Influence of anti-TNF-α infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis

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Abstract

Objective

Chronic systemic inflammation plays a pivotal role in the development of atherosclerosis in rheumatoid arthritis (RA). Soluble (s) adhesion molecules were found significantly increased in RA patients with active disease. Since increased levels of some adhesion molecules were closely linked to the development of endothelial dysfunction and atherosclerosis and administration of anti-TNF-α-infliximab resulted in a rapid and dramatic improvement of endothelial function in long-term infliximab treated RA patients, we assessed whether infusion of the chimeric anti-TNF-α infliximab might also yield a rapid and favorable effect on serum levels of soluble adhesion molecules in RA patients periodically treated with this drug because of severe disease.

Methods

We recruited patients with RA refractory to conventional therapy seen over a period of 2 months at Hospital Xeral-Calde, Lugo, Spain, who were on periodical treatment with infliximab for at least 14 weeks. Blood samples for determination of sICAM-1, sICAM-3, sVCAM-1, sE-selectin, and sP-selectin levels by ELISA were taken immediately before and after infliximab infusion.

Results

Thirty-four RA patients (25 women; mean age: 55.4 years; mean DAS28: 4.27) fulfilled the inclusion criteria. Following infliximab infusion a reduction of the overall mean values of the five adhesion molecules was observed. However, when a Wilcoxon signed-rank test was used, only significant differences for sICAM-3 and sP-selectin were observed. In this regard, sICAM-3 and sP-selectin levels fell in 26 (77%) and 28 (82%) of the 34 patients.

Conclusions

Our study confirms a rapid and beneficial effect of infliximab infusion on expression of some adhesion molecules in RA patients treated periodically with this anti-TNF-α monoclonal antibody because of severe disease.

Key words

Rheumatoid arthritis, inflammation, endothelial dysfunction, atherosclerosis, anti-TNF-α antibody-infliximab, adhesion molecules, sICAM-3 and sP-selectin.

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Received on October 26, 2005; accepted in revised form on February 28, 2006.

Introduction

Rheumatoid arthritis (RA) is a disease associated with increased cardiovascular (CV) morbidity and mortality (1). The augmented risk of CV events in patients with RA is a consequence of atherosclerosis (2). As reported in Japan and Korea (3, 4), we have also confirmed the presence of severe subclinical atherosclerotic disease in actively treated Caucasian RA patients from Northwest Spain without clinical evidence of atherosclerosis or cardiovascular risk factors (5).

Chronic systemic inflammation is considered of major importance for the development of atherosclerosis in RA (6). In this regard, in RA patients without classic atherosclerosis risk factors the presence of increased carotid intima-media thickness was associated with increasing quartiles of C-reactive protein (CRP) (7). This observation supports a direct relationship between the magnitude of the systemic inflammation and the development of CV events in RA (7).

Chronic increase of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 causes deleterious effects including a proatherogenic lipid profile, insulin resistance and endothelial dysfunction in RA (6).

Endothelial dysfunction, an early step in the atherosclerosis process, has been observed in young RA patients with low disease activity and in long-term actively methotrexate (MTX) treated RA patients without clinically evident cardiovascular disease (8, 9). Several mechanisms that link systemic inflammation may promote the development of endothelial dysfunction in RA (6).

Targeted TNF-α antagonists have had a significant impact on the treatment of patients with RA. In general, the benefit/ risk ratio for these agents has been quite favorable (10). Both short-term and long-term TNF-α blockade using the antagonist drug infliximab reduced disease activity and significantly improved endothelial function in RA patients (11, 12). We have recently observed that RA patients with severe disease on periodical treatment with the chimeric anti-TNF-α monoclonal antibody infliximab, which specifically and with high affinity binds to TNF-α and neutralizes this cytokine, experienced a rapid and dramatic reduction in the serum insulin levels and insulin/-glucose index (13). Also, following infusion of this drug a rapid improvement of insulin resistance and insulin sensitivity was observed (13). Interestingly, in another recent study that assessed RA patients before and after 6 month's treatment with infliximab, Kiortsis *et al.* also observed an improvement of insulin resistance and insulin sensitivity (14).

