Matrix metalloproteinases involvement in rheumatoid arthritis

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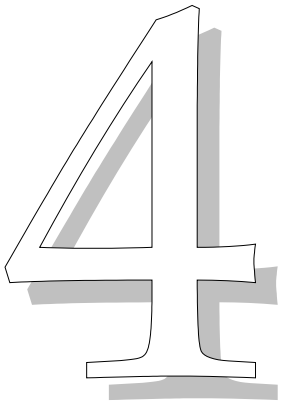
CHAPTER



MMP PROFILE IN PAIRED SERUM AND SYNOVIAL FLUID SAMPLES OF RHEUMATOID ARTHRITIS PATIENTS

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Abstract

Objective. To analyze Matrix Metalloproteinases (MMPs) and Tissue Inhibitor-1 of MMPs (TIMP-1) levels in the systemic circulation and synovial fluid (SF) of rheumatoid arthritis patients and to compare these levels to inflammatory and collagen degradation markers.

Methods. The measurements were performed in paired SF and serum samples from 15 RA and 13 OA patients and compared to normal levels. ProMMP-1, -2, -3, -8, -9, TIMP-1, levels of MMP/α_2Macroglobulin complexes and collagen degradation products were measured using sandwich ELISA, activity assays and HPLC.

Results. MMPs were highly increased in SF of RA patients as compared to OA or control groups. MMP levels in SF of OA patients were higher than in control group. In serum, levels of proMMP-3, -8 and -9 were increased in RA patients as compared to OA or controls, whereas only proMMP-8 and -9 were increased in serum of OA patients as compared to controls. A strong correlation was observed between serum and SF levels of MMP-8 and -9 in RA. Further, increased levels in MMP/α_2Macroglobulin complexes indicated presence of MMP/TIMP imbalance in serum and SF of RA patients. SF Hypdroxyproline level (Hyp, used as a marker of joint collagen degradation) was significantly correlated with SF levels of proMMP-9 in RA.

Conclusions. Present study shows that systemic MMP-8 and -9 levels represent the situation in the inflamed joint and suggest that MMP-9 is likely to be involved in degradation of joint collagen. The results also strengthen the hypothesis of MMP/TIMP imbalance in RA.

Introduction

Matrix Metalloproteinases (MMPs)[I] are a group of Zn^{2+} dependent extracellular enzymes that play a key role in a normal and pathological tissue remodeling. The whole group can be divided into subclasses, such as collagenases, gelatinases, stromelysins and membrane types MMPs.[1] In rheumatoid arthritis (RA), MMPs are indicated to be involved in excessive degradation of joint tissue.

MMP-3 (stromelysin-1) can degrade various components of joint tissue as well as to activate proMMPs.[1] MMP-3 is suggested to be a joint derived marker of synovitis since it is produced by synovial fibroblasts of the rheumatoid joints and not by normal synovial cells[1] and is correlated with inflammatory markers in the systemic circulation such as C-reactive protein (CRP).[2] Other MMP subclasses such as gelatinases and collagenases are expressed by the cells of the pannus-cartilage or bone junction and by infiltrating inflammatory cells such as neutrophils and macrophages. Increased levels of collagenases and gelatinases are found not only at tissue level,[3] but also in the synovial fluid (SF)[4] and in the systemic circulation.[1] Moreover, high tissue levels of MMP-2 and -9 and systemic levels of MMP-1 in RA patients were indicated to be associated with development of joint erosions.[5,6]

The above mentioned studies show that a significant progress is made on identification of MMPs involved in the disease process in RA. However, studies on multiple MMP subclasses and/or the relationship between local and systemic levels of the different MMP subclasses are scarce.

The present study was designed to provide a broad analysis of the MMPs involved in the RA pathology and to investigate the relation between local and systemic levels of MMPs and their Tissue Inhibitor. Furthermore, the relation between studied MMPs and systemic marker of inflammation (CRP) and joint collagen degradation product (hydroxyporline) in RA patients was investigated.

