

## MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in patients with rheumatoid arthritis and psoriatic arthritis

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### ABSTRACT

**Objective.** *Rheumatoid arthritis (RA) and psoriatic arthritis (PA) are both chronic rheumatic inflammatory diseases characterized by disruption of the extra-cellular matrix (ECM) protein of the cartilage, likely induced by proteolytic enzymes such as matrix metalloproteases (MMPs). The goal of this study was to quantify the expression of MMPs such as MMP-2 and MMP-9, and their physiological tissue inhibitors TIMP-2 and TIMP-1, respectively, in serum and synovial fluid.*

**Methods.** *Serum and synovial fluid from 24 RA patients and 17 PA patients were studied to determine the levels of MMP-2 and MMP-9 proteolytic activity using a modified gelatin zymography procedure. TIMP-1 and TIMP-2 were measured by a commercially available ELISA kit.*

**Results.** *Our results show that MMP-2 was detected in the latent form only, while MMP-9 was present in latent and active form. Both gelatinases were more concentrated in synovial fluid than in serum, and TIMP-1 and TIMP-2 concentrations were also more elevated in synovial fluid than in serum.*

**Conclusions.** *To investigate the remodeling of cartilage ECM proteins, the evaluation of synovial fluid concentrations of MMP-2, MMP-9, TIMP-1 and TIMP-2 is more reliable than that determined in serum. In view of these data, MMPs inhibitors might represent a possible target for new therapies delivered directly in the joint space.*

### Introduction

Rheumatoid arthritis (AR) and psoriatic arthritis (PA) are both chronic inflammatory rheumatic diseases featuring disruption of the cartilage hampering joint functionality (1). The underlying molecular basis responsible for the breakdown of extracellular matrix components (ECM) of the cartilage is still unclear, but a role for proteolytic enzymes released by either chondrocytes or infiltrated inflammatory cells has been suggested (1).

Among the different classes of proteolytic enzymes, matrix metalloproteases (MMPs) are believed to play a key role in the remodelling of joint tissue.

MMPs are a family of Zinc (Zn) endopeptidases consisting of at least 20 different members classified in six subgroups based on different substrate specificity and/or structure homology: interstitial collagenases, gelatinases, membrane type MMPs (MT-MMPs), stromelysin, stromelysin-3 and metalloelastase. Gelatinases, also known as MMP-2 and MMP-9, are the best characterized and investigated type of MMPs (2). They are secreted in a latent form as pro-MMP-2 and pro-MMP-9, of 72 and 92 Kd, respectively. To acquire their proteolytic activity they require a proteolytic processing that commonly occurs at the cellular surface by other types of MMPs such as MT1-MMP, and as a consequence their molecular weights becomes 62 and 82 Kd, respectively (3). Gelatinase proteolytic activity is directed towards a number of different ECM proteins including Collagens, Laminins, Fibronectin etc. and is balanced by the tissue inhibitors of MMPs (TIMPs) (4). TIMP-1 and TIMP-2 avoid too extensive degradation of ECM proteins but they also take part in the activation process of MMP-9 and MMP-2, respectively (3).

Breakdown of ECM tissue boundaries is required in a number of physiological and pathological situations including tissue morphogenesis, organ differentiation and cancer metastasis (5). Elevated levels of MMP-2 and/or MMP-9 have been detected in the synovial fluid of RA patients, and this could have a role in the joint tissue damage (6). In this study we investigate the expression of MMP-2, MMP-9 and their inhibitors, TIMP-1 and TIMP-2, in synovial fluid and in the serum of patients with RA and PA.

### Materials and methods

#### Patients

We tested paired sera and synovial fluids from 41 patients with chronic arthritides. Mean age was 45.1 (14 yrs (range 23-72); 29 females and 12 males and mean disease duration was 9.1 (5 yrs). None of the patients had either severe concomitant illness such as cancer, infectious diseases, or extra-articular systemic manifestations. Twenty-four patients fulfilled the 1987 Ameri-

can College of Rheumatology (ACR) criteria for RA(7) with a mean age was 50.3 (10 yrs (range 23-72)).

Thirty-five out of 41 patients were rheumatoid factor positive (all the patients with RA and 11 with PA, none of the patients had both diseases). All RA patients had erosive polyarthritis and active disease, assessed by considering the erythrocyte sedimentation rate (ESR) (Westergren), C-reactive protein, number of tender and swollen joints and a 100 mm Visual Analogue Scale (VAS) for general health status. Rheumatoid nodules were present in 20 out of 24 RA patients.

Seventeen patients had active PA with a mean age of  $41.3 \pm 12$  yrs (range 25-61). Nine out of 17 patients had polyarticular PA, 5 had the oligoarticular and 3 had the axial PA subset. All of the PA patients, including those with RF, fulfilled the international criteria for the diagnosis of PA (8). Patients with either disease, RA and PA, had a similar disease duration. Synovial fluid was obtained from the knee, and the leucocyte cell count and chemical analysis were diagnostic for arthritic fluid. Furthermore, in our patients based on their clinical history and outcome we had ruled out the possibility of knee osteoarthritis involvement.

