

Indicators of Collagen Degradation in a Canine Model of Osteoarthritis

Summary: In this canine experimental model of osteoarthritis (OA), created by transecting the cranial cruciate ligament (CCL) within the stifle joint, collagenase generated fragments of types I and II collagen in synovial fluid increased significantly after CCL transection. This study demonstrated that type I and/or type II collagen degradation is/are key features of this experimental model of OA in the dog. It also demonstrates that this breakdown can be quantified and monitored in body fluids.

Collagen is an abundant protein in the mammalian body. There are many different types of collagen present throughout the body. Within joints, type II collagen is important because it is the most abundant collagen in articular cartilage. Type I collagen is also important because it is present in the menisci, ligaments, and supporting bone. These collagens are responsible for providing strength and structure to these tissues. Therefore, breakdown of collagen in the joint compromises the integrity of each tissue, decreases its functionality and results in ultimate breakdown of the joint. This is what occurs during osteoarthritis (OA).

One way to study OA is to create an animal model by transecting the cranial cruciate ligament (CCL) within the stifle joint. Transection of the CCL in dogs causes instability and inflammation of the joint that leads to articular cartilage breakdown, as well as damage to the menisci, joint capsule, and supporting bone. The initial inflammatory response results in the release of enzymes, called collagenases, which can cleave the collagen molecule into fragments (Figure 1). These fragments are eventually released into the surrounding synovial fluid and eventually reach the blood. These fragments have a portion of the collagen molecule exposed that cannot be detected when the molecule is intact.

A specific antibody, called COL 2-3/4C_{short}, can measure the levels of this newly exposed portion (neopeptide) of the collagen molecule in the synovial fluid and blood using an assay. Therefore, this antibody can be used as a direct indicator of type I and II collagen breakdown. The purpose of this study, done by Dr. Troy Trumble with Drs. Clark Billingham and Wayne McIlwraith and student Stephen Davis, was to use this COL 2-3/4C_{short} antibody to measure collagen degradation products in the synovial fluids and bloods of dogs that had the CCL transected to create osteoarthritis. The researchers hypothesized that the amount of type I and II collagens in synovial fluid and blood will increase over the values present before transection.

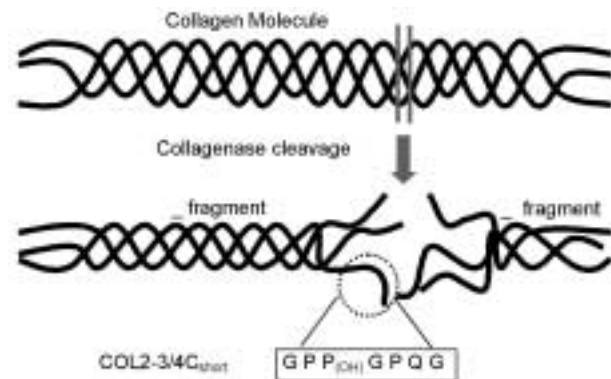


Figure 1. Schematic representation of an intact collagen molecule (top) that gets cleaved during inflammation into two fragments by collagenases (large arrow). On the end of the 3/4 fragment where the cleavage occurred, there is a peptide sequence (highlighted in the box) that can be recognized by the COL 2-3/4C_{short} antibody. This sequence is specific for type I and II collagen degradation.

The markers were examined in 19 mature male Walker Hounds with experimental OA. Blood samples were collected from the jugular vein prior to surgery (Baseline), one week post-operatively, and every two weeks thereafter until day 126. Synovial fluid samples were collected from the right stifle using a lavage method where 5 ml of saline was injected into the joint prior to aspiration. Fluid was collected prior to surgery, and then again at 14, 70, and 126 days post-operatively. Combined cleavage of types I and II collagen were assayed in synovial fluid and blood using the COL2-3/4C_{short} antibody in an inhibition ELISA. The concentrations of collagen fragments bearing the COL2-3/4C_{short} neopeptide were compared to baseline using a Kruskal-Wallis test with significance set at $p < 0.05$. All procedures were approved by CSU's Animal Care and Use Committee.