Materials and Methods

Hydroxyproline measurements

Hydroxyproline (Hyp) was measured in diluted SF samples by HPLC after acid hydrolysis according to the method described by Bank *et al.*[7]

ProMMPs AND TIMP-1

ProMMP-3 and -13 and TIMP-1 were measured using the sandwich ELISA (Amersham Biosciences, Little Chalfont, UK). ProMMP-1, -2, -8, and -9, were detected using specific MMP activity assays (Biotrak activity assay, Amersham Biosciences, Little Chalfont, UK).[8]

[I] Abbreviations: α_2M: alpha$_2$Macroglobulin; CRP: C-reactive protein; MMPs: Matrix Metalloproteinases; OA: osteoarthritis; proMMPs: pro-Matrix Metalloproteinases; RA: rheumatoid arthritis; RF: rheumatoid factor; SF: synovial fluid; TIMPs: Tissue Inhibitors of Metalloproteinases.

MMP activity in MMP/α_2Macroglobulin complexes

MMP activity in α_2Macroglobulin complexes was determined using small fluorogenic substrates according to the modified method described by Beekman *et al.*[9], Riley *et al.*[10] and DeGroot *et al.*[11]

Paired serum/SF samples

Paired SF and serum samples from RA (N = 15) and OA (N = 13) patients were collected during therapeutic arthrocentesis of a knee joint. *Post mortem* obtained SF samples of traffic casualties were used as controls (N = 9, material was obtained with informed consent of the relatives), healthy volunteers (N = 15) provided control serum samples. Basic characteristics of the patients groups are shown in **Table 1**.

***Table 1**. Basic characteristics of the patients groups. CRP = C-reactive protein; RF = rheumatoid factor.*

	RA	*OA*	*Controle serum*
Age, years (mean ± SD)	53 ± 13.7	76.8 ± 10	47.5 ± 11.9
Male/Female	3/12	3/8	9/6
Disease duration, years (mean ± SD)	6.7 ± 6.3	5.2 ± 2.5	
CRP, mean ± SD	28.9 ± 23.7	3 ± 3.4	
RF positive	11 of 15		

Statistical analysis

The Kruskal-Wallis test was used to assess differences between study groups, which were further analyzed using the Mann-Whitney U test. Correlations were evaluated using the Pearson's correlation or Spearman rank correlation coefficients with SPSS software (Chicago, IL, USA). $P \leq 0.05$ was considered significant.

Results

The median values of the Synovial Fluid and serum levels of the studied MMPs and TIMP-1 are shown in the **Table 2**. As expected, highly increased MMP levels were found in SF of RA patients as compared to control group. Also OA SF MMP levels were significantly higher than in control population, although they were lower than in RA. In contrast to SF, only proMMP-3, -8 and -9 levels were increased in serum of RA patients when compared to control group or OA patients. ProMMP-13 levels were below the detection limit in both compartments in all groups.

Significant correlations were found between SF and serum proMMP-9 ($r = 0.718$, $P < 0.003$) and proMMP-8 ($r = 0.664$, $P < 0.007$) levels in RA patients. Further, levels of the studied MMPs were higher in SF than in the systemic circulation ($P < 0.001$) in RA group except for proMMP-9 levels ($P = 0.78$). To assess the degree of the on-going joint tissue degradation during acute joint effusion, Hydroxyproline (Hyp) levels in SF were analyzed. No significant difference was found with regard to the Hyp levels in RA and OA SF (median [25^{th} - 75^{th} percentiles]: 14.4 [10.8 - 17.2] vs. 11.8 [9.3 - 19.8], $P = 0.68$).

