INCREASED LEVELS OF SYNTHESIS AND DEGRADATION MARKERS OF TYPE II COLLAGEN IN THE SYNOVIAL FLUID OF A CANINE CRANIAL CRUCIATE TRANSECTION MODEL OF OSTEOARTHRITIS

INTRODUCTION: Type II collagen is the primary collagen within articular cartilage and provides the necessary tensile properties required to maintain normal joint function. In a normal joint environment, homeostasis occurs between synthesis and degradation of type II collagen. However, when there are abnormal biomechanical stresses applied to the joint due to injury, such as damage to the cranial cruciate ligament (CCL), osteoarthritis (OA) often develops (1). In the initial stages after injury, there is a critical loss of proteoglycans within the cartilage matrix exposing the triple helix of type II collagen. This leads to an increase in synthesis and degradation of type II collagen in an attempt to maintain a normal joint environment. Presumably, without joint stabilization, this initial balance diminishes over time, leading predominantly to catabolism of type II collagen from a combination of proteolytic enzymes acting on the type II triple helix itself as well as continued biomechanical trauma. During formation of the type II collagen fibrils, the carboxy- (C-) terminal propeptide (CPII) is enzymatically cleaved from the C-terminal of the procollagen molecule. CPII content and release from the cartilage matrix has been directly correlated with type II collagen synthesis (2). In human OA, CPIII levels have been shown to increase in the synovial fluid (3) and decrease in the serum following injury (2). Degradation of type II collagen is presumed to be initiated by enzymatic cleavage of the triple helix. This intrahelical digestion of the α chains of each triple helical molecule is initiated by matrix metalloproteinases (MMPs), specifically the mammalian collagenases MMP-1, MMP-8, and MMP-13 (4). The 234CEQ antibody has been developed to detect equine specific collagenase-generated neoepitopes that are the C-termini of the cleaved type II collagen α chains (4). An assay using this antibody has been employed to identify increased type II collagen degradation in the serum of young horses with osteochondrosis (5). The purpose of this study was to evaluate concentrations of biomarkers of type II collagen synthesis (CPII) and degradation (234CEQ) in the serum and synovial fluid of a canine CCL transection model of OA.

MATERIALS AND METHODS: An experimental model of osteoarthritis was created in 19 mature male Walker Hounds that were used as controls in another larger study. The right CCL was transected arthroscopically in all dogs. Synovial fluid samples were collected from the right stifle using a lavage technique (5 ml of sterile saline) at surgery (Day 0), and the again at 14, 70, and 126 days post-operatively. Serum samples were collected from the jugular vein pre-operatively (Day 0), and then again at day 7, 14, and every two weeks thereafter until day 126 post-operatively. Type II collagen synthesis was assayed by measuring the levels of type II specific carboxy propeptides (CPIII) in the serum and synovial fluid using a commercially available two-step competitive ELISA. Specific degradation products of type II collagen from collagenase cleavage were measured in the serum and synovial fluid using the 234CEQ antibody in an inhibition ELISA (3). Western blotting confirmed cross-reactivity of the 234CEQ antibody with the Ⅲ C-terminal fragment of purified type II collagen from canine articular cartilage digested with collagenase-3. Post-operative CPII and 234CEQ synovial fluid and serum concentrations were compared to baseline. Significant differences in concentrations of each biomarker between time points were determined using an ANOVA with Tukey’s analysis for multiple comparisons. A value of p<0.05 was considered significant for all analyses. All procedures were approved by the university’s Animal Care and Use Committee.

RESULTS: Synthesis (CPII): The concentration of CPII in the serum significantly decreased from baseline at 28 days (p<0.05), 42 days (p<0.05), 56 days (p<0.001), and 70 days (p<0.01) post-CCL transection (Fig. 1A). The CPII concentrations in synovial fluid were 2.3 times higher (p<0.001) at 14 days, 1.9 times higher (p<0.001) at 70 days, and 1.8 times (p<0.001) at 126 days post-CCL transection, compared to baseline at day 0 (Fig. 1A).

Degradation (234CEQ): The concentrations of type II collagen degradation products in the serum peaked at different points throughout the study period but was 3.2 times higher (p<0.01) at day 126 post-CCL transaction compared to baseline at day 0 (Fig. 1B). The 234CEQ levels in the synovial fluid increased from baseline over time, but not significantly (p<0.07) (Fig. 1B).

DISCUSSION: During normal joint homeostasis, there is a balance between synthesis and degradation of type II collagen within articular cartilage. However, after joint injury, it is presumed that the chondrocytes only have a limited potential for repair through the synthesis of type II collagen. During human OA, increased levels of products of type II collagen synthesis have been identified in the synovial fluid (3). CPII analysis of the synovial fluid in this study demonstrates that type II collagen synthesis significantly increases above baseline by day 14 and throughout the post-operative period. The type II collagen degradation products in the synovial fluid also increase from baseline, peaking at 126 days post-operatively. Therefore, type II collagen synthesis and degradation appear to be stimulated in the early stage of OA following CCL transection in this experimental model. In the serum, type II collagen propeptide levels decreased during the first two months after injury and then slowly increase back toward baseline. Dilution of the propeptides, as well as additional proteolytic processing of the propeptide in the serum may prevent detection of elevation in CPII levels by the immunoassay. This study illustrates through the assaying of biomarker levels in the synovial fluid that type II collagen synthesis and degradation are both stimulated after joint injury. However, unlike synthesis, degradation of type II collagen increases in the serum and synovial fluid over time, demonstrating ultimate catabolism of the type II collagen network in articular cartilage that is the hallmark of OA. To the best of our knowledge, this is the first study to use biomarkers in the serum and synovial fluid to compare synthesis and degradation of type II collagen in a canine CCL transection model of OA, illustrating the value of using biomarker levels of products of skeletal tissue metabolism in body fluids of patients with early OA.

REFERENCES: