MMP-3 ACTIVITY MEASUREMENTS IN SERUM AND PLASMA OF OA AND RA PATIENTS

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Introduction: Matrix Metalloproteinases (MMPs) play a key role in normal and pathologic connective tissue remodeling, including cartilage degradation in Rheumatoid Arthritis (RA) and Osteoarthritis (OA) [1]. Especially serum MMP-3 protein levels have been suggested to have a prognostic value for cartilage destruction in RA [2,3].

In the normal situation a tight balance exists between the amount of activated MMPs and the level of Tissue Inhibitors of Matrix metalloproteinases (TIMPs). In inflammatory joint diseases an imbalance between MMPs and TIMPs is present which has been implicated in cartilage degradation in RA and OA [4]. Inasmuch as MMPs are also known to form complexes with the in plasma abundantly present protease inhibitor α2Macroglobulin (α2M) activated MMPs that are not neutralized by TIMPs are expected to result in α2M/MMP complexes in the systemic circulation [5,6]. The first aim of the present study was to provide evidence for the formation of such α2M/MMP complexes in the systemic circulation. The second aim was to measure active MMP-3, including α2M-bound MMP-3, in human serum or plasma, and to compare RA and OA patients and healthy subjects.

Methods: To provide evidence for α2M/MMP complex formation activated collagenase-3 (MMP-13) was spiked to human plasma and fractionated using FPLC. MMP activity, measured with a low molecular weight fluorogenic substrate, was compared in serum and plasma of RA and OA patients and healthy controls (n=8, 6, and 15, resp.). Each sample was measured in duplicate using TNO003-F substrate in combination with CP138,521 (inhibits all MMPs except MMP-3) or BB94 (inhibits all MMPs) [7,8]. MMP-3 related substrate conversion was calculated as the difference in increase of fluorescence between samples measured in presence of CP138,521 or BB94.

To minimize general background protease activity, all measurements were performed in presence of Complete™, a general protease inhibitor cocktail. Similarly, MMP-3 activity was determined in synovial fluid samples of RA patients and healthy control subjects (n=7 and 14, resp.).

Results: After FPLC separation of α2M/MMP-13 complexes (reconstituted in buffer) enzyme activity measured with fluorogenic substrate showed a peak at the position of α2M (Mw ~750 kDa). Active free MMP-13 (Mw 48 kDa) resulted in enzyme activity at the tail of the albumin peak (Mw 64 kDa). Spiking of MMP-13 to plasma only revealed MMP enzyme activity at the α2M and α2M/MMP position. Enzyme activity was successfully inhibited by BB94 but not by TIMP-1 (this ~20 kDa protein is unable to enter the α2M/MMP complex). Altogether, these findings indicate that upon entering the systemic circulation active MMPs form complexes with α2M and that the fluorogenic assay allows measurement of α2M-bound MMP-3.

MMP-3 activity could satisfactorily be determined in paired plasma and serum samples. MMP-3 activity in serum and plasma were highly correlated (r=0.797, p=0.01, Fig. 1). MMP-3 activity was 22±38 % higher in serum than in plasma. This may be due to activation of proMMPs, or due to excretion (and activation) of MMPs from white blood cells during coagulation of blood [9].

In healthy controls, low levels of MMP-3 activity were found in serum. In serum of OA patients, MMP-3 activity was not significantly higher than in control subjects. In RA patients, MMP-3 activity was 2.4-fold higher than in OA or control subjects (p=0.002; t-test, Fig. 2).

In synovial fluids, MMP-3 activity determined with this assay was also higher in RA patients than in fluid from healthy subjects (p=0.001, Fig. 3). As such the higher MMP-3 activity in RA subjects than in control subjects is consistently found in serum and synovial fluid.

Discussion: Use of serum MMP levels as a readout parameter for disease activity or as a prognostic parameter is currently a subject of investigation and seems to reflect not only the inflammation but also cartilage degradation in RA [2,3]. The 2-fold higher MMP-3 activity in serum of RA patients compared to healthy controls is consistent with MMP-3 protein levels as determined by ELISA [3].

Inasmuch as several new clinically applied anti-arthritic drugs [10] alter gene expression of proMMPs and TIMPs, our newly developed assay allows assessment of the overall effect of these drugs on joint degradation, i.e. their effect on net proteolytic activity.

In conclusion, the present study indicates that active MMPs are converted into α2M/MMP complexes upon entering the systemic circulation, that α2M/MMP-3 can be measured in the systemic circulation, and that the MMP-3 activity in serum is higher in RA patients than in healthy controls.

References:

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Poster Session - Matrix Metalloproteinases - Hall E