Infliximab therapy does not modify MMP-2 and MMP-9 serum concentrations in chronic arthritis

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Abstract

Objective

Matrix metalloprotease-2 (MMP-2) and matrix metalloprotease-9 (MMP-9) play a key role in tissue remodelling after processes such as joint destruction in rheumatoid arthritis. Their expression may reflect the disease activity and they could therefore represent a useful marker to assess the efficacy of therapy. In this study MMP-2 and MMP-9 serum concentrations were evaluated in patients with chronic arthritis during therapy with the anti-TNFα mAb, infliximab.

Methods

Fifty patients with chronic arthritis, 26 with rheumatoid arthritis and 24 with undifferentiated chronic arthritis, were recruited and treated with infliximab (3 mg/kg). Serum concentrations of MMP-2 and MMP-9 were serially measured by gelatine zymography at baseline and after two and fourteen weeks of infliximab therapy. DAS-28 and ACR response criteria were applied to assess disease activity and clinical improvement. Twenty-four healthy donors were included in the study as controls.

Results

Although therapy with infliximab induced a statistically significant reduction of the DAS-28 score and improvement of the ACR clinical response, MMP-2 and MMP-9 serum concentrations were not modulated during therapy with infliximab.

Conclusions

Our study provides further evidence that blocking TNFα by infliximab is a powerful tool in the management of chronic arthritis. Nevertheless, infliximab does not seem to be able to modify the serum expression of MMP-2 and MMP-9, probably because modification of these enzymes is restricted to the site of joint inflammation and serum detection cannot truly mirror the local situation. Additional soluble factors correlating with joint damage should be investigated as possible markers for monitoring anti-TNFα therapy.

Key words

MMP-2, MMP-9, infliximab, chronic arthritis, TNF-alpha, matrix metalloproteases.

Introduction

There is a growing body of evidence that tumor necrosis factor α (TNFα) blocking agents induce a remarkable clinical benefit in patients with rheumatoid arthritis (RA) and undifferentiated seronegative chronic polyarthritis (SCP). The efficacy of TNFα inhibitors is not confined to inducing symptomatic relief but also alters the key biological mechanisms causing chronic inflammation of the joints. Recently, it has been shown that infliximab, a chimeric monoclonal anti-TNFα antibody, can affect the immunopathological processes occurring in the synovial membrane of patients with RA and SCP. A reduction of TNFα, IL-18, and inflammatory cells has been demonstrated in the synovial membrane in RA patients following infliximab therapy (1). Likewise, serial analyses of synovial biopsies in patients with spondyloarthropathy have shown a decrease of synovial hyperplasia, endothelial activation, and inflammation after TNFα blockade by infliximab (2). Indeed, TNFα inhibitors seem to be able to modify the course of RA disease and prevent joint damage (cartilage breakdown and bone erosions), suggesting that infliximab can hamper the process directly involved in tissue destruction (3).

It has been largely reported that inflammatory cytokines such as TNFα upregulate the expression of matrix metalloproteases (MMPs) (4), a family of enzymes with a proteolytic activity towards a number of different extracellular matrix (ECM) proteins (5). These enzymes, secreted in a latent form, are activated at the cellular surface by the complex membrane type (MT)1-MMP, together with the tissue inhibitor of MMPs (TIMPs) (6-8). TIMPS are also involved in limiting the proteolytic activity so that too extensive and uncontrolled a degradation of the ECM components is avoided; therefore, proteolysis occurs as an imbalance between the proteolytic and inhibitory activity (9, 10). MMP-2 and MMP-9 are two members of this family involved in physiological and pathological conditions such as development, cancer invasion and tissue remodelling (5). In RA synovitis, whereby synovial cells invade the cartilage and the bone tissue, an imbalance between MMP-2 and its inhibitor TIMP-2 has been detected (11). Interestingly, in RA synovitis an increased expression of MMP-2 and MT1-MMP, together with a reduced expression of TIMP-2, correlate with the occurrence of early bone erosions (11).

The goal of this study is to investigate the expression of MMP-2 and MMP-9 serum levels in patients with RA and SPC before and during infliximab therapy.

Patients and methods

Patients

A series of patients with chronic polyarthritis was enrolled in an open non-placebo study. The study was approved by the local ethics committee and written informed consent was obtained from each patient.

