Development of a Novel Clinical Biomarker Assay to Detect and Quantify Aggrecanase-Generated Aggrecan Fