Serum Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Patients with Early Rheumatoid Arthritis

MALGORZATA FIEDORCZYK, PIOTR ADRIAN KLIMIUK, STANISLAW SIERAKOWSKI, EWA GINDZIENSKA-SIESKIEWICZ, and JUSTYNA CHWIECKO

ABSTRACT. ***Objective.*** To analyze serum concentrations of matrix metalloproteinases (MMP) MMP-1, MMP-3, MMP-9, MMP-13, tissue inhibitors of MMP (TIMP) TIMP-1 and TIMP-2, and MMP/TIMP ratios in patients with early rheumatoid arthritis (RA) before and after 6 months of treatment with methotrexate (MTX).

Methods. The study group consisted of 30 patients with RA, not treated with disease modifying antirheumatic drugs or corticosteroids, with disease duration < 3 years. Twenty patients with osteoarthritis (OA) served as a control group. Analysis of serum concentrations of MMP and TIMP was based on a quantitative sandwich ELISA.

Results. Serum concentrations of MMP-1, MMP-3, MMP-9, and MMP-13 were higher in untreated patients with early RA than in OA patients ($p < 0.001$ in all cases). Serum levels of TIMP-1 and TIMP-2 dominated in the serum of RA patients compared with controls ($p < 0.01$ and $p < 0.05$, respectively). Ratios of MMP to TIMP were significantly higher in patients with early RA versus controls. Six months' treatment with MTX downregulated serum concentrations of MMP-1 ($p < 0.001$), MMP-3 ($p < 0.001$), MMP-9 ($p < 0.001$), MMP-13 ($p < 0.01$), and TIMP-1 ($p < 0.05$) in patients with RA. These changes were accompanied by significantly reduced ratios of MMP to TIMP. MTX treatment decreased markers of RA activity such as the number of painful and swollen joints, erythrocyte sedimentation rate, Disease Activity Score, and C-reactive protein.

Conclusion. Patients with early RA are characterized by high serum concentrations of tissue-degrading metalloproteinases. Therapy with MTX resulted in clinical improvement and reduced serum MMP levels in patients with RA, confirming effectiveness of MTX in patients in early stages of the disease. (J Rheumatol 2006;33:1523–9)

Key Indexing Terms:
MATRIX METALLOPROTEINASES TISSUE INHIBITORS OF METALLOPROTEINASES
METHOTREXATE EARLY RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease with progressive articular damage often associated with systemic manifestations, resulting in a significant morbidity and irreversible disability. Lymphocytes, macrophages, and synoviocytes infiltrating synovial membrane are involved in this process. Histomorphological changes include neoangiogenesis and proliferation of the synovial lining layer[1-3]. Tissue destruction is caused by several mechanisms including the production of cytokines and matrix metalloproteinases (MMP), which include collagenases, stromelysins, and gelatinases[1,3-5]. Production of these proteases in fibroblasts, macrophages, and chondrocytes is regulated by synovial macrophages and lymphocytes[5-7]. MMP are the proteases that participate in irreparable proteolytic degradation and remodelling of the extracellular matrix. The action of MMP is controlled by their natural inhibitors, known as tissue inhibitors of metalloproteinases (TIMP), which under normal conditions neutralize the activity of these proteases[4,5,8]. Thus, MMP and TIMP and especially the imbalance between activated enzymes and inhibitors are thought to play an important role in the destruction and remodelling of articular tissues in patients with RA.

The upregulation of MMP in RA is well documented. However, little is know about the MMP and their tissue inhibitors and MMP/TIMP ratios in patients with early-stage RA. We analyzed serum concentrations of MMP and their tissue inhibitors and MMP/TIMP ratios in patients with early RA not treated with disease modifying antirheumatic drugs (DMARD) or corticosteroids, and then after 6 months of treatment with methotrexate (MTX).

From the Department of Rheumatology and Internal Diseases, Medical University of Bialystok, Bialystok, Poland.

M. Fiedorczyk, MD, PhD, Assistant; P.A. Klimiuk, MD, PhD, Assistant; S. Sierakowski, MD, PhD, DSc (Med), Professor and Chairman; E. Gindzienska-Sieskiewicz, MD, PhD, Assistant; J. Chwiecko, MA, PhD, Principal Technician.

Address reprint requests to Dr. P.A. Klimiuk, Department of Rheumatology and Internal Diseases, Medical University of Bialystok, M.C. Sklodowskiej 24a, 15-276 Bialystok, Poland. E-mail: klimp@amb.edu.pl

Accepted for publication March 16, 2006.