A total of 50 patients were recruited; 26 patients fulfilled the American College of Rheumatology 1987 revised criteria for RA (12) and 24 patients had undifferentiated seronegative chronic polyarthritis (SCP) according to the European Spondylarthropathy Study Group criteria (13). Demographic and clinical data of the patients are described in Table I. Furthermore, 90% of RA patients had articular erosions at standard plain X-rays. The inclusion criteria were (1) resistance to 2 or more DMARDs (disease modifying antirheumatic drugs) after at least 6 months administration; (2) "active disease" as measured by the Disease Activity Score (DAS), including 28 joints (DAS-28) (14). This composite index takes into account the number of swollen joints, the number of tender joints, patients' global assessment of disease activity measured on a visual analogue scale (VAS, range 0-100 mm) and the erythrocyte sedimentation rate (ESR, mm/1st hour). A DAS28 score ranging from 2.6 to 3.2 defines low disease activity, from 3.2 to 5.1 moderate activity, and > 5.1 high activity. All patients were screened for tuberculosis by chest X-ray and skin Mantoux's test prior to the treatment.

All patients received methotrexate (range 10-15 mg/week) for more than 4 weeks. Stable dosages of steroids

Table I. Clinical and demographic data of the patients (mean ± SD).

	All patients	RA	SCP
Number:	50	26	24
Age (yrs.)	44.6 ± 15	44.7 ± 15	44.5 ± 16
Female/male	35/15	23/3	12/12
Disease duration (yrs.)	9.9 ± 7	9.8 ± 7	10.1 ± 8
DAS 28 score	6.02 ± 1.4	6.4 ± 1.3	5.5 ± 1.4

RA: rheumatoid arthritis; SCP: seronegative chronic polyarthritis.

(prednisone equivalent, range 5-10 mg/day) and NSAIDs (non-steroidal anti-inflammatory drugs) were reached at least 4 weeks before enrolment. The above therapy was continued unchanged throughout the study. Infliximab (3 mg/Kg) was infused at weeks 0, 2, 6, 14. The efficacy of the therapy was assessed on the basis of the ACR 20%, 50%, and 70% clinical response (15). Serum samples were collected prior to infusion at weeks 0, 2, 14 and stored at $-20°C$ until use. Serum samples of twenty-four healthy donors (HD) were included in the study as controls.

Methods
All the samples were normalized for protein concentration as measured by the bicinchoninic acid method (Pierce Chemical Co, Rockford, IL). MMP-2 and MMP-9 were analyzed by gelatine zymography as previously reported (16). The gels were incubated over-night, stained with Coomassie blue, and destained with a Methanol Acetic acid solution until gelatinolytic areas appeared evident as unstained bands in a blue stained gel. The quantification of both latent and active gelatinases was performed as described (17). Briefly, HT1080 conditioned medium containing known concentrations of MMP-2 and MMP-9 was used with serial dilutions to obtain a standard curve. Serial dilutions of the sample were loaded in each gel, yielding a linear relationship between sample dilution and gelatinolytic activity. In each experiment, a standard curve of HT1080 conditioned medium was loaded into the gels, and then gelatin zymography results were acquired and quantified with an image-analysis-software system (Image Master 1D Prime, Pharmacia Biotech, UK).
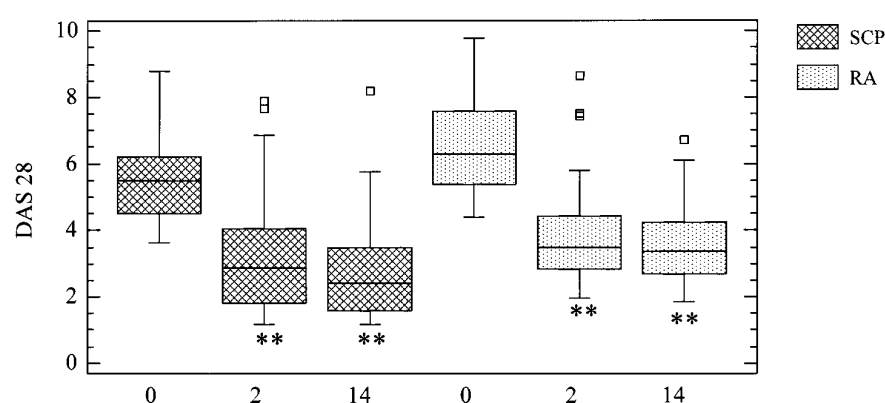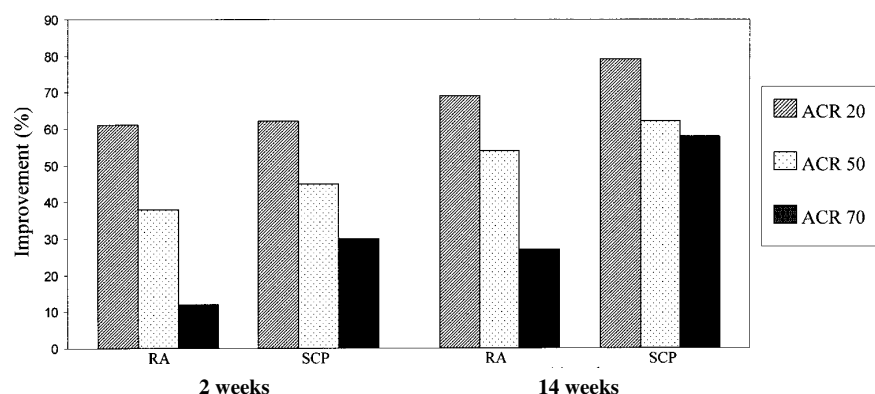
Statistical analysis
For statistical comparison the Kruskall-Wallis test was used. Correlation analysis was carried out using Spearman's test for non-parametric data. The significance level was set at $p < 0.05$.

Results
The evaluation of DAS-28 showed a median disease activity of both RA and SCP patients at the baseline of 6.3 and 5.5, respectively; after two weeks it had decreased to 3.5 ($p < 0.01$) and to 2.8 ($p < 0.01$), respectively (Fig. 1). A sustained reduction was observed after 14 weeks (RA: median 3.3 $p < 0.01$; SCP: median 2.4 $p < 0.01$) (Fig. 1).
Consistently, infliximab induced a rapid therapeutic effect according to ACR response criteria in both RA and SCP patients. Specifically, after two weeks, the percentage of RA patients with an ACR20, 50, 70 was 61%, 38%, 12%, respectively, while after fourteen weeks the percentage of patients with ACR20, 50 and 70 was 69%, 54%, 27%, respectively (Fig. 2). A similar pattern of response was observed in SCP patients both after 2 weeks (62% ACR 20, 45% ACR 50, and 30% ACR 70) and after 14 weeks (79% ACR 20, 62% ACR 50, and 58% ACR 70) (Fig. 2).
A significant difference was observed between RA ($p < 0.05$) but not SCP ($p > 0.05$) at baseline levels and HD as

Fig. 1. Disease activity, measured by DAS-28, in rheumatoid arthritis (RA) and seronegative chronic polyarthritis (SCP) patients at weeks 0, 2, 14 during infliximab therapy. Box plots represent the median with 25th and 75th percentiles and whiskers 10th and 90th percentiles. **$p < 0.01$ vs week 0.

Fig. 2. Percent improvement in ACR2 20, 50, and 70 response criteria at 2 and 14 weeks of treatment with infliximab in rheumatoid arthritis (RA) and seronegative chronic polyarthritis (SCP) patients.
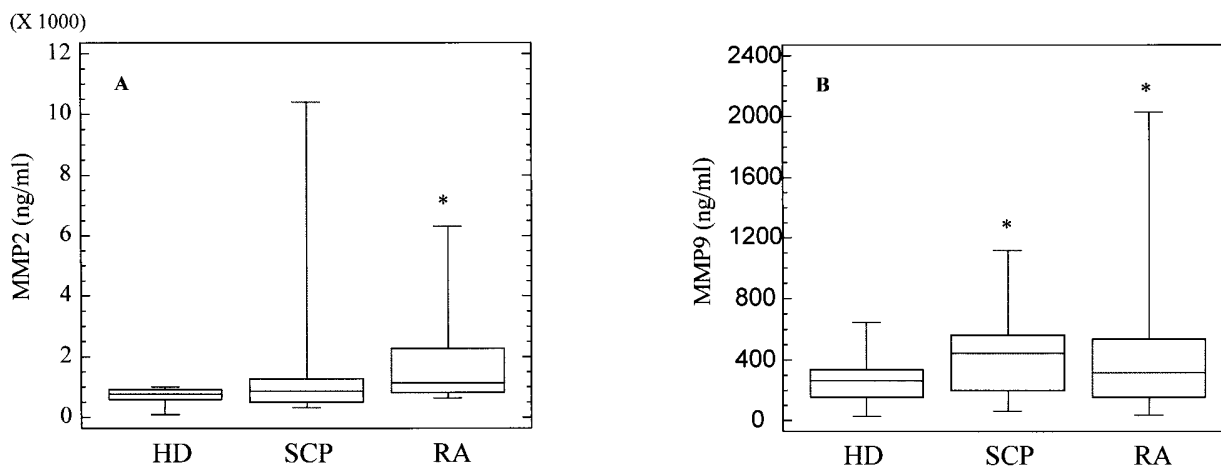
Fig. 3. (**A**) Serum levels of MMP-2 and MMP-9 in healthy donors (HD) and rheumatoid arthritis (RA), seronegative chronic polyarthritis (SCP) patients at baseline prior to therapy with infliximab. Box plots represent the median with 25th and 75th percentiles and whiskers 10th and 90th percentiles. *p < 0.05 vs HD. (**B**) Serum levels of MMP-9 in healthy donors (HD) and rheumatoid arthritis (RA), seronegative chronic polyarthritis (SCP) patients at baseline prior to therapy with infliximab. Box plots represent the median with 25th and 75th percentiles and whiskers 10th and 90th percentiles. *p < 0.05 vs HD.

