**THE RELEASE OF CROSS-LINKED PEPTIDES FROM TYPE II COLLAGEN INTO JOINT FLUID AND SERUM IS INCREASED IN OSTEOARTHRITIS AND AFTER JOINT INJURY**

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**Introduction** Osteoarthritis (OA) and joint injury are characterized by degradation and remodeling of cartilage and other joint tissues. Increased turnover of articular cartilage matrix has been shown both in animal models and in the human (1). This increase in tissue turnover is reflected by the increased release into joint fluid, serum and urine of molecules and molecular fragments resulting from degradation and synthesis of matrix. For example, increased concentrations of fragments of aggregcan (AGN) and cartilage oligomeric matrix protein (COMP), and matrix metalloproteinases-1 and –3 have been found in increased concentrations in joint fluid in these conditions, and of e.g. hyaluronan and COMP in serum (1). The increased synthesis of type II collagen in some disease phases is consistent with increased release of type II collagen C-propeptide to joint fluid (2), and the elevated synthesis of an AGN subpopulation is suggested by the increased release of 846 epitope into joint fluid (3). While there is evidence for an increased degradation of type II collagen in both animal injury and OA models and in human OA cartilage (4,5), no biomarker has been available to show the increased degradation of cross-linked type II collagen and release of soluble type II collagen-specific fragments to body fluids. The objective of this study was to determine, in patients with OA or joint injury, the concentrations in joint fluid and serum of a C-telopeptide cross-linking domain of type II collagen.

**Patients and methods** In this cross-sectional study, each individual supplied one sample of knee synovial fluid (SF) and serum at one time-point at varying times after knee injury or onset of symptoms. Study groups were: knee-healthy volunteers (REF), acute pyrophosphate arthritis (pseudogout, PPA), anterior cruciate ligament rupture and/or tear of meniscus (INJ), primary knee OA (POA) (Table 1). Diagnosis was by arthroscopy, radiography, and SF and clinical exam. No patient was treated by surgery before sampling. Pharmacological treatment prior to sampling was limited to analgesics or nonsteroidal anti-inflammatory drugs (NSAIDs). Procedures were approved by the Ethics Review Board.

**Table 1** [Age, volume, weeks as given as median (range)]

<table>
<thead>
<tr>
<th>Group</th>
<th>N (males)</th>
<th>Age</th>
<th>SF Vol.</th>
<th>Weeks fr. onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>9 (7)</td>
<td>28 (21-40)</td>
<td>3 (0.5-10)</td>
<td>-</td>
</tr>
<tr>
<td>PPA</td>
<td>48 (32)</td>
<td>64 (41-92)</td>
<td>54 (15-140)</td>
<td>48</td>
</tr>
<tr>
<td>INJ</td>
<td>260 (200)</td>
<td>33 (14-76)</td>
<td>16 (0.5-145)</td>
<td>105</td>
</tr>
<tr>
<td>POA</td>
<td>46 (32)</td>
<td>60 (36-84)</td>
<td>15 (0.5-80)</td>
<td>160</td>
</tr>
</tbody>
</table>

**Results** Concentrations of Col2CTx in joint fluid were significantly increased in PPA, INJ and POA over the REF group, p<0.001 (Fig. 1). Likewise, serum concentrations of Col2CTx were increased in the same groups, p<0.018 (data not shown). When the INJ samples were ordered by time after injury, concentrations of Col2CTx in SF were increased at all times after injury, p<0.001 (Fig. 2). Concentrations were highest early after injury, and increased within hours after trauma, but elevated average levels over REF levels were also noted for many years after injury. The average ratio between Col2CTx concentrations in joint fluid and serum was 2.2, suggesting local joint production of this fragment. Higher ratios were noted in acute injury and pyrophosphate arthritis, and lower in primary OA.

**Conclusions** This is the first report on the release into joint fluid and serum of a soluble molecular fragment specific for degradation of mature, crosslinked, type II collagen in human osteoarthritis and joint injury. Concentrations were increased in both joint fluid and serum in OA, after joint injury (meniscus and/or cruciate ligament), and in pyrophosphate arthritis, compared with knee-healthy volunteers. The higher average concentrations of Col2CTx in joint fluid compared with serum, suggests local production of these fragments in the diseased joint. Synovial fluid concentrations were highest in ‘active’ conditions with clinical signs of synovitis. High concentrations of Col2CTx in joint fluid were noted immediately after injury, suggesting that significant type II collagen degradation is an early event in the post-injury disease process. In fact, a rise in type II collagen fragments was observed as early as we have previously observed an increase in aggregan fragment levels in these samples (8). The degradation of type II collagen is thus an early event in human arthritis. An assay of soluble type II collagen fragments may, with appropriate validation, be of value for monitoring the progression and treatment of human joint disease.

**Figure 2.** Joint fluid concentrations of Col2CTx in relation to time after injury. Injury samples (260) were divided into time windows by time after injury, with the same number of samples in each. Symbols represent average, bars 95% ci, and shaded zone 95% ci for REF. Logarithmic time axis.

**References**


**Figure 1.** Joint fluid concentration of Col2CTx in diagnostic groups. Boxplot bars represent 90th percentiles, symbols are outliers.

Crosslinked peptides from the C-telopeptide domain of type II collagen (Col2CTx) were measured by competition ELISA (6,7). The assay is based on a monoclonal antibody raised against the peptide, EKGPDP, a matrix metalloproteinase-generated sequence from the C-telopeptide cross-linking domain of human type II collagen. Chemically cross-linked EKGPDP was used to calibrate the assay. Prior to assay, SFs were digested overnight at 37°C with testicular hyaluronidase (5 U/ml). Statistical comparisons were by ANOVA on ranks with Dunn’s correction for multiple tests, or by Mann-Whitney.

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