The TNF–MMP–matrix network and serum markers for cartilage synthesis and degradation

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TNF has been shown to affect articular matrix integrity by different mechanisms; directly by inhibiting proteoglycan aggrecan synthesis and indirectly by up-regulating matrix metalloproteinases (MMPs) mediating collagen and proteoglycan aggrecan degradation. The net effect of TNF on articular matrix remodeling / degradation in rheumatoid arthritis and the effect of synovial TNF inhibition may be reflected in patients by changes in serum profiles of immunochemical markers which represent important research tools in the clinical development of novel anti-rheumatic agents.

Our aim was to investigate in patients with rheumatoid arthritis whether correlations exist between synovial fluid levels of TNF / sTNFRs and pro-MMP-1 / pro-MMP-3, the extra-synovial concentrations of these molecules and between serum TNF / sTNFR and pro-MMP levels and immunochemical markers of articular matrix remodeling and to explore the effects of synovial TNF trapping on the TNF–matrix network.

Patients and methods

Paired serum / synovial fluid samples from 11 patients with RA (1987 revised ACR criteria) were analyzed for TNF, sTNFR 55, sTNFR 75, pro-MMP-1 and pro-MMP-3 using ELISA techniques. In a separate exploratory study serial serum measurement of pro-MMP-1, pro-MMP-3, TNF, sTNFR 75, keratan sulfate, CS 846, CP II and hyaluronic acid was done in parallel to clinical and laboratory assessment of disease activity before and after synovial TNF trapping following the administration of a TNF receptor fusion protein (Fenner 1994).

Results

1. Simultaneous measurement of TNF / TNF receptors and the latent forms of stromelysin (pro-MMP-3) and collagenase (pro-MMP-1) in synovial fluid:

A linear relationship exists between synovial fluid TNF concentrations and pro-MMP expression / release (TNF / pro-MMP-3: R = 0.77, TNF / pro-MMP-1: R = 0.61).

There was also a good correlation between synovial fluid concentrations of soluble TNF receptors and pro-MMPs (sTNFR 55 / pro-MMP-1: R = 0.58; sTNFR 55 / pro-MMP-3: R = 0.58; sTNFR 75 / pro-MMP-1 :R = 0.63; sTNFR 75 / pro-MMP-3: R = 0.80) and between pro-MMP-1 and pro-MMP-3 synovial fluid concentrations (R = 0.86).

2. Serum / synovial fluid correlations: pro-MMP-1; R = 0.66, pro-MMP-3;R = 0.79

3. Changes in pro-MMP levels upon synovial TNF trapping: Circulating pro-MMP-1 and pro-MMP-3 levels significantly decline, in many patients more than 50% compared to baseline values. Persisting down-regulation of pro-MMPs corresponds with persistent synovial TNF inhibition.

4. Effects of pro-MMP–down-regulation on immunochemical serum markers for cartilage metabolism: type II procollagen C-propeptide (CP II) levels decline in parallel to pro-MMP-1 inhibition, the levels of the cartilage aggrecan chondroitin sulfate epitope 846 follow the decline of pro-MMP levels, keratan sulfate decreases as well as the CS 846 / KS ratio.

Discussion

Synovial up-regulation of pro-MMP-1 and pro-MMP-3 correlates with local TNF production suggesting a synovial TNF concentration–response relationship. Both latent MMP forms are up-regulated simultaneously indicating a coordinated regulation of collagenase and stromelysin genes. Serum pro-MMP levels reflect their linear distribution from the synovial system into circulation, where they can be measured by routine ELISA procedures. It is apparent that serum pro-MMPs represent a probe for synovial TNF- induced activation of effector cells which can be reversed by synovial TNF trapping as demonstrated in patients after TNF inhibitor administration. Clearance of the synovial system from TNF has a rapid and profound effect on the imbalance between synthesis and degradation. These preliminary results on the effects of TNF inhibition indicate a pivotal role of TNF for articular matrix degradation and suggest that long-term treatment with agents targeting TNF has the potential for lowering the rate of progression of structural damage in RA. Research on immunochemical markers for cartilage metabolism will have significant benefit from data generated in patients treated with TNF inhibitors since this mechanism based intervention provides a unique opportunity to

follow changes in marker profiles and the underlying mechanisms which may reflect a restored balance in synthesis and degradation of matrix components.

Reference

Fenner H. TNF as a target of therapeutic intervention:The immunopharmacological profile of a TNF soluble receptor fusion protein. Rheumatology in Europe (Suppl 2) 1994; 23: 17–18.

Measurement of chondroitin sulphate disaccharides in pathological joint fluids

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A range of extracellular matrix components have been investigated as 'markers' of joint tissue metabolism and many of them have proved to be useful for this purpose. Chondroitin sulphate is the major sulphated glycosaminoglycan present in the extracellular matrix of joint tissues and is an integral component of many proteoglycans. The different molecular forms of proteoglycan (decorin, biglycan, versican, aggrecan etc.,) have specific chondroitin sulphation patterns which vary with age and disease.

The purpose of this study was to detemine whether chondroitin sulphate disaccharides could discriminate between disease groups and be useful indicators of joint disease severity and progression.

Methods

Normal synovial fluids were obtained from healthy volunteers with no evidence of joint disease and from patients with osteoarthritis (OA) or rheumatoid arthritis (RA) of the knee. Patients with knee OA were further subdivided according to the presence of calcium pyrophosphate (CPPD) and the presence of nodal OA of the hands (NGOA). In patients with OA or RA, knee inflammation was assessed as active or inactive using a summated clinical score (Doherty et al. 1988). Synovial fluids were sequentially digested with *streptomyces* hyaluronidase and chondroitin ABC lyase and the resultant unsaturated chondroitin disaccharides were analysed by capillary zone electrophoresis.

Results

The only sulphated chondroitin disaccharide species detected in synovial fluid were Δdi-4S and Δdi-6S. The mean ± SEM values of Δdi-4S and Δdi-6S together with the ratios of Δdi-6S to Δdi-4S for OA and RA patients are shown in Table 1. The total chondroitin sulphate concentration was estimated from the sum of Δdi-4S and Δdi-6S and was higher in normal fluids than in OA, CPPD and RA fluids. When the distribution of chondroitin sulphate isomers in each group were compared, Δdi-6S was the predominant disaccharide, particularly in the normal synovial fluids. The Δdi-6S content of all OA groups also appeared to be higher than in RA, but the presence of CPPD or clinical inflammation in the OA groups did not affect the synovial fluid disaccharide composition. Although the concentration of Δdi-4S was dis-

Table 1. The chondroitin sulphate disaccharide composition of normal and pathological human synovial fluids, mean ± SEM

Synovial Fluid	n	Mean age	Δdi 6S ng/mg	Δdi 4S ng/mg	Δdi6S/ Δdi 4S
Normal	14	(22–65)	21.43±2.12	3.93±0.41	5.49
OA active	10	72.4 (59–88)	9.54±1.51	3.40±0.52	2.98
OA inactive	10	71.8 (61–79)	11.30±2.22	4.30±0.45	2.46
CPPD active	5	8.7 (59–76)	11.90±2.18	4.80±0.79	2.53
CPPD inactive	12	69.5 (66–92)	13.72±1.77	4.59±0.72	3.26
NGOA active	5	70.3 (66–73)	9.26±1.10	6.06±0.39	1.50
NGOA inactive	8	74.3 (60–87)	10.90±0.76	6.47±0.25	1.69
RA active	15	60.1 (28–85)	6.09±1.02	5.69±0.63	1.11