Fig. 4. Serum levels of MMP-2 in rheumatoid arthritis (RA) and seronegative chronic polyarthritis (SCP) patients at weeks 0, 2, 14 of therapy with infliximab. Box plots represent the median with 25th and 75th percentiles and whiskers 10th and 90th percentiles.
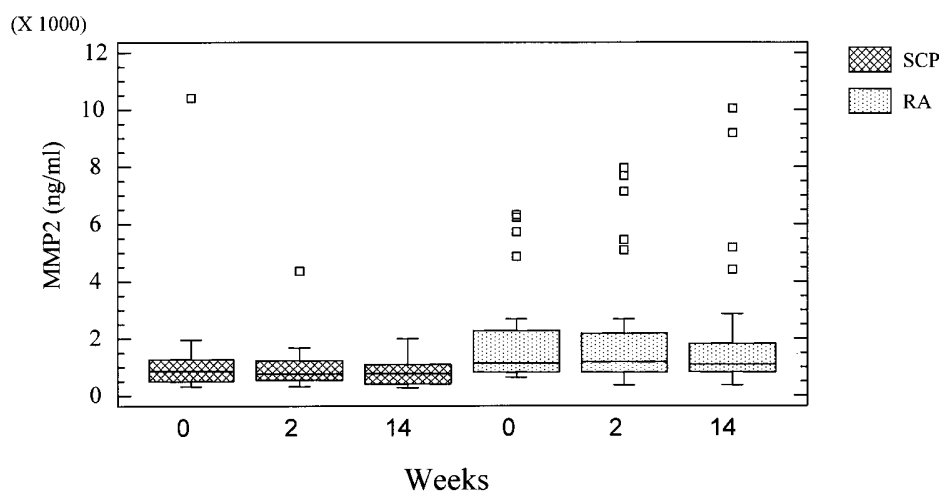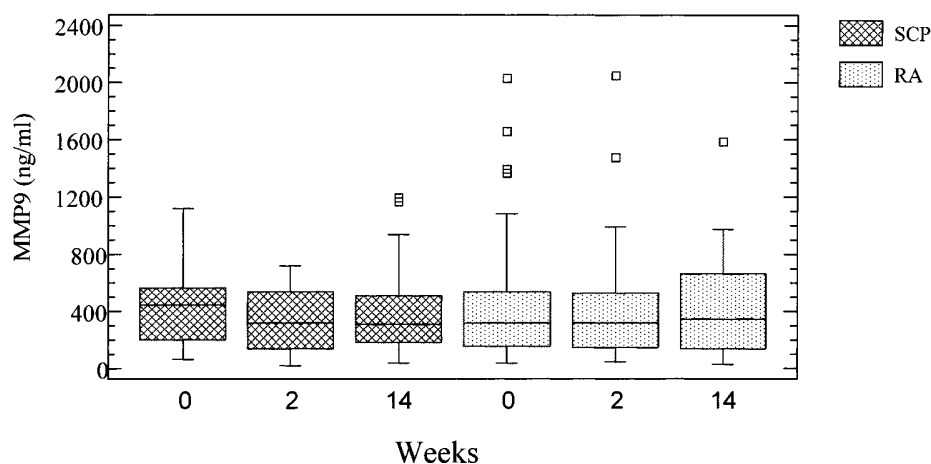

