Early Changes In Articular Cartilage MMP-3 Expression In Response To Mechanical Unloading As Assessed By Laser Capture Microdissection

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Introduction: Physiological joint loading is a critical factor for cartilage homeostasis. Mechanical overload due to altered joint loading [1] or to an acute joint injury [2] can lead to cartilage degradation and osteoarthritis (OA). In addition, absence of mechanical load [3] can also lead to atrophic changes in cartilage, including increased tissue breakdown. However, the mechanisms by which mechanical unloading affects the early catabolic response in cartilage is unclear. Matrix metalloproteinase-3 (MMP-3), which breaks down cartilage extracellular matrix components and activates various proMMP’s, may play a key role in cartilage degeneration [4]. MMP-3 levels are increased in human cartilage after mechanical damage [5], and MMP-3 has been implicated in the development of OA [6]. However, articular cartilage is not uniformly organized, and chondrocytes from each tissue zone exhibit metabolic differences [7]. Moreover, cartilage breakdown in OA does not occur uniformly throughout the tissue; thus information about the spatial and temporal changes in response to overloading or disuse may be especially important in understanding how OA develops. Here we used Laser Capture Microdissection (LCM) to specifically isolate chondrocytes from the superficial, middle, and deep zones of the lateral and medial femoral condyles of immobilized rats. We then used real-time PCR to perform a gene expression analysis on chondrocytes specifically obtained from each zone.

Materials and Methods: Rat Immobilization. Under an IACUC-approved protocol, right hindlimbs of five to six-month-old male Sprague-Dawley rats were immobilized in full flexion with a cast made of cotton and steel mesh [8] for 0, 1, 3, 6, 24 hours, and 7, and 21 days (n=3 per time point). Tissue Preparation for LCM. Distal femurs were decalcified in Morse’s solution, fixed in methacarn and embedded in paraffin [9,10]. LCM. Sections of 5-7μm thickness were cut and mounted on Superfrost/Plus slides. The slides were deparaffinized and then air-dried in a fume hood. Cell isolation by LCM was performed with the Arcturus Pixcell IIe. Chondrocytes were microdissected from the superficial, middle, and deep zones of the lateral and medial condyles with the following settings: Power – 85mW, Spot size – 7.5μm, Duration – 600μs-2.5ms. Captured cells (~500 per isolate) were placed in a microwell with lysis buffer. RNA isolation, reverse-transcription, and real-time PCR. Total RNA was extracted, reverse transcribed, and real-time PCR for MMP-3 carried out with SYBR Green. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were used as internal controls to determine the relative gene expression levels. Data were analyzed using one-way ANOVA and Tukey’s test for post hoc analysis with significance set at p < 0.05.

Results: Fig 1a shows the time course of MMP-3 expression averaged over all tissue regions. Starting at 6 hours MMP-3 expression increased throughout the 21 day experiment. Comparison of average expression in the lateral and medial condyles showed no differences throughout the experiment (Fig 1b). The only spatial differences observed were increases in MMP-3 expression in the medial superficial zone, seen after 7 days of unloading. At 21 days, no differences in MMP-3 expression could be detected in the different zones (Fig 1c).

Discussion: This study used LCM and real-time PCR to analyze spatial and temporal changes in the mRNA levels of MMP-3 after a catabolic stimulus, mechanical unloading. Considering that the earliest morphological changes in cartilage usually appear only after 7 days of immobilization [11], it was surprising to find a significant upregulation of MMP-3 after only 6 hours. This shows that an altered mechanical environment in vivo can provoke immediate changes in cartilage gene expression. The rate of increase in MMP-3 expression slowed after 7 days of immobilization, suggesting that early changes in cartilage following immobilization may be particularly important in establishing the later course of cartilage degradation. Interestingly, the pattern of MMP-3 expression corresponded in general to changes observed in cartilage in OA. The only significant increase in MMP-3 expression was seen in the medial femoral condyle, the same compartment where cartilage degradation more commonly occurs in OA [12]. That immobilization and overuse (a likely trigger for OA) lead to degradative changes in this zone suggest that the medial region may be particularly sensitive to mechanical changes. In addition, MMP-3 expression at 7 and 21 days was highest in the superficial zone, followed by the middle and deep zones. This finding also is consistent with the concept that the initial stages of OA are characterized by disorganization and loosening of the collagen fibers from the superficial and upper middle zones [13]. In summary, LCM was able to document acute changes in MMP-3 expression in a spatial manner reflecting the development of degradative changes previously demonstrated in this animal model [11], and consistent with changes observed clinically in OA.

Figure 1. a. Relative gene expression of MMP-3 after immobilization. *compared to control. b. Lateral and medial condyles relative gene expression. c. Relative gene expression in the different zones of cartilage. *compared to medial middle and deep zones. L=medial condyle, M=medial condyle.


Acknowledgements: NIH Grants AR47628 and AR52743; Dr. Shen Yao for LCM support.