MATERIALS AND METHODS

Patients. The study group consisted of 30 patients with RA and disease duration < 3 years, as measured from the first clinical signs of arthritis. All patients met the American College of Rheumatology 1987 criteria for the diagnosis of RA[9]. Patients were receiving nonsteroidal antiinflammatory drugs (NSAID) in stable doses at least 1 month before and through the 6 month study period. No patient was using DMARD or corticosteroids before the study. After enrollment into the study all patients were receiving MTX in a mean dose of 8.9 mg/week (range 7.5–12.5 mg/wk) and folic acid 15 mg/week. During the 6 months of the study, 8 RA patients were taking prednisone in a mean dose of 7.8 mg/day (range 5–10 mg/day). In the last 3 months of the study patients were receiving MTX and corticosteroids in stable doses. Twenty patients with osteoarthritis (OA) taking stable dose of NSAID for at least 1 month were used as a control group. The study protocol was approved by the local ethics committee and patients' written consent was obtained. Table 1 shows the characteristics of patient populations.

Clinical and laboratory evaluation. Analysis included duration of morning stiffness, number of tender joints, Ritchie Articular Index[10], number of swollen joints, erythrocyte sedimentation rate (ESR), Disease Activity Score (DAS)[11], C-reactive protein (CRP), and rheumatoid factor level. Assessment was performed before and after 6 months' treatment with MTX.

Serum sample preparation. Blood specimens were clotted for 30 min and then centrifuged for 5 min at 2000 g. Serum aliquots were frozen at –80°C immediately after sample collection.

Serum MMP and TIMP measurements. Analysis of serum concentrations of MMP-1, MMP-3, MMP-9, MMP-13, TIMP-1, and TIMP-2 was based on a quantitative sandwich ELISA (Amersham Biosciences UK Ltd., Little Chalfont, UK) according to the manufacturer's instructions. Assessment was performed before and after 6 months' treatment with MTX. MMP and TIMP concentrations were expressed as ng/ml. The sensitivities were as follows: 1.7 ng/ml for MMP-1, 2.35 ng/ml for MMP-3, 0.6 ng/ml for MMP-9, 0.032 ng/ml for MMP-13, 1.25 ng/ml TIMP-1, and 3.0 ng/ml for TIMP-2.

Statistical analysis. Statistical comparison of normally distributed data between RA patients and controls was by unpaired Student t test. Mann-Whitney U test was employed to analyze differences between abnormally distributed values. The statistical comparison of data from RA patients before and after 6 months' treatment with MTX was performed using paired Student t test or Wilcoxon test. The probability of differences in frequency distributions was assessed by chi-square test; p values < 0.05 were considered statistically significant.

RESULTS

Serum concentrations of MMP and TIMP. Before treatment with MTX, serum levels of MMP-1, MMP-3, MMP-9, and MMP-13 were significantly increased in patients with early RA compared with OA ($p < 0.001$ for all comparisons; Figures 1-4). Similarly to MMP concentrations, serum levels of TIMP-1 and TIMP-2 were higher in the serum of RA patients than in controls ($p < 0.01$ and $p < 0.05$, respectively; Figures 5 and 6). As shown in Table 2, the calculated ratios of MMP to TIMP were significantly higher in patients with early RA than in controls. After 6 months' treatment with MTX, serum concentrations of MMP-1, MMP-3, MMP-9, MMP-13, and TIMP-1 diminished in patients with RA ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; Figures 1-5). Serum levels of TIMP-2 also decreased in RA patients; however, not significantly (Figure 6). These changes were accompanied by significantly reduced ratios of measured MMP to TIMP, especially in the case of MMP-1 and MMP-3 (Table 2).

Clinical response. MTX treatment was followed by decreases in the markers of RA activity such as the number of painful and swollen joints, Ritchie Index, ESR, CRP, and DAS (Table 3). Serum concentration of studied proteases correlated with these clinical markers of disease activity, such as the number of painful and swollen joints, Ritchie Index, DAS, ESR, and CRP, especially before MTX treatment (data not shown).

DISCUSSION

MMP and TIMP have been identified as key agents in the remodelling of articular tissues in RA[3,4,12]. MMP are involved in the proteolytic degradation of extracellular matrix components. Their production is regulated by cytokines such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α)[5,13,14]. The action of MMP is controlled by their tissue inhibitors, the production of which is regulated by cytokines

Table 1. Characteristics of patient populations (data are mean ± SD).