Table 2. *MMP, TIMP and MMP activity in MMP/α_2Macroglobulin activity (MMP/α_2M) levels in serum and synovial fluid of RA and OA patients and in controls. Values shown are median [25th - 75th percentiles]. *, † and $^{\#}$ indicate statistical significance between the study populations.*

SF	*Controls*	*OA*	*RA*
MMP-1, U/ml	0.0 [0.0 - 0.01]	0.28 [0.21 - 1.23] $^{\#}$	5.9 [3.6 - 11.3] $^{\ddagger\,**}$
MMP-8, U/ml	0.0 [0.0 - 0.01]	0.75 [0.15 - 2.2] $^{\#}$	10 [4.7 - 30.0] $^{\ddagger\,**}$
MMP-3, ng/ml	177 [149 - 249]	869 [625 - 7401] $^{\#}$	22044 [14700 - 25800] $^{\ddagger\,**}$
MMP-2, U/ml	17 [13 - 19]	30 [25 - 35] $^{\#}$	31 [30 - 34] **
MMP-9, U/ml	0.1 [0.07 - 0.18]	0.7 [0.4 - 1.7] $^{\#}$	4.0 [2.3 - 6.3] $^{\ddagger\,**}$
MMP-13	Not detectable	Not detectable	Not detectable
MMP/α_2M, U/ml	37 [34 - 41]	55 [43 - 124] $^{\#}$	470 [334 - 1321] $^{\ddagger\,**}$
TIMP-1, ng/ml	322 [0 - 1478]	8961 [5287 - 16252] $^{\#\#}$	5717 [1951 - 9666] **
Serum			
MMP-1, U/ml	0.06 [0.02 - 0.1]	0.06 [0 - 0.1]	0.06 [0.06 - 0.1]
MMP-8, U/ml	0.64 [0.6 - 1]	1.12 [0.7 - 2.6] $^{\#\#}$	4.4 [1.0 - 6.6] $^{\dagger\,**}$
MMP-3, ng/ml	16 [11 - 25]	17 [12 - 28]	85 [36 - 117] $^{\ddagger\,**}$
MMP-2, U/ml	5.6 [4.9 - 6.4]	6 [4.9 - 7.0]	6.15 [5.6 - 8.0]
MMP-9, U/ml	2.1 [1.6 - 2.6]	3.0 [2.0 - 3.6] $^{\#\#}$	4.6 [3.0 - 6.4] $^{\dagger\,**}$
MMP-13	Not detectable	Not detectable	Not detectable
MMP/α_2M, U/ml	86 [77 - 128]	88 [80 - 145]	172 [71 - 198] *
TIMP-1, ng/ml	445 [428 - 627]	495 [412 - 1368]	345 [266 - 1088] †

$^{\ddagger}P < 0.001$ RA vs. OA $^{**}P < 0.001$ RA vs. Controls $^{\#\#}P<0.001$ OA vs. Controls
$^{\dagger}P < 0.05$ RA vs. OA $^{*}P < 0.05$ RA vs. Controls $^{\#}P<0.05$ OA vs. Controls

A significant correlation was found between SF proMMP-9 levels and Hyp in RA group, **Table 3**. Also, a trend towards a correlation was seen between SF proMMP-8 and Hyp, whereas no correlation was found between Hyp and SF proMMP-3 or systemic CRP levels. In SF, the following distribution pattern of the proMMPs was observed: RA > OA > Controls. However, TIMP-1 showed a different distribution pattern: RA = OA > Controls, which implies an imbalance within the proteolytic system in favour of the MMPs. Moreover, higher levels of MMP in complex with α_2Macroglobulin (activated, but not-TIMP-inhibited MMPs are likely to form complexes with α_2M[12]) were found in RA patients as compared to control or OA groups, strengthening the hypothesis of an MMP/TIMP imbalance.

