DETERMINATION OF COLLAGEN TYPE II PEPTIDES IN THE SYNOVIAL FLUIDS OF NON-INFECTIONOUS SYNOVITIS AND OSTEOARTHRITIS PATIENTS USING A NOVEL SANDWICH ELISA

INTRODUCTION
Osteoarthritis (OA) is characterized by the loss of articular cartilage. Previous studies have shown that matrix metalloproteinases (MMPs) play a significant role in the degradation (1). Collagen type II degraded peptides and proteoglycan monomers were previously detected in OA synovial fluids (2). Acute knee injuries have been associated with the subsequent development of OA (3). Our objective is to compare patterns of CII peptide release among traumatic synovitis, OA, and normal synovial fluid groups using a novel CII specific sandwich ELISA assay in order to understand the biochemical processes that may lead to the development of postratumatic secondary osteoarthritis.

Patients
Non-infectious synovitis patient group (48 patients) suffered an injury ranging from the same day to two weeks and a pain severity from mild to severe. Osteoarthritis patient group (72 patients, 54% female and 46% males) with an average age of 74 and a Noyes criteria grade III or IV OA. Normal human synovial fluids were obtained from human subjects post-mortem following fatal accidents that showed no signs of gross degenerative joint disease.

METHODS
Synovial Fluid Hyaluronidase Treatment: Synovial fluid samples were treated with 5mM EDTA and centrifuged at 10,000 rpm for 15 minutes to remove cells, aliquoted and stored at –20°C. Aliquots were thawed prior to use. At 56°C, 200 µl synovial fluid and 200 µl hyaluronidase (50 units in 1 ml PBS) were incubated for 40 minutes and subsequently at 80°C for 20 minutes. The hyaluronidase-treated synovial fluid was centrifuged at 10,000 rpm for 20 minutes and the supernatant was transferred to an EDTA-containing centrifuge tube.

Sandwich ELISA: Two CII specific monoclonal antibodies, 18:6:D6 and 14:7:D8 were developed and used in the assay. Micro titer plates were coated with a 1:50 dilution of 18:6:D6 antibody in PBS overnight. Each well received 100 µl of the antibody solution. Hyaluronidase-pre-treated synovial fluid samples (serial dilutions in PBS-Tween 20), cyanoogen bromide cleaved collagen II spiked synovial fluid samples and standards (different dilutions of cyanogen bromide cleaved collagen II peptides) were incubated on the pre-coated micro titer plates for 60 minutes at room temperature. Antibody 14:7: D8 conjugated with horseradish peroxidase in PBS-Tween, 1:1000 dilution, was subsequently added for 60 minutes at room temperature. Pierce Quantablu fluorescence substrate developing solution (100 µl) was added for 60 minutes and the fluorescence was measured at an excitation wavelength 430 nm and an emission wavelength 460 nm.

Total SGAG: Sulfated GAG released to the synovial fluid was quantified with the DMMB binding assay.

Hyaluronic Acid: HA was determined by the carbazole method for uronic acid (4).

RESULTS
The results of ELISA assays indicate that 74% of non-infectious synovitis synovial fluids had detectable quantities of CII degradation peptides compared to 90% in OA samples, while normal samples had undetectable levels. There were significantly higher levels of CII degradation peptides (P<0.005) in the synovial fluids of non-infectious synovitis compared to osteoarthritic patients. Two synovial fluid markers were also quantified, hyaluronic acid and total protein levels. Hyaluronic acid levels showed no significant difference (α=0.05) between the two patient groups. Total protein levels were significantly higher in non-infectious synovitis (p<0.005) compared to OA samples. When CII levels were normalized to total protein levels, the significant elevation in non-infectious synovitis compared to osteoarthritits (P<0.005) was still apparent (Fig. 1). 5-D-4 epitope levels in OA were significantly higher (P<0.005) than non-infectious synovitis levels, indicating that enzymatic degradation of proteoglycans is more pronounced in osteoarthritic conditions. This is supported by our measurements of total sulfated GAG, which indicates that GAG levels in OA synovial fluids are significantly higher (p<0.005) than synovitis synovial fluids.

Fig. 1. Collagen II Synovial Fluid Peptide Levels by ELISA

The results of these assays indicate that CII peptides and proteoglycans are released following knee traumatic injury. Elevated levels of CII in acute synovitis indicate early signs of cartilage network damage. The release of CII peptides may contribute the predisposing factor to developing postratumatic OA. Detection of SGAG and 5-D-4 epitopes in the synovial fluids of synovitis patients also support that cartilage network is damaged following acute injury. Previous research has shown that apoptosis plays a prominent role in proteoglycans release from articular cartilage explants following mechanical loading (5). It is not clear yet how acute knee injury may lead to CII degradation. Our future research will focus on investigating possible mechanisms that may contribute to CII degradation following acute injury.

REFERENCES

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