Characteristic	Patients with OA	Patients with RA	p
Female/male	16/4	25/5	NS
Mean age, yrs	54.0 ± 14.1	52.1 ± 13.4	NS
Mean disease duration, mo	30.6 ± 23.3	16.5 ± 10.1	NS
ESR, mm/h	17.5 ± 11.6	62.8 ± 25.2	< 0.001
CRP, mg/l	5.4 ± 4.2	41.9 ± 35.7	< 0.001
No. painful joints	—	14.1 ± 7.7	—
Ritchie Index	—	7.8 ± 3.0	—
No. swollen joints	—	11.2 ± 7.3	—
Disease Activity Score	—	3.8 ± 0.8	—
Morning stiffness, min	—	118.7 ± 69.7	—
RF-positive patients, %	—	66.7	—
Patients with radiological stage I*, %	—	53.3	—
Patients with radiological stage II*, %	—	46.7	—

* Radiological stage of RA according to Steinbrocker. NS: differences not significant; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

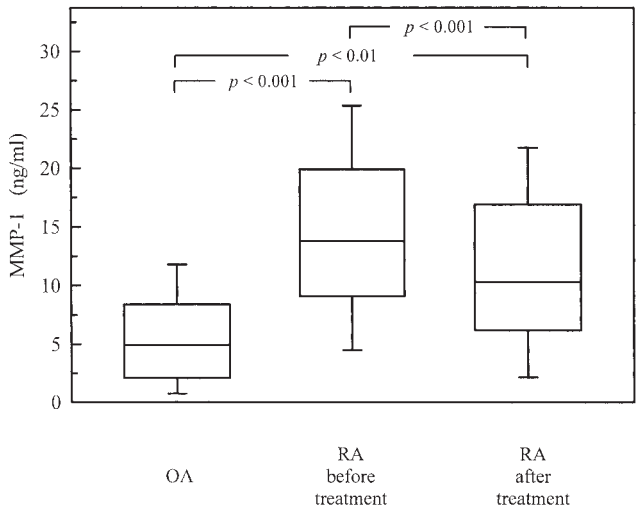

Figure 1. Serum concentrations of interstitial collagenase MMP-1 in patients with early RA compared to controls with OA, assessed by ELISA. Blood samples were obtained before and after 6 months' treatment with MTX (7.5-12.5 mg/wk). Box plots represent median (line), 25th and 75th percentiles (box), and 10th and 90th percentiles (whiskers).

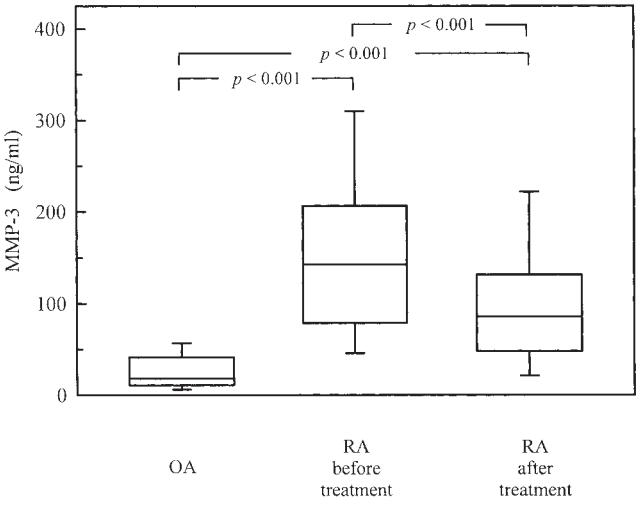

Figure 2. Serum concentrations of stromelysin-1 (MMP-3), determined and shown as described in the legend to Figure 1.

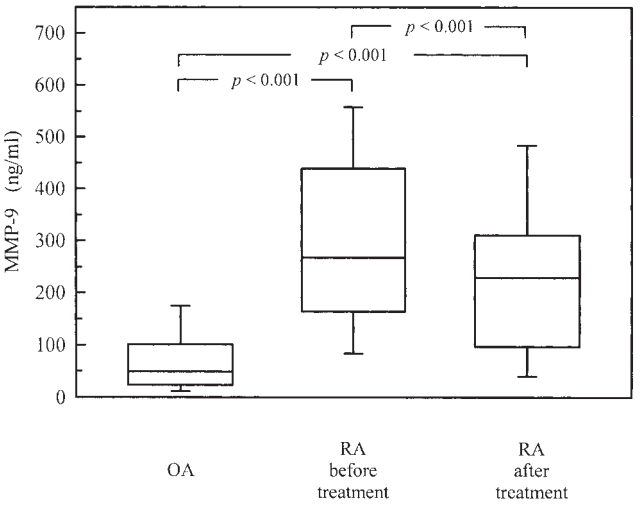

Figure 3. Serum concentrations of gelatinase B (MMP-9), determined and presented as described in the legend to Figure 1.

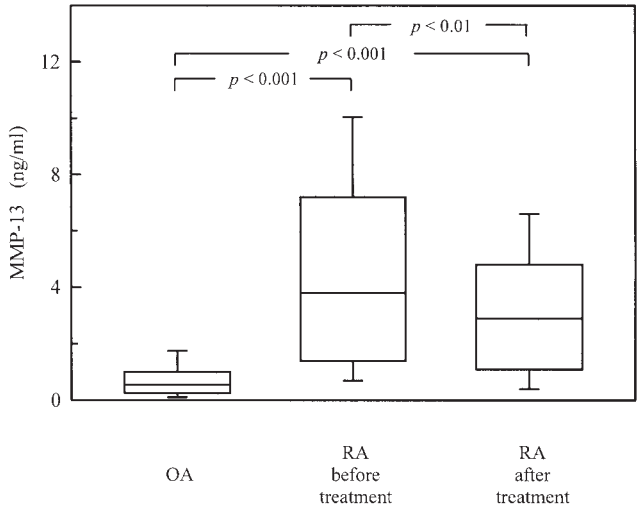

Figure 4. Serum concentrations of collagenase-3 (MMP-13), determined and shown as described in the legend to Figure 1.

such as transforming growth factor-ß, IL-6, and IL-11[4,5,15]. Our aim was to analyze serum concentrations of MMP-1, MMP-3, MMP-9, MMP-13, TIMP-1, and TIMP-2, and MMP/TIMP ratios in patients with early RA who were previously untreated with DMARD, and after 6 months treatment with MTX.

Interstitial collagenase (collagenase-1, MMP-1) produced mainly by synovial fibroblasts[16,17] is a prominent member of the MMP family and mediates the degradation of articular cartilage and synovium[4,5,8]. Expression of MMP-1, not only in synovia of patients with established erosive RA but also in patients with early arthritis, implies that the potential for joint destruction already exists at the early stage of the disease[18,19]. Previous investigators have shown that MMP-1 in RA is present in serum of patients with long-standing disease[12,17,20]. Recently, it was shown that serum concentrations of MMP-1 correlate with progression of joint destruction not only in patients with long-standing RA[21,22], but also in patients with early-stage disease[23]. Our study revealed that the level of MMP-1 in serum is already elevated in patients with early RA compared to OA patients. It is worthwhile emphasizing that our patients were previously untreated with DMARD or corticosteroids. Further, we observed decreases of serum MMP-1 levels during treatment with MTX. However, after 6 months' treatment with MTX, serum MMP-1 levels were still higher than in controls, suggesting that doses of MTX used in our

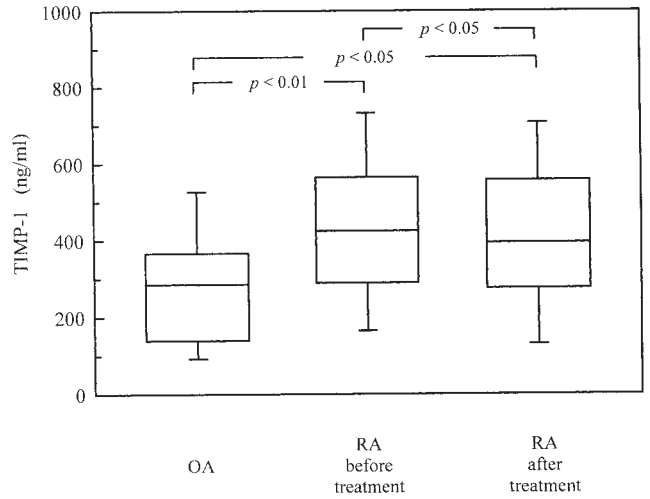

Figure 5. Serum concentrations of TIMP-1, determined and shown as described in the legend to Figure 1.

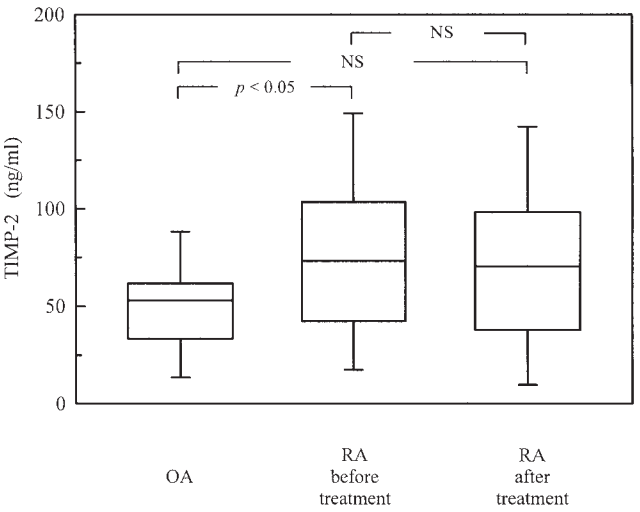

Figure 6. Serum concentrations of TIMP-2, determined and shown as described in the legend to Figure 1.

early RA patients were too low to suppress MMP-1 production. Our study also showed that this protease may serve as a marker of the very early stage of the disease.

Stromelysin-1 (MMP-3) is known to degrade components of the extracellular matrix including proteoglycans, gelatin, fibronectin, laminin, and various types of collagens[4,5,8,17]. It is also an activator of pro-MMP such as MMP-1 or MMP-9. This protease is mainly produced by fibroblasts[16]. MMP-3 was found in increased amounts in sera of patients with long-standing RA[12,17,24,25] and was correlated with progression of joint destruction[22,26,27]. Further, it was shown that the concentration of MMP-3 in RA synovial fluid correlated with that in serum[25,28,29]. Therefore, serum concentration of MMP-3 may be a useful marker of inflammation activity in the joints of patients with RA. In our study, similarly to MMP-1, the serum levels of MMP-3 were also increased in patients with early-stage disease. Levels of MMP-3 in serum of RA patients, even in the early stages, have been shown by others to be higher than in controls, and appeared to increase as the disease progressed[23,25,30,31]. Similarly to MMP-1, after treatment with MTX, serum MMP-3 levels were still higher than in controls, suggesting that inadequate doses of MTX were used in therapy of our patients in the early stages of RA. All these findings suggest that the measurement of serum MMP-3 may be an important tool in the diagnosis of early onset of RA.

The serum concentration of gelatinase B (MMP-9), produced mainly by granulocytes[6,32], was found to be elevated in serum of patients with long-standing RA[6,12,21,33]. Gelatinase B, besides digesting gelatins, participates in degradation of elastin, aggrecans, and link protein[34]. Therefore, almost all components of articular tissue are readily degraded if 2 or more MMP act together. We observed abundant presence of MMP-9 in the sera of patients with early RA. Although increased serum levels of MMP-9 diminished after treatment with MTX, they remained elevated compared to controls. Such observations also suggest that higher doses of MTX are needed to control the presence of gelatinase B in early RA.

Table 2. Concentration ratios of MMP to TIMP in RA patients before and after 6 months' treatment with MTX, and in OA patients.

	OA	RA Before Treatment	RA After Treatment	p		
	C	A	B	C vs A	C vs B	A vs B
MMP-1/TIMP-1	0.028	0.042	0.031	< 0.01	NS	< 0.001
MMP-1/TIMP-2	0.148	0.352	0.237	< 0.01	NS	< 0.05
MMP-3/TIMP-1	0.124	0.474	0.322	< 0.001	< 0.01	< 0.001
MMP-3/TIMP-2	0.746	2.865	2.061	< 0.001	< 0.001	NS
MMP-9/TIMP-1	0.274	0.903	0.711	< 0.001	< 0.001	< 0.05
MMP-9/TIMP-2	2.280	7.631	6.465	< 0.001	< 0.01	NS
MMP-13/TIMP-1	0.004	0.016	0.012	< 0.001	< 0.01	< 0.01
MMP-13/TIMP-2	0.018	0.099	0.094	< 0.001	< 0.01	NS
MMP/TIMP*	0.332	1.161	0.870	< 0.001	< 0.001	< 0.001

* Total measured MMP/total measures TIMP. MMP: matrix metalloproteinases; TIMP: tissue inhibitors of metalloproteinases; MTX: methotrexate; NS: differences not significant.

Table 3. Disease activity in RA patients before and after 6 months' treatment with MTX. Results presented as means ± SD and medians (25–75 percentiles).