#### Gelatin zymography

MMP-2 and MMP-9 were investigated in the serum and synovial fluid of each patient. Protein concentrations were measured by the bicinchoninic acid method (Pierce Chemical Co, Rockford, IL); each sample was measured in duplicate and with two serial dilutions to avoid any possible technical problem. Gelatin zymography was performed as previously described. Gelatinase quantification was carried out by zymography using HT1080 conditioned medium as the internal standard control, as previously reported (9).

#### Detection of TIMP-2 and TIMP-1 by ELISA

We quantified the concentrations of both TIMP-2 and TIMP-1 in the samples already processed for zymography. Serum and synovial concentrations of TIMP-2 and TIMP-1 were determined

by ELISA kits (Amersham Pharmacia biotech, UK), based on a double sandwich system.

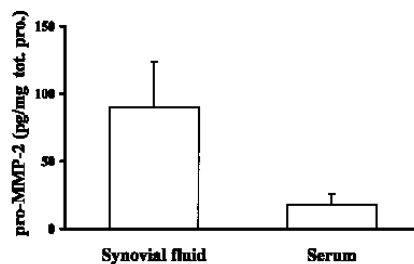
#### Statistical analysis

Student's t-test was used to determine the 99% confidence intervals (CI) for the MMP-2, MMP-9 and TIMP-2 levels in the serum and synovial samples. A probability of  $<0.05$  was considered to be statistically significant.

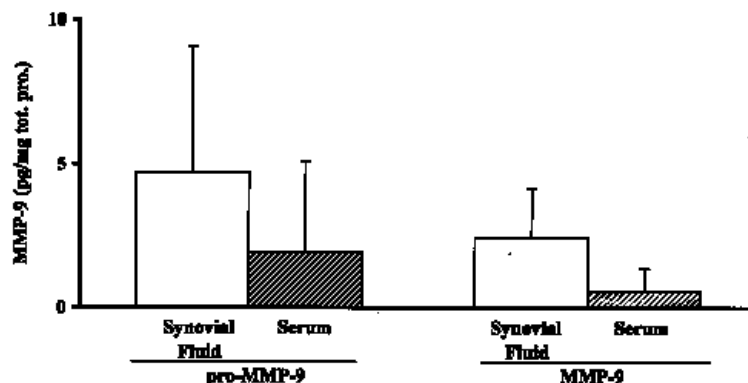
#### Results

No differences were observed in patients with rheumatoid versus psoriatic arthritis in terms of serum and synovial levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 (data not shown).

As shown in Figure 1, MMP-2 was present only in latent form (pro-MMP-2) in both the serum and synovial fluid. However, a statistically significant difference was observed between the two biological fluids, since the MMP-2 concentration was  $89.50 \pm 34.34$  pg/mg of



**Fig. 1.** MMP-2 concentrations in the synovial fluid and serum. MMP-2 was detected only in the latent form, whereas the active form was absent in both sample preparations. A significant difference was found between the synovial and serum concentrations ( $p < 0.0001$ ).



**Fig. 2.** MMP-9 concentrations in the synovial fluid and serum. Pro-MMP-9 and MMP-9 concentrations were higher in the synovial fluid than in the serum, ( $p = 0.02$ ) and ( $p = 0.013$ ) respectively.

total protein in the synovial fluid versus  $17.76 \pm 7.80$  pg/mg of total protein in the serum ( $p < 0.0001$ ).

MMP-9 was present in the inactive and active forms in both serum and synovial fluid (Fig.2). The concentration of latent form MMP-9 was  $4.70 \pm 4.36$  pg/mg of total protein in the synovial fluid versus  $2.42 \pm 1.71$  pg/mg of total protein in the serum ( $p = 0.02$ ), while the active form concentration was  $1.90 \pm 3.17$  pg/mg and  $0.8 \pm 0.57$  pg/mg of total protein in synovial fluid and serum, respectively ( $p = 0.013$ ).

As shown in Figure 3, TIMP-2 concentrations were  $2.84 \pm 2.46$  ng/mg of the total protein in the synovial fluid versus  $1.19 \pm 1.38$  ng/mg of the total protein in the serum; the difference was statistically significant ( $p = 0.02$ ). Consistently, TIMP-1 concentrations were  $69.10 \pm 31.42$  ng/mg of total protein in the synovial fluid versus  $14.30 \pm 8.61$  in the serum ( $p < 0.001$ ) (Fig. 4).