Cellular adhesion molecules are widely expressed and function to mediate cell-cell and cell-matrix interactions. They have been identified as potential participants in atherogenesis. Elevated circulating levels of adhesion molecules such as intercellular cell adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1) are associated with CV risk factors and predict atherosclerosis and CV events (15-17). Also the selectins, a family of lectin-like molecules expressed on the surfaces of endothelial cells, leukocytes, and platelets, have also been implicated in atherogenesis by mediating leukocyte rolling on the vascular endothelium and by facilitating platelet-leukocyte interaction. E-selectin is expressed exclusively by activated vascular endothelium whereas P-selectin mediates platelet-neutrophil interactions, which may be a means by which inflammation promotes a prothrombotic state (18). Levels of soluble (s) P-selectin are elevated during acute coronary syndromes, and also independently predict risk of future cardiac events in women (19, 20).

Levels of soluble adhesion molecules were found significantly increased in RA patients with active disease (18, 21). Since increased levels of some soluble adhesion molecules such as VCAM-1 were closely linked to the development of endothelial dysfunction and atherosclerosis (21), and administration of anti-TNF-α–infliximab resulted in a rapid and dramatic improvement of endothelial function in long-term RA patients treated with this drug (12), we assessed whether infu-

sion of the chimeric anti-TNF-α infliximab might also yield a rapid and favorable effect on serum levels of soluble adhesion molecules in RA patients periodically treated with this drug because of severe and refractory disease.

Patients and methods

Patients

Patients who met the 1987 American College of Rheumatology classification criteria for RA (22) and were treated by the same group of rheumatologists (MAG-G, CG-P and AS-A) were recruited from Hospital Xeral-Calde, Lugo, Northwest Spain. The cohort constituted a series of patients attending hospital outpatient clinics seen over a period of 2 months (February-March 2004).

Since the purpose of this study was to assess the potential immediate effect of anti-TNF-α infliximab administration on serum levels of soluble adhesion molecules in RA patients on periodical treatment with this drug, only infliximab treated patients for at least 14 weeks due to severe and refractory disease were included. For ethical reasons, patients included in the present study were not randomized to a placebo group. The same procedure has been found acceptable and followed in a recent study on the effect of infliximab therapy on the lipid profile in patients with RA (23).

In all cases RA patients were switched from standard therapy to anti-TNF-alpha-infliximab treatment because of severe and active disease (DAS28 greater than 5.1) (24). In all patients, treatment with a modifying antirheumatic drug (DMARD) had been initiated when a diagnosis of RA was made. Prior to anti-TNF-α therapy patients were required to have been treated with at least two DMARD drugs including chloroquine, sulphasalazine, gold, MTX (at least 15 mg/week), leflunomide, and cyclosporine A (3 mg/Kg/day). Infliximab therapy (in all cases an initial dosage of 3 mg/kg) was administered intravenously at 0, 2, 6 weeks and then every 8 weeks. However, in some patients, because disease severity, dosage was increased to 5 mg/Kg and if required interval between infliximab infusions was shortened to 6 weeks.

Besides non-steroidal anti-inflammatory drugs, all had received treatment with low doses of prednisone (generally 5 mg bid) immediately after disease diagnosis. When this study was performed all patients were on treatment with MTX (range 15 to 25 mg/week) or leflunomide (20 mg/day) with or without chloroquine (250 mg/day) and prednisone (range 2.5 to 7.5 mg/day) plus infliximab 3 or 5 mg/kg/intravenously every 6 or 8 weeks according to disease severity.

The local institutional committee approved anti-TNF-α therapy. Also, patients gave informed consent to participate in this study. Neither this study nor the former ones (12, 13) were supported by any pharmaceutical drug company.

Study protocol

In each patient a disease activity score (DAS)28 (24) was assessed by the same rheumatologist (MAG-G) prior to infliximab infusion (the same day). In all cases the drug was given at 8 a.m. as intravenous infusion in saline solution over 120 minutes. None of the patients received any nutrient before and during infusion.