	OA	RA Before Treatment	RA After Treatment	p		
	C	A	B	C vs A	C vs B	A vs B
No. painful joints	—	14.1 ± 7.7 13.0 (7.0–20.0)	5.8 ± 3.8 5.0 (3.0–8.0)	—	—	< 0.001
Ritchie Index	—	7.8 ± 3.0 8.0 (5.0–10.0)	3.6 ± 2.2 3.0 (2.0–4.0)	—	—	< 0.001
No. swollen joints	—	11.2 ± 7.3 8.0 (6.0–15.0)	3.8 ± 3.3 3.0 (1.0–6.0)	—	—	< 0.001
Disease Activity Score	—	3.8 ± 0.8 3.7 (3.0–4.4)	2.5 ± 0.6 2.4 (2.1–2.9)	—	—	< 0.001
ESR, mm/h	17.5 ± 11.6 12.0 (8.0–29.0)	62.8 ± 25.2 59.0 (40.0–80.0)	26.2 ± 12.0 22.5 (16.0–36.0)	< 0.001	< 0.05	< 0.001
CRP, mg/l	5.4 ± 4.2 4.0 (2.2–8.8)	41.9 ± 35.7 31.0 (15.7–53.7)	14.6 ± 11.0 11.7 (5.7–21.5)	< 0.001	< 0.001	< 0.001

Collagenase-3 (MMP-13) is produced by several cells such as chondrocytes, osteoblasts, fibroblasts, macrophages, and synoviocytes[35]. Although both collagenases we studied (MMP-1 and MMP-13) degrade fibrillar collagens, MMP-1 preferentially hydrolyzes type III collagen, whereas MMP-13 preferentially digests type II collagen[34]. Further, collagenase-3 cleaves type II collagen not only at the same bond as MMP-1 but also at 2 other bonds[36]. MMP-13 was found in synovium and synovial fluid of patients with long-standing RA, but not in their blood[37]. We detected MMP-13 in sera of patients with early RA and found its level was higher than in controls. Serum concentrations of MMP-13 decreased during treatment with MTX; they did not, however, reach the levels of the control group. Because concentrations of MMP-13 that we measured were rather low, these data should be considered with caution.

It is known that the activity of MMP is controlled by proenzyme production and/or by activation of their endogenous inhibitors. There are at least 2 TIMP; TIMP-1, the most widely distributed TIMP, suppresses the activity of all MMP[4,5]. The presence of TIMP-1 in increased amounts in serum of patients with long-standing RA was reported from other studies[12,17,25,38]. However, some researchers detected very similar levels of TIMP-1 in sera from RA and OA patients[24,39]. We showed that the serum levels of TIMP-1 were elevated in patients with early RA. Upregulation of TIMP-1 synthesis in patients with RA seems to be important for the suppression of synovium and cartilage destruction in early-stage disease. Serum TIMP-1 levels decreased slightly during treatment with MTX.

Higher serum concentration of TIMP-2 in patients with RA than in controls has been reported[12,38]. In our study, similarly to TIMP-1, serum concentrations of TIMP-2 were also increased in patients with early RA compared to patients with OA. TIMP-2 levels remained almost unchanged after MTX treatment.

Some investigators suggest that the articular damage in RA may be caused by alteration in the balance between MMP and TIMP in favor of destructive metalloproteinases[6,28,40]. In our study the concentration ratios of MMP-1, MMP-3, MMP-9, MMP-13, and total MMP to TIMP-1, TIMP-2, and total TIMP were higher in patients with early RA than in controls. The analysis of ratios of individual or total measured MMP to individual or total measured TIMP suggests that relative production of TIMP is already inadequate in early RA. These data should be taken with caution because other proteinases involved in the process of articular tissue destruction and other inhibitors were not measured.

Radiological analysis showed that patients with more advanced joint destruction had higher serum levels of MMP and TIMP-1 (data not shown). Other investigators also observed correlations of serum concentrations of MMP-1, MMP-3, and TIMP-1 with disease progression[21,22,25]; however, those studies were mainly in patients with long-standing disease.

MTX has been shown to reduce MMP-1 and MMP-3[41-43] and even to increase TIMP-1 production[44]. On the other hand, some studies suggest that MTX has no significant influence on MMP-3[41] or TIMP-1 production[41,43]. We found decreased serum levels of all studied MMP (MMP-1, MMP-3, MMP-9, and MMP-13) and TIMP-1 during treatment with MTX. Further, these changes were accompanied by decreased concentration ratios of MMP to TIMP. However, a significant decrease was observed mainly in the case of TIMP-1. And except for MMP-1, concentration ratios of MMP-3, MMP-9, and MMP-13 to TIMP-1 and TIMP-2 after MTX therapy were still significantly higher than in controls even after 6 months' treatment with MTX. Such findings may suggest that higher doses of MTX should be used in therapy of patients with early RA.

