Endogenous Aggrecanase-1 activity in Synovial Fluids in Patients with Joint Disease

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Introduction
Proteolytic degradation of the aggrecan core protein is known to be a key event in cartilage degeneration, leading to loss of proteoglycan, swelling pressure and function. Aggrecanases, which cleave the interglobular domain of aggrecan between the residues Glu<sup>373</sup>-Ala<sup>374</sup>, are members of the ADAM-TS (a disintegrin and metalloproteinase with thrombospondin-like repeats) family (Bondeson et al 2008). Two members, known as aggrecanase-1 (ADAM-TS 4) and aggrecanase-2 (ADAM-TS 5) have been identified in humans, with aggrecanase-1 being a major protease in human osteoarthritic cartilage (Naito et al 2007). Most investigations of aggrecanase in degenerate joint diseases have involved identifying aggrecanase-generated fragments rather than aggrecanase activity per se. In this study we have measured the extent of aggrecanase activity in synovial fluid from a cohort of patients with different degrees of joint pathology, ranging from macroscopically normal to end stage osteoarthritis (OA).

Method
Aggrecanase activity was measured in synovial fluid (SF) using the Sensolyt<sup>TM</sup> 520 Aggrecanase-1 assay kit (Anaspec). This utilizes a substrate for fluorescence resonance energy transfer (FRET), 5-FAM and TAMRA as the donor-acceptor pair. This acts as a substrate for aggrecanase-1 with active ADAMTS-4 cleaving it into two separate fragments resulting in an increase of 5-FAM fluorescence which can be monitored at excitation and emission of 490nm and 520nm, respectively. Synovial fluid was collected from 163 patients presenting with clinical symptoms who were undergoing arthroscopy of the knee. These were classified into 3 groups according to the appearance at arthroscopy (Table 1):

(i) macroscopically normal knees with no obvious abnormality (n=35, age range 15.9-54.6 years, mean 29.6),
(ii) those with injury of the meniscus, anterior cruciate ligament or chondral/osteochondral lesions (n=79, age range 16-57.8, mean 32.9 years) and
(iii) those with OA (n=49, age range 16.3-78.5, mean 47.0 years).

The presence of inflammation was also noted in any of the joint tissues at arthroscopy.

After a washout of the synovial cavity with 20mls of saline the SF was collected, centrifuged for 15 minutes at 3000g and the supernatant stored at -80°C until use. Plasma was collected at the same time and a dilution factor as 50% of the joints had visual signs of inflammation noted but

Discussion
Aggrecanase-1 found in synovial fluid could be synthesized by cells of several joint tissues including articular cartilage, meniscus, joint capsule, tendons and synovium, all of which have been shown to have the capability to produce aggrecanases. The modulation and influence of their production by cytokines differs between tissues (Yamamishi et al 2002) and also possibly between different disease processes. For example, whilst aggrecanase-2 is expressed constitutively, aggrecanase-1 production is influenced by cytokines and growth factors, with increased levels being produced by synovial tissue in rheumatoid arthritis but not in OA (Yamamishi et al 2002). The very high levels of aggrecanase-1 seen in the patients with synovitis or septic arthritis may reflect a response to such stimulatory factors, with septic arthritis affecting many or all tissues within the joint so resulting in exceptionally elevated levels. Inflammation per se does not seem to be a stimulatory factor as 50% of the joints had visual signs of inflammation noted but aggrecanase-1 was only measurable in 38% of the joints, with no apparent correlation between its presence and inflammation.

This study reports on aggrecanase activity in synovial fluid within a fairly large cohort of patients with various knee disorders. Since there appears to be different levels of activity within different patient groups it suggests that aggrecanase-1 might prove useful as a discriminatory biomarker of different types or phases of joint pathologies.

References


Acknowledgements
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Table 1

<table>
<thead>
<tr>
<th>Macroscopically Normal</th>
<th>n</th>
<th>Age (years)</th>
<th>Aggrecanase (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury</td>
<td>79</td>
<td>32.9±10</td>
<td>1.4±2</td>
</tr>
<tr>
<td>OA / OA + Injury</td>
<td>49</td>
<td>47±12.7</td>
<td>3.1±4.4</td>
</tr>
</tbody>
</table>

Inflammation was present in the knee joint of 23% of ‘normals’, 47% of the injury group and 70% of those in the OA group. The 2 patients with the very high levels of aggrecanase-1 had either synovitis (562mg/ml) or septic arthritis (3270mg/ml).