#### Discussion

The healthy functioning of joints mainly depends on a correct turnover of the cartilage ECM components. To maintain the homeostasis of cartilage tissues, MMP proteolytic activity is finely balanced by TIMP activity, but in a number of different conditions, including chronic inflammatory diseases, this regulation can be altered (1).

In our study, MMP-2, MMP-9, TIMP-2 and TIMP-1 were measured in the serum and synovial fluid of the same group of patients. For the first time, we provide a quantification of gelatinase proteolytic activity using a modified

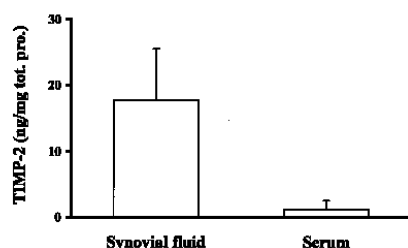


Fig. 3. TIMP-2 concentrations in the synovial fluid and serum. TIMP-2 concentrations were higher in the synovial fluid than in the serum ( $p = 0.02$ ).

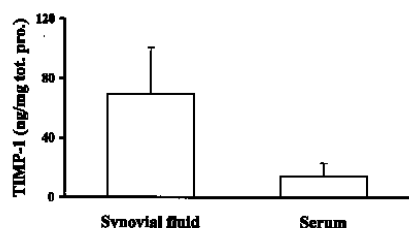


Fig. 4. TIMP-1 concentrations in the synovial fluid and serum. TIMP-1 concentrations were higher in the synovial fluid than in the serum ( $p = 0.001$ ).

gelatine zymography method (9), whereas the concentration of their physiological inhibitors, TIMP-1 and TIMP-2, was measured by ELISA. Independently of the type of arthritis – RA or PA – the concentrations of MMP-2 and MMP-9 were significantly higher in the synovial fluid than in the serum. The absence of the active form of MMP-2 even in synovial fluid is not surprising since it mainly depends on the amount of protein loaded in the zymography analysis (9). Furthermore, in this study TIMP-1 and TIMP-2 concentrations were also significantly higher in the synovial fluid than in the serum and these results are consistent with other studies evaluating different types of MMPs and/or different TIMPs in patients with RA (10). Here we confirm the findings of another study (11, 12) and in addition we provide clear cut evidence that in PA patients, serum and synovial fluid concentrations of MMP-2, MMP-9, TIMP-1 and TIMP-2 have a trend similar to that observed for RA patients.

Therefore, the high levels of gelatinases could be responsible for the proteolysis of cartilage ECM components, while the high levels of TIMP-1 and TIMP-2 could be involved in the gelatinase ac-

tivation process and therefore contribute to cartilage damage. While this explanation seems reasonable and is also well accepted in the literature (1), the cellular source of TIMPs and gelatinases is still in doubt. One possibility is that the inflammatory cells invading the synovial fluid may release elevated amounts of either gelatinases and TIMPs (6,13) or cytokines, which are in turn responsible for a paracrine stimulation of other cells, including synovial cells (1). However, gelatinases are also able to degrade the ECM components of the blood vessel basement membrane and then facilitate the invasion of other inflammatory cells. The elevated recruitment to the joint site of neutrophils, macrophages and other inflammatory cells could explain the higher levels of both gelatinases and TIMPs in the synovial fluid than the serum, despite the fact that RA and PA are both systemic diseases.

Based on these data, it seems evident that inhibition of the MMPs proteolytic activity could preserve joint tissue remodelling and prevent cartilage disruption. Like many other disorders, RA and PA are candidates for targeted therapies to inhibit MMPs. In the last few years, the production of synthetic inhibitors of MMPs has generated great interest (14). Batimastat, and more recently marimastat, mimic the collagen cleavage site, competing with other ECM substrates for MMP proteolytic activity. These inhibitors have been shown to inhibit growth and/or metastasis of a number of different malignancies in animal models both *in vitro* and *in vivo* (15,16). However, in humans their application has been limited owing to side effects mainly affecting joint functionality, probably because MMP proteolytic activity is required to ensure a correct turnover of the ECM protein under physiological conditions. This has also been confirmed by other studies where matrix type 1-MMP deficient mice showed arthritis and fibrosis of the soft tissues due to the absence of proper collagenolytic activity (17).

However, in chronic arthritis patients the use of MMPs inhibitors, preferably delivered into the joint space rather than as systemic therapy, could represent a

new therapeutic approach. For this purpose, more recently epigallocatechin-3-gallate, a flavonol present in green tea, has been reported to be a potent MMP inhibitor (18). Another possibility is a genetic approach to inhibit MMPs through TIMP-2 directly delivered into cancer cells; this has shown encouraging results (16).

In conclusion, the quantification of MMP-2, MMP-9, TIMP-2 and TIMP-1 in the synovial fluid rather than in the serum is a reliable indicator of ECM cartilage proteolysis, and this could guide the use of MMP inhibitors in the therapy of chronic arthritis inflammatory diseases.

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