Fasting blood samples, including full blood cell count, routine biochemistry profile, ESR (Westergren) and CRP (by latex immunoturbidity method) were taken and determined the same day of infliximab infusion. In addition, blood sampling for further determination of levels of soluble adhesion molecules were performed immediately prior to the onset of infliximab infusion (time 0) and just at the end of infliximab infusion (time 120).

Adhesion molecule determination was performed as follows:

Blood was taken into a tube containing coagulant beads, left for one hour at 37ºC and then centrifuged at 3500 rpm for 15 minutes at 4ºC. The serum was then removed, aliquoted and stored at -70ºC. Afterwards sICAM-1, sICAM-3, sVCAM-1, sE-selectin, and sP-selectin were all measured in serum using ELISA kits from Bender MedSystems (Vienna, Austria). The intra-assay coefficient of variation and sensitivity (respectively) were 4.1% and 3.3 ng/ml for sICAM-1, 2.5% and 0.58 ng/ml for sICAM-3, 3.1% and 0.9 ng/ml for sVCAM-1, 5.4% and 0.5 ng/ml for sE-selectin, and 2.4% and 1.06 ng/ml for sP-selectin.

Statistical analyses

Clinical data were expressed as mean ± standard deviation (SD), median and range or interquartile (IQ) range. CRP and ESR values were shown as median and IQ range. Considering the whole group of patients overall data on each soluble adhesion molecule prior (time 0) and after infliximab infusion (time 120) were expressed as mean ± SD, range, median and IQ range.

Paired serial results for each soluble adhesion molecule in individual RA patients before (time 0) and postinfusion (time 120) were compared using the Wilcoxon signed-rank test for matched observations. All results were presented as two-tailed values. Correlations were sought using the Spearman Rank correlation test. Statistical significance was accepted at $p < 0.05$.

Results

Thirty-four patients fulfilled the inclusion criteria. The main features of the patients included in the study are summarized in Table I.

In all cases when they were switched from standard conventional therapy to infliximab treatment DAS28 was greater than 5.1. However, at the time of this study and following periodical infliximab therapy most of them had experienced clinical improvement manifested by reduction in DAS28 score compared to that found prior to the onset of anti-TNF-α–infliximab treatment. Because of that, at the time of the study, only 7 (20.6%) of the 34 patients had severe disease (DAS28 > 5.1). Nonetheless, despite periodical treatment with infliximab, most patients still had active disease and only 3 exhibited a DAS28 < 2.6 (Table I).

As shown in Table II, following infliximab infusion a reduction of the overall mean values of the five soluble adhesion molecules was observed when baseline data (time 0) were compared

Table I. Description of 34 patients on periodical treatment with anti-TNF-α (infliximab) because of RA refractory to at least two DMARDs.

Women	25 (73.5%)
Age (years) (mean ± SD)	55.4 ± 12.6
median (range)	56.5 (24 –74)
BMI (Kg/m^2) (mean ± SD)	25.5 ± 4.3
median (range)	24.1 (22.6– 28.9)
BMI < 25	18 (52.9%)
BMI 25-30	10 (29.4%)
BMI > 30	6 (17.6%)
DAS28 (mean ± SD)	4.27 ± 1.18
median (IQ range)	4.38 (3.17–5.02)
CRP (mg/l)	
Median (IQ range)	5.5 (4.0 – 23.4)
ESR (mm/1st hour)	
Median (IQ range)	26.5 (14.0 – 39.0)
Infliximab dose at the time of the study	
3 mg / 8 weeks	20 (58.8%)
5 mg / 6 weeks	8 (23.5%)
5 mg / 8 weeks	6 (17.7%)

IQ: Interquartile.

Table II. Differences between basal (time 0) and postinfusion (time 120 minutes) serum levels of soluble adhesion molecules in 34 patients with RA periodically treated with anti-TNF-α (infliximab).

