IN VIVO ASSESSMENT OF GAG SYNTHESIS IN A RODENT MODEL OF OSTEOARTHRITIS
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Introduction: Osteoarthritis (OA) is characterized by degeneration of articular cartilage, resulting in a net loss of extracellular matrix molecules, such as chondroitin sulfate (CS). The mechanical basis of OA has been studied in a variety of animal models, in which joint degeneration is induced following joint instability. In large animals, cartilage can respond to experimental OA with a sustained increase in matrix biosynthesis⁴,⁶. A similar repair response may occur in rodent models of OA, such as the medial meniscal tear model (MMT)². CS synthesis can be directly measured in vivo by using the stable isotope deuterium as a metabolic tracer¹. The objectives of this study were to determine CS synthesis in the articular cartilage of rat knees following MMT by quantifying deuterium incorporation into CS from either cartilage or synovial fluid.

Materials and Methods: Medial Meniscal Tear (MMT)²: Male Lewis rats, 66-80 days of age, were studied with IACUC approval. OA was induced by transecting the medial meniscus in the right knee. The left knee served as an unoperated control. In some animals, the right knee joint was opened, but the meniscus was not torn (sham). CS biosynthesis¹: CS biosynthesis was assessed in 4 day intervals at various times following surgery. To label newly-synthesized CS, 2H2O was administered to animals, first as an IP priming bolus, then as 8% drinking water. Animals were sacrificed 4 days later, and hind limbs were stored frozen until use. To sample synovial fluid (SF), knee joints were exposed, and 100 µl saline was used to rinse the articular surfaces and collect SF constituents. Cartilage was then scraped from the surface of medial (MTP) and lateral tibial plateau (LTP). Both SF and cartilage scrapings were solubilized with proteinase K and CS disaccharides were isolated as chondroitinase ABC cleavage products. Deuterium incorporation was quantified by gas chromatography / mass spectrometry. Specifically, fractional synthesis (f, the fraction of CS that was newly-synthesized) was determined from the 2H-enrichment of N-acetyl galactosamine. Data are presented as mean±SD and were compared with 2-way ANOVA and Tukey. Additionally, linear regression was performed to analyze the correlation between the fractional synthesis of CS from cartilage and SF.

Results: MMT resulted in cartilage lesions in the articular surface of the medial tibial plateau (MTP) within 4 days, while the lateral tibial plateau (LTP) was not torn (sham). CS biosynthesis in the MTP (0.001). However, by 16 days, CS synthesis in the LTP of osteoarthritic knees had reverted to baseline, while the MTP continued to exhibit enhanced matrix biosynthesis relative to controls, although the level of stimulation declined (to 2.1X and 1.6X at day 16 and 26, respectively). Sham surgery did not have a detectable effect on CS biosynthesis as measured from synovial fluid (SF) and cartilage scrapings were solubilized with proteinase K and CS disaccharides were isolated as chondroitinase ABC cleavage products. Deuterium incorporation was quantified by gas chromatography / mass spectrometry. Specifically, fractional synthesis (f, the fraction of CS that was newly-synthesized) was determined from the 2H-enrichment of N-acetyl galactosamine. Data are presented as mean±SD and were compared with 2-way ANOVA and Tukey. Additionally, linear regression was performed to analyze the correlation between the fractional synthesis of CS from cartilage and SF.

Discussion: The regulatory factors that stimulate the biosynthesis of GAG in the osteoarthritic joint immediately following joint destabilization remain to be clarified, and the release of anabolic soluble factors is likely to play a role. However, these stimulatory factors continued to exert influence in the MTP only, as the initial stimulatory effects rapidly dissipated in the LTP cartilage harvested on the earliest timepoint. This model is the first to model the joint destabilization and subsequent joint degeneration, both factors that may maintain chondrocytes in a repair phenotype.

The majority of CS in SF is bound to aggrecan, indicating that it originates from articular cartilage. In this study, the increased CS biosynthesis in response to mechanical instability was reflected in the fractional synthesis of CS collected in SF, suggesting that breakdown products found in the SF can provide qualitative information as to the kinetics of the underlying cartilage. Quantitatively, the CS from SF had fractional synthesis values that were significantly greater than that in the articular cartilage, suggesting that the CS in the SF was preferentially derived from newly-synthesized CS, rather than from degraded matrix. Since labeling methods similar to those used here have been applied to human patients, the ability to infer cartilage GAG synthesis from a SF aspirate without necessitating a cartilage biopsy would have significant clinical implications as a minimally-invasive kinetic biomarker.


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Figure 1. CS synthesis in the cartilage of the medial and lateral tibial plateaus following induction of OA in the MMT rat model.

Figure 2. a) CS synthesis measured from synovial fluid (SF) and b) correlated with kinetic CS measurements from the corresponding cartilage.