We also demonstrated that MTX treatment was followed by decreases in the markers of RA severity such as the number of painful and swollen joints, Ritchie Index, Disease

Activity Score, ESR, and CRP levels. Serum concentrations of studied proteases correlated with these clinical markers of disease activity, such as the number of painful and swollen joints, Ritchie Index, DAS, ESR, and CRP levels, especially before MTX treatment.

We conclude that patients with early RA are characterized by high serum levels of tissue degrading metalloproteinases such as MMP-1, MMP-3, MMP-9, and MMP-13. Therapy with MTX results in clinical improvement and reduced serum MMP concentrations in patients with RA, confirming the effectiveness of MTX in patients with early-stage disease. Serum levels of matrix metalloproteinases and their tissue inhibitors seem to be useful markers of disease activity in early RA.

REFERENCES

1. Bresnihan B. Pathogenesis of joint damage in rheumatoid arthritis. J Rheumatol 1999;26:717-9.
2. Hale LP, Haynes BF. Pathology of rheumatoid arthritis and associated disorders. In: Koopman WJ, editor. Arthritis and allied conditions. A textbook of rheumatology. Baltimore: Williams & Wilkins; 1997:993-1016.
3. Harris ED. Rheumatoid arthritis. Philadelphia: W.B. Saunders Company; 1997.
4. Firestein GS. Etiology and pathogenesis of rheumatoid arthritis. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, editors. Textbook of rheumatology. Philadelphia: W.B. Saunders Company; 1997:851-97.
5. Nagase H, Okada Y. Proteinases and matrix degradation. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, editors. Textbook of rheumatology. Philadelphia: W.B. Saunders Company; 1997:851-97.
6. Ahrens D, Koch AE, Pope RM, Stein-Picarella M, Niedbala MJ. Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. Arthritis Rheum 1996;39:1576-87.
7. Klimiuk PA, Yang H, Goronzy JJ, Weyand CM. Production of cytokines and metalloproteinases in rheumatoid arthritis is T cell dependent. Clin Immunol 1999;90:65-78.
8. Matrisian L. Metalloproteinases and their inhibitors in matrix remodelling. Trends Genet 1990;6:121-5.
9. 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.
10. Ritchie DM, Boyle JA, McInnes JM, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 1968;37:93-406.
11. van der Heijde DMFM, van 't Hof MA, van Riel PLCM, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49:916-20.
12. Klimiuk PA, Sierakowski S, Latosiewicz R, Cylwik B, Skowronski J, Chwiecko J. Serum metalloproteinases and tissue inhibitors of metalloproteinases in different histological variants of rheumatoid arthritis. Rheumatology Oxford 2002;41:78-87.
13. Klimiuk PA, Sierakowski S, Domyslawska I, Chwiecko J. Effect of repeated infliximab therapy on serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with rheumatoid arthritis. J Rheumatol 2004;31:238-42.
14. Lefebvre V, Peeters-Joris C, Vaes G. Modulation by interleukin 1 and tumor necrosis factor α of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta 1990;1052:366-78.
15. Hermann JA, Hall MA, Maini RN, Feldman M, Brennan FM. Important immunoregulatory role of interleukin-11 in the inflammatory process in rheumatoid arthritis. Arthritis Rheum 1998;41:1388-97.
16. Tetlow LC, Lees M, Woolley DE. Comparative studies of collagenase and stromelysin-1 expression by rheumatoid synoviocytes in vitro. Virchows Archiv 1995;425:569-76.
17. Manicourt DH, Fujimoto N, Obata K, Thonar EJMA. Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. Arthritis Rheum 1995;38:1031-9.
18. Cunnane G, FitzGerald O, Hummel KM, Gay RE, Gay S, Bresnihan B. Collagenase, cathepsin B and cathepsin L gene expression in the synovial membrane of patients with early inflammatory arthritis. Rheumatology Oxford 1999;38:34-42.
19. Katrib A, Tak PP, Bertouch JV, et al. Expression of chemokines and matrix metalloproteinases in early rheumatoid arthritis. Rheumatology Oxford 2001;40:988-94.
20. Maeda S, Sawai T, Uzuki M, et al. Determination of interstitial collagenase (MMP-1) in patients with rheumatoid arthritis. Ann Rheum Dis 1995;54:970-5.
21. Goldbach-Mansky R, Lee JM, Hoxworth JM, et al. Active synovial matrix metalloproteinase-2 is associated with radiographic erosions in patients with early synovitis. Arthritis Res 2000;2:145-53.
22. Green MJ, Gough AKS, Devlin J, et al. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. Rheumatology Oxford 2003;42:83-8.
23. Cunnane G, FitzGerald O, Beeton C, Cawston TE, Bresnihan B. Early joint erosions and serum levels of matrix metalloproteinase 1, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinases 1 in rheumatoid arthritis. Arthritis Rheum 2001;44:2263-74.
24. Keyszer G, Lambiri I, Nagel R, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. J Rheumatol 1999;26:251-8.
25. Yoshihara Y, Obata K, Fujimoto N, Yamashita K, Hayakawa T, Shimmei M. Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. Arthritis Rheum 1995;38:969-75.
26. Sasaki S, Iwata H, Ishiguro N, Obata K, Miura T. Detection of stromelysin in synovial fluid and serum from patients with rheumatoid arthritis and osteoarthritis. Clin Rheumatol 1994;13:228-33.
27. Tchetverikov I, Lard LR, DeGroot J, et al. Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. Ann Rheum Dis 2003;62:1094-9.
28. Ishiguro N, Ito T, Obata K, Fujimoto N, Iwata H. Determination of stromelysin-1, 72 and 92 kDa type IV collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. J Rheumatol 1996;23:1599-604.
29. Taylor DJ, Cheung NT, Dawes PT. Increased serum proMMP-3 in inflammatory arthritides: a potential indicator of synovial inflammatory monokine activity. Ann Rheum Dis 1994;53:768-72.
30. Posthumus MD, Limburg PC, Westra J, et al. Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. Rheumatology Oxford 1999;38:1081-7.
31. Yamanaka H, Matsuda Y, Tanaka M, et al. Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early