	Basal (time 0) Mean ± SD** (range)	Postinfusion (time120) Mean ± SD (range)	p*
sICAM-1 (ng/ml)	351.6 ± 102.1(166 – 712)	338.5 ± 112.5 (156 – 776)	0.103
sICAM-3 (ng/ml)	59.2 ± 15.6 (36 – 100)	55.2 ± 15.1 (36 – 100)	< 0.001
sVCAM-1 (ng/ml)	1109.0 ± 370.3 (597 – 2442)	1080.2 ± 345.3 (528 – 1930)	0.301
sE-selectin (ng/ml)	53.0 ± 27.5 (15 – 126)	52.0 ± 29.8 (12 – 130)	0.383
sP-selectin (ng/ml)	285.6 ± 352.0 (20 – 2110)	252.1 ± 327.7 (20 – 1775)	< 0.001
	Basal (time 0) Median (IQ range)	**Postinfusion (time120) Median (IQ range)**	
sICAM-1 (ng/ml)	351 (288 – 410)	329 (257 – 380)	
sICAM-3 (ng/ml)	54 (49 – 65)	52 (43 – 62)	
sVCAM-1 (ng/ml)	1016 (882 – 1317)	1010 (842 – 1230)	
sE-selectin (ng/ml)	46 (33 – 67)	45 (28 – 66)	
sP-selectin (ng/ml)	218 (133 – 316)	175 (97 – 248)	

* Wilcoxon signed-rank test for matched observations.
** For the whole group of 34 RA patients.
IQ: Interquartile.

with those found immediately after infliximab infusion (time 120).

However, when a Wilcoxon signed-rank test was used to compare differences between levels of soluble adhesion molecules before (time 0) and immediately after infliximab infusion (time 120) for each patient, only significant differences for sICAM-3 and sP-selectin were observed. In this regard, whereas sICAM-1 and sVCAM-1 levels after infliximab infusion compared to baseline levels only fell in 21 (62%) and 20 (59%) of the 34 patients, levels of sICAM-3 experienced a reduction after infliximab infusion in 26 (77%) of the 34 RA patients. Likewise, although only 18 (53%) of the 34 patients had reduction in sE-selectin levels, 28 (82%) of the 34 RA patients experienced reduction of sP-selectin levels following infliximab infusion. The high SD values observed in the study of sP-selectin were due to a single patient (number 15) that had 2110 ng/ml at time 0 and 1775 ng/ml at time 120 (postinfusion) (Table II).

Figures 1 and 2 show the reduction of levels of sICAM-3 and sP-selectin (respectively) observed in most patients following anti-TNF-α infliximab infusion.

As expected, a strong direct correlation between CRP serum levels and ESR was observed ($r = 0.704$; $p < 0.001$). It was also the case when the relationship between DAS28 and CRP ($r = 0.549$; $p < 0.001$) or ESR ($r = 0.616$; $p < 0.001$) was assessed.

Since sP-selectin in the circulation derives from more than one source including platelets and vascular endothelial cells, we also assessed the potential correlation between platelet count determined the same day of infliximab administration, before the onset of infliximab infusion, and adhesion molecules.

However, no significant correlation between DAS28 and serum levels of soluble adhesion molecules observed before or after infliximab infusion was found. In this regard, correlations between DAS28 and serum adhesion molecules before infliximab infusion ranged from 0.12 ($p = 0.51$) for sE-selectin to -0.25 ($p = 0.15$) for sP-selectin. Correlations between DAS28 and change in serum adhesion molecule levels after infliximab infusion ranged from 0.18 ($p = 0.30$) for sVCAM-1 to -0.28 ($p = 0.10$) for sE-selectin; these ranges are within the random variability. Likewise, no correlation between CRP, ESR or platelet count and levels of soluble adhesion were found (data not shown).

Discussion

Some inflammatory mediators such as

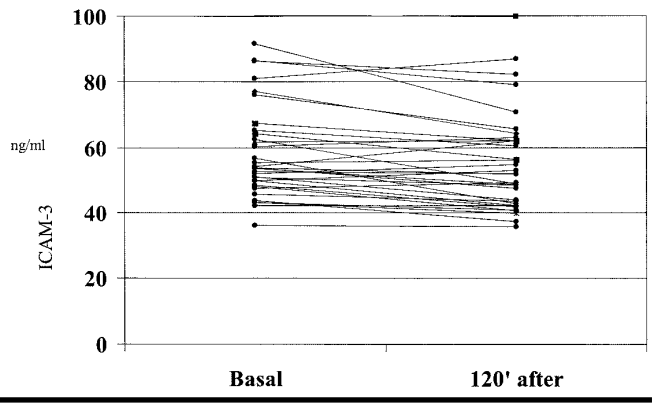

Fig. 1. Improvement of sICAM-3 in most patients following anti-TNF-α-infliximab infusion.

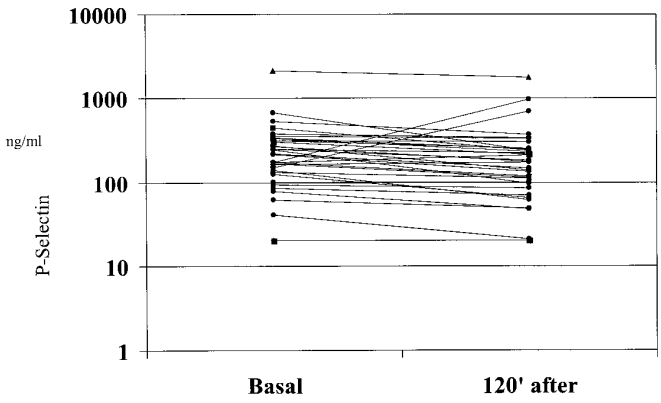

Fig. 2. Improvement of sP-selectin in most patients following anti-TNF-α-infliximab infusion.

adhesion molecules play a pivotal role in the atherogenesis and plaque vulnerability (25). RA is associated to accelerated atherogenesis and increased incidence of CV events (1, 2).

Increased serum levels of soluble adhesion molecules have been observed in RA patients (18, 21, 26-28).

Reduction of adhesion molecule serum levels following conventional DMARD therapy has previously been reported (18). With respect to this, in a series of 13 patients with active RA of recent onset, Veale *et al.* found a significant reduction of sICAM-1 and sP-selectin after 12 weeks of therapy with sulphasalazine (18). In this regard, over the 12 weeks of study sICAM-1 and sP-selectin fell in 8 and 7 of the 13 patients respectively. Also, the mean levels of sICAM-1 fell from 345.0 at baseline to 335.5 ng/ml at week 12 (18). An even greater reduction of the mean sP-selectin levels at 12 week (116.2 ng/ml) compared to baseline values (332.8 ng/ml) was observed (18). However, no significant differences in the sE-selectin and sVCAM-1 levels between values observed at baseline or at week 12 were observed (18). However, to our knowledge, there are no data in the literature assessing whether an immediate effect on reduction in some adhesion molecules may be observed following the infusion of anti-TNF-α–infliximab.

Chimeric monoclonal anti-TNF-α antibody-infliximab alone or in combination with low-dose MTX is an effective therapy in RA (10, 29). We previously reported that patients with severe RA on periodical treatment with infliximab, which specifically and with high affinity binds to TNF-α and neutralizes this cytokine, experienced a rapid improvement of endothelial function and insulin resistance (12, 13). However, the improvement of endothelial function after infliximab infusion was transient as values of endothelial dependent vasodilatation in RA patients returned to baseline levels by 4 weeks after infliximab infusion (12).

Taken together, in the present study we specifically sought to assess whether there was a change in serum levels of soluble adhesion molecules following infliximab infusion in RA patients receiving periodically this medication because of severe disease refractory to conventional therapy. Our results confirm a significant decrease of sICAM-3 and sP-selectin levels after infliximab infusion in most RA patients treated periodically with this drug. Although the reduction of some adhesion molecules observed in this study following infliximab therapy might be considered an epiphenomenon, considering that sulphasalazine therapy also yielded clinical improvement and reduction of some adhesion molecule serum levels, we feel that the reduction observed in sICAM-3 and sP-selectin in our series may be due to a direct effect of TNF-α blockade.

