction of coagulation (12) described in RA patients treated with infliximab, may contribute in reducing the whole thrombotic risk. In contrast in active RA patients, the inhibition of fibrinolysis may have two main consequences. On one hand, it may play a role in joint damage because it has been demonstrated that extravasation of plasma fibrinogen in RA synovial tissues rapidly clotted and led to a local deposition of fibrin which may contribute to perpetuate synovial inflammation and joint damage (5, 6). On the other hand, inhibition of fibrinolysis may have important consequences in the systemic circulation; in fact, elevated plasma levels of PAI-1 are generally considered a CVD risk factor (11). Clinical and experimental evidence suggests that long-term effects of PAI-1 are crucial in vascular diseases, and therefore, the known increased risk for cardiovascular events in RA can be partially attributed to the inhibition of fibrinolytic system and endothelial dysfunction (10). Thus, the decrease in the inhibition of fibrinolysis related to anti-TNF-α therapy may act both locally, reducing the joint inflammation/damage, and at a systemic level reducing the thrombotic risk. This latter aspect is further supported by the

previous observation that anti-TNF-α therapy reduces prothrombotic markers of coagulation activation and fibrin degradation (12).

Key messages

Active rheumatoid arthritis in the absence of other atherosclerosis risk factors shows fibrinolysis inhibition mainly due to an increase of PAI-1.
Infliximab reduces inflammation and fibrinolysis inhibition, thus potentially reducing thrombotic risk.

References

1. MARADIT-KREMERS H, CROWSON CS, NICOLA PJ *et al*.: Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005; 52: 402-11.
2. SOLOMON DH, CURHAN GC, RIMM EB, CANNUSCIO CC, KARLSON EW: Cardiovascular risk factors in women with and without rheumatoid arthritis. *Arthritis Rheum* 2004; 50: 3444-9.
3. WÅLLBERG-JONSSON S, JOHANSSON H, OHMAN ML, RANTAPÄÄ-DAHLQVIST S: Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. *J Rheumatol* 1999; 26: 2562-71.
4. CUGNO M, INGEGNOLI F, GUALTIEROTTI R, FANTINI F: Potential effect of anti-tumour necrosis factor-alpha treatment on reducing the cardiovascular risk related to rheumatoid arthritis. *Curr Vasc Pharmacol* 2010; 8: 285-92.
5. BUSSO N, HAMILTON JA: Extravascular coagulation and the plasminogen activator/plasmin system in rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 2268-79.
6. SEBBAG M, MOINARD N, AUGER I *et al*.: Epitopes of human fibrin recognized by the rheumatoid arthritis-specific autoantibodies to citrullinated proteins. *Eur J Immunol* 2006; 36: 2250-63.
7. LAU CS, MCLAREN M, HANSLIP J, KERR M, BELCH JJ: Abnormal plasma fibrinolysis in patients with rheumatoid arthritis and impaired endothelial fibrinolytic response in those complicated by vasculitis. *Ann Rheum Dis* 1993; 52: 643-9.
8. KOPEIKINA LT, KAMPER EF, KOUTSOUKOS V, BASSIAKOS Y, STAVRIDIS I: Imbalance of tissue-type plasminogen activator (t-PA) and its specific inhibitor (PAI-1) in patients with rheumatoid arthritis associated with disease activity. *Clin Rheumatol* 1997; 16: 254-60.
9. WÅLLBERG-JONSSON S, DAHLEN GH, NILSSON TK, RANBY M, RANTAPÄÄ-DAHLQVIST S: Tissue plasminogen activator, plasminogen activator inhibitor-1 and von Willebrand factor in rheumatoid arthritis. *Clin Rheumatol* 1993; 12: 318-24.
10. MEDCALF RL: Fibrinolysis, inflammation, and regulation of the plasminogen activating system. *J Thromb Haemost* 2007; 5 (Suppl. 1): 132-42.
11. AGIRBASLI M, INANC N, BAYKAN OA, DIRESKENELI H: The effects of TNF alpha inhibition on plasma fibrinolytic balance in patients with chronic inflammatory rheumatical disorders. *Clin Exp Rheumatol* 2006; 24: 580-3.
12. INGEGNOLI F, FANTINI F, FAVALLI EG *et al*.: Inflammatory and prothrombotic biomarkers in patients with rheumatoid arthritis: effects of tumor necrosis factor-alpha blockade. *J Autoimmun* 2008; 31: 175-9.
13. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
14. SAAG KG, TENG GG, PATKAR NM *et al*.: American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis Rheum* 2008; 59: 762-84.
15. MANNUCCI PM, BERNARDINELLI L, FOCO L *et al*.: Tissue plasminogen activator antigen is strongly associated with myocardial infarction in young women. *J Thromb Haemost* 2005; 3: 280-6.
16. WÅLLBERG-JONSSON S, CEDERFELT M, RANTAPÄÄ-DAHLQVIST S: Hemostatic factors and cardiovascular disease in active rheumatoid arthritis: an 8-year follow-up study. *J Rheumatol* 2000; 27: 71-5.
17. CHARLES P, ELLIOTT MJ, DAVIS D *et al*.: Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. *J Immunol* 1999; 163: 1521-8.