Fig. 5. Serum levels of MMP-9 in rheumatoid arthritis (RA) and seronegative chronic polyarthritis (SCP) patients at weeks 0, 2, 14 of therapy with infliximab. Box plots represent the median with 25th and 75th percentiles and whiskers 10th and 90th percentiles.

regarding MMP-2. Serum concentrations of MMP-2 (Fig. 3A) were similar in the RA and SCP patient groups, but no statistically significant differences were observed in either group during infliximab treatment. In particular, in SCP patients, median MMP-2 was 857.0 ng/ml at baseline, 740.0 ng/ml after 2 weeks, and 751.0 ng/ml after 14 weeks (p > 0.05). In RA patients, median MMP-2 was 1134.0 ng/ml at baseline, 1153.0 ng/ml after 2 weeks, and 1056.0 ng/ml after 14 weeks (p > 0.05), (Fig. 4).

Similar results were observed as re-

gards MMP-9 serum concentrations. However, a significant difference was observed between RA (p < 0.05), SCP (p < 0.05) and HD as regarding MMP-9 (Fig. 3B). In particular, in SCP patients, median MMP-9 was 444.0 ng/ml at baseline, 313.0 ng/ml after 2 weeks, and 305.0 ng/ml after 14 weeks (p > 0.05). In RA patients, median MMP-9 was 320.0 ng/ml at baseline, 313.0 ng/ml after 2 weeks, and 341.0 ng/ml after 14 weeks (p > 0.05). No statistically significant correlation was found between MMP-2 and/or MMP-9 and disease activity (DAS-28) at any time of treatment (data not shown), (Fig. 5). Finally, no correlation was found between both MMP-2 and MMP-9 and baseline disease activity including bone erosions.

Discussion

The molecular mechanisms underlying the bone erosion tissue damage occurring in RA and SCP are not fully understood, although growing evidence suggests that an imbalance between MMPs and TIMPs likely takes place (11). MMP-2 and MMP-9 proteolytic activity is required to break down ECM proteins tissue boundaries allowing the invasion of synovial cells and hence bone erosion. Recently, a reduction of MMPs serum levels in RA patients treated with infliximab has been reported by Klimiuk et al. (18). This result seems to be consistent with the fact that TNFα upregulates the expression of MMPs, and therefore has very promising clinical implications (18). On the contrary, in our study, no substantial differences were observed in chronic arthritis before and during infliximab therapy, although we observed a therapeutic efficacy of the drug in terms of both DAS-28 and ACR response criteria. These results are consistent with a previous study reporting that both MMP-2 and MMP-9 are increased at the synovial levels, where they correlate with tissue erosion, whereas in the serum they are not altered and do not correlate with any clinical manifestation (11). These findings are also consistent with those in our previous study of RA and psoriatic arthritis patients, in whom MMP-2, MMP-9, as well as their inhibitors

TIMP-2 and TIMP-1, were increased or decreased, respectively, in the synovial fluid but not in the serum (19). The focalization of proteolysis is a finely regulated process, restricted to the site where tissue remodelling occurs, such as the advancing edge of a tumor that is metastasising in the surrounding tissue (20). However, an altered proteolytic balance has been reported to be necessary for several types of cancer, to correlate with the clinical outcome (5). In RA patients it is possible that the inflammatory process is limited to the microenvironment implicated in the tissue damage, unlike in cancer, where more systemic alterations are involved. This could justify the fact that in our study and others the MMP-2 and MMP-9 serum concentrations failed to correlate with the clinical response to therapy and/or to the therapeutic efficacy of the treatment (11). Nevertheless, the study by Klimiuk et al. represents a potential important finding that should be more extensively investigated and confirmed.

In conclusion, our study provides further evidence that blocking TNFα by infliximab is a powerful tool in the management of chronic arthritis. Nevertheless, infliximab does not seem to be able to modify the serum expression of MMP-2 and MMP-9, probably because modification of these enzymes is restricted to the site of joint inflammation and serum detection cannot truly mirror the local situation. More studies are needed to assess whether serum evaluation of the proteolytic imbalance is useful to further clarify mechanistic aspects of bone erosion in RA patients, and additional soluble factors correlating with joint damage should be investigated as possible markers for monitoring anti-TNFα therapy.

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