rheumatoid arthritis. Arthritis Rheum 2000;43:852-8.
32. Blaser J, Triebel S, Maasjosthusmann U, et al. Determination of metalloproteinases, plasminogen-activators and their inhibitors in synovial fluids of patients with rheumatoid arthritis during chemical synoviorthesis. Clin Chim Acta 1996;244:17-33.
33. Gruber BL, Sorbi D, French DL, et al. Markedly elevated serum MMP-9 (gelatinase B) levels in rheumatoid arthritis: a potentially useful laboratory marker. Clin Immunol Immunopathol 1996;78:161-71.
34. Okada Y. Matrix-degrading metalloproteinases and their roles in joint destruction. Mod Rheumatol 2000;10:121-8.
35. Murphy G, Knäuper V, Atkinson S, et al. Matrix metalloproteinases in arthritic disease. Arthritis Res 2002;4 Suppl:S39-S49.
36. Leeman MF, Curran S, Murray GI. The structure, regulation, and function of human matrix metalloproteinase-13. Crit Rev Biochem Mol Biol 2002;37:149-66.
37. Tchetverikov I, Ronday HK, Van El B, et al. MMP profile in paired serum and synovial fluid samples of patients with rheumatoid arthritis. Ann Rheum Dis 2004;63:881-3.
38. Baker T, Tickle S, Wasan H, Docherty A, Isenberg D, Waxman J. Serum metalloproteinases and their inhibitors: markers for malignant potential. Br J Cancer 1994;70:506-12.
39. Keyszer G, Lambiri I, Keysser M, et al. Matrix metalloproteinases, but not cathepsins B, H, and L or their inhibitors in peripheral blood of patients with rheumatoid arthritis, are potentially useful markers of disease activity. Z Rheumatol 1998;57:392-8.
40. Yoshihara Y, Nakamura H, Obata K, et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis 2000;59:455-61.
41. Firestein GS, Paine MM, Boyle DL. Mechanisms of methotrexate action in rheumatoid arthritis. Selective decrease in synovial collagenase gene expression. Arthritis Rheum 1994;37:193-200.
42. Kraan MC, Reece RJ, Barg EC, et al. Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. Findings in a prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. Arthritis Rheum 2000;43:1820-30.
43. Martel-Pelletier J, McCollum R, Fujimoto N, Obata K, Cloutier JM, Pelletier JP. Excess of metalloproteases over tissue inhibitor of metalloprotease may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. Lab Invest 1994;70:807-15.
44. Seitz M, Dayer JM. Enhanced production of tissue inhibitor of metalloproteinases by peripheral blood mononuclear cells of rheumatoid arthritis patients responding to methotrexate treatment. Rheumatology Oxford 2000;39:637-45.