The concentrations of types I and II collagen cleavage products in synovial fluid were significantly increased from baseline, with day 14 samples peaking at levels 11.4 times higher ($p < 0.001$), day 70 samples 7.5 times higher ($p < 0.001$),

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and day 126 samples 5.7 times higher ($p < 0.01$) than baseline concentrations (Figure 2a). The concentrations of types I and II collagen cleavage in blood were not significantly different from baseline, but did peak at day 7 post-operatively (Figure 2b). The concentrations of types I and II collagen cleavage products in blood decreased at all measurement periods after day 7 post-operatively, with no significant differences from baseline values.

TYPE I and II COLLAGEN DEGRADATION

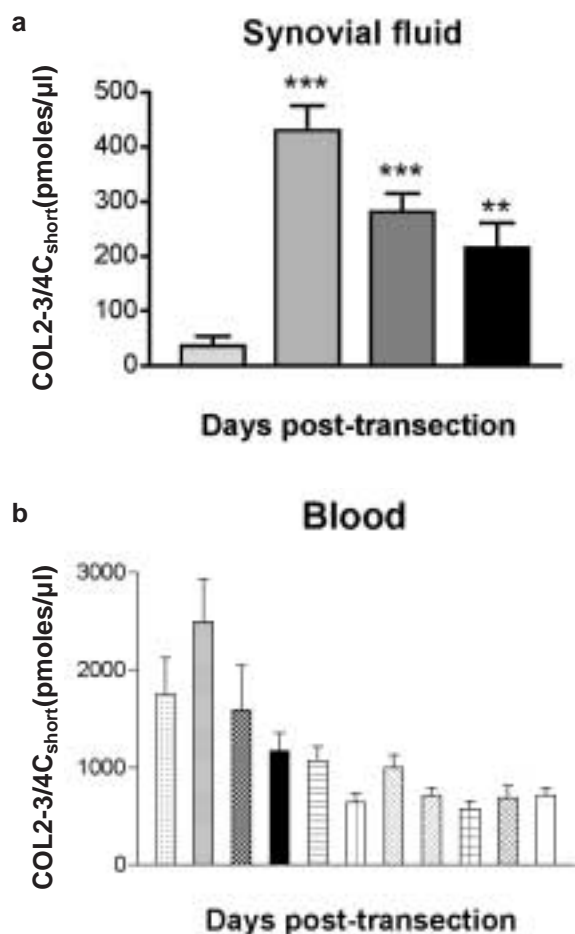


Figure 2. Concentrations (pmoles/μl) of type I and II collagen degradation products in synovial fluid (a) and blood (b) from dogs with CCL transection. All post-transection samples were compared to baseline (Day 0), and significant differences are represented as *** $p < 0.001$, ** $p < 0.01$. Error bars represent the standard error of the mean (SEM). Notice that the concentrations of degradation products peak above baseline within the first two weeks after CCL transection.

Type I and II collagen degradation is a key feature of our CCL deficient canine OA model that can be monitored by measurement of specific cleavage products of types I and II collagen in synovial fluid and blood. Type II collagen cleavage is derived mainly from degradation of articular cartilage, whereas the source of type I cleavage products likely varies with the stage of the disease process. Type I degradation in the acute stages is likely due to the fibrotic response within the joint, including adhesions to the CCL, and thickening of the joint capsule, synovial membrane, and fat pad. However, in the later stages, type I collagen degradation products may derive more from the menisci or the fibrocartilaginous repair of cartilage lesions.

In this canine experimental model of osteoarthritis (OA), collagenase generated fragments of types I and II collagen in synovial fluid significantly increased after CCL transection, compared to baseline. Since the levels of collagen fragments peaked at day 14 post-operatively, it appears that these degradation products correlate with the early inflammatory process, when collagenase levels are likely to be their highest in the synovial fluid. Similarly, blood levels of type I and II collagen cleavage products increased acutely, and then progressively decreased to levels below baseline. This decrease is likely due to further digestion of the primary collagenase cleavage products of collagen. This study does not distinguish between the relative amounts of type I and II collagen cleavage by the collagenases, but it does demonstrate that type I and/or type II collagen degradation is/are key features of this experimental model of OA in the dog. It also demonstrates that this breakdown can be quantified and monitored in body fluids.

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