Table 3. *Correlations between synovial fluid (SF) proMMP-3, -8 and -9 levels, Hydroxiproline levels in SF and systemic levels of C-reactive protein (CRP). Values shown are correlation coefficient (P-value). Pearson's correlation was used to analyze proMMP-3 levels; Spearman's rank correlation test was used to analyze proMMP-8 and -9 levels.*

	Hyp	CRP
MMP-3	0.305 (*0.27*)	0.696 (*0.004*)*
MMP-8	0.49 (*0.06*)	0.196 (*0.48*)
MMP-9	0.59 (*0.02*)*	0.147 (*0.602*)
CRP	0.116 (*0.68*)	

** MMP-3: Pearson's correlation; MMP-8 and -9: Spearman's rank correlation test*

Discussion

The present study provides an extensive analysis of MMPs, TIMP-1 and MMP/α_2Macroglobulin complex in paired serum and synovial fluid samples of RA and OA patients and knee-healthy controls.

The highest MMP levels in SF of RA patients were found for proMMP-1, -3, -8 and -9. Whereas MMP-3 and -9 were also detectable in control SF, MMP-1 and -8 were found in control SF only at extremely low concentrations. MMP levels in SF of OA patients were lower than in RA, but significantly higher than in control SF, indicating that use of OA material as control for RA patients may not always be appropriate. Interestingly, MMP-8 and MMP-9 levels were correlated with each other in SF of both RA and OA groups. Also MMP-1 and MMP-3 levels were correlated with each other in RA and OA groups, probably indicating the predominant cell source of these MMPs: macrophages and neutrophils for MMP-8 and -9 and synovial cells for MMP-1 and -3.[4] MMP analysis in the systemic circulation of RA and OA patients indicated that not all MMP locally involved (as shown by the increased SF levels) are also elevated in serum. High serum levels of MMP-3, -8 and -9 were found in RA, whereas only MMP-8 and -9 were also found to be increased in serum of OA patients.

Furthermore, strong correlations between serum and SF levels of proMMP-8 and -9, which were both elevated in SF and serum of RA patients were found, suggesting that serum levels of these enzymes may be used to assess the situation in the inflamed joints.

To elucidate the involvement of different MMP subclasses in the joint inflammation and tissue degradation, MMP levels were compared to markers of systemic inflammation (CRP) and collagen degradation marker Hydroxyproline (Hyp). Only proMMP-3 levels in SF were correlated with CRP levels, which is consistent with findings by others.[6] SF Hyp levels were not correlated with proMMP-3, whereas a significant correlation with proMMP-9 levels and a trend towards correlation with proMMP-8 were found. These results are in line with *in vitro* data on collagen degradation: collagen, one of the main components of the articular cartilage, can be specifically cleaved by collagenases into the characteristic ¾ and ¼ fragments,[13] which can be further degraded by gelatinases.[1] Thus, the results provide *in vivo* indication of MMP-9 involvement in collagen degradation in joints of RA patients. Taken together, these results show that MMP-8 and -9 are likely to be involved in degradation of collagenous network of joint tissue, whereas proMMP-3 levels are likely to reflect the inflammatory component of the disease process.

Ample body of evidence suggests presence of MMP/TIMP imbalance in arthritic diseases.[4,14] Based on molar ratios, TIMPs levels seem be insufficient to counteract increased MMP production levels in rheumatoid arthritis. As such, our results of equally increased TIMP-1 levels in serum and SF of OA and RA patients and higher levels of proMMPs in RA are in line with this hypothesis. Furthermore, in SF and serum of RA patients high levels of active MMPs in complex with α_2Macroglobulin are present, also indicating MMP/TIMP imbalance in RA as compared to the normal situation or OA patients. The importance of MMP/TIMP imbalance in RA is also indicated by higher MMP activity levels at the inflammation site (SF) as compared to the serum levels, since no difference were seen between local and systemic levels in OA.

In conclusion, the present study provides an extensive analysis of systemic and SF levels of major MMP subclasses in RA and OA patients. Furthermore, the results (i) show that systemic levels of MMP-8 and -9 are representative for the levels of these enzymes in the inflamed joints, (ii) may indicate involvement of MMP-9 in generation of collagen degradation products in SF of RA patients and (iii) are in accordance with the hypothesis of MMP/TIMP imbalance in RA.

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