It is known that after activation by proinflammatory cytokines, cell adhesion molecules are shed from the surface of endothelial cells and leukocytes and levels of circulation cell adhesion molecules can be measured in serum. Due to this, these soluble adhesion molecules may be useful as markers of endothelial activation and dysfunction and vascular inflammation. In the present study a significant decrease of sICAM-3 but not of sICAM-1 was found. Although ICAM-3 shares considerable sequence homology with ICAM-1, it is not expressed by the by the endothelium of normal or inflamed synovial vessels. Intense expression of ICAM-3 by rheumatoid synovial lymphocytes and macrophages suggests that this adhesion molecule may play a role in processes requiring cell-cell contact, such as antigen presentation and homotypic aggregation (30). ICAM-3 can play a pivotal role in initiating a cascade of adhesion events, which may be crucial in immune activation and in the development of inflammatory lesions. (31). A study on different autoimmune diseases yielded significantly high levels of circulation forms of ICAM-3 in RA, systemic lupus erythematosus, Guillain-Barre syndrome, and multiple sclerosis, but not in type I diabetes, Grave's disease, chronic autoimmune thyroiditis, ulcerative colitis, or Crohn's disease (32). Interestingly, ICAM-3 levels were significantly higher in systemic lupus erythematosus patients with active disease (32). Although ICAM-3 and ICAM-1 bind to the same integrin receptor, there is no correlation of serum levels of circulating forms of ICAM-3 and ICAM-1 in either healthy individuals or in patients with immune-mediated diseases (32). Most patients with these conditions have either elevated ICAM-3 or ICAM-1 levels but not both (32). This independent behavior may explain why we observed a decrease of sICAM-3 but not of sICAM-1 in most of our RA after infliximab infusion.

Levels of sP-selectin were found to be an independent predictor of future CV risk in a large-scale prospective study of almost thirty thousand healthy women enrolled in the Women's Health Study (20). The overall mean levels of sP-selectin were significantly higher at baseline among those women who later suffered CV events (20). The risk of future CV events among women in the highest quartile of sP-selectin levels was 2.2 times higher than those in the lowest quartile. This was independent of the presence of traditional risk factors of atherosclerosis (20). According

to that, the presence of increased serum levels of sP-selectin in RA patients should be considered as a predictor of future CV complications. Interestingly, in our series infliximab infusion yielded a reduction of sP-selectin levels in most patients periodically treated with this therapy. However, we did not find a correlation between sP-selectin levels and CRP, ESR or DAS28 in our study. This was also the case for the other adhesion molecules assessed in the present study. Since we specifically aimed to determine the immediate effect of infliximab in patients receiving this drug periodically, it is possible that the lack of correlation between adhesion molecule serum levels and classic parameters of inflammation in our series of RA patients might be due to the fact that RA patients assessed in the present study were periodically on treatment with anti-TNF-α therapy at the time that serum adhesion molecule levels were determined. In keeping with our observations, Veale *et al.* did not find a correlation between the highly significant reduction of sP-selectin and CRP over a 12 week treatment with sulphasalazine (18).

In conclusion, as observed in clinical ultrasonographic studies that confirmed a rapid and positive effect of anti-TNF-α blockade in chronically infliximab treated RA patients (12), administration of this drug also yielded a positive effect in terms of decrease of some adhesion molecules. However, among the selectin adhesion molecules investigated in the present study, only P-selectin that is closely related to endothelial cells and their activation was significantly reduced following infliximab administration. In contrast, E-selectin, one of the most specific endothelial adhesion molecules was not affected by the treatment. Due to this, further studies are required to support a protective effect of anti-TNF-α–infliximab therapy on endothelium through its inhibition of the up-regulation of adhesion molecules. Also, additional studies are required to know the duration of the biological effect of infliximab administration on the reduction of the markers of endothelial damage in RA. The search for TNF-α antagonists with long lasting effects on endothelial function is required to decrease the high incidence of CV complications associated with this chronic disease.

Acknowledgements

The authors thank Mrs. Susana Escandon, a nurse from the Rheumatology Outpatient Clinic, and Ms. Pilar Ruiz, a nurse from the Hematology Department (Hospital Xeral-Calde, Lugo, Spain) for their valuable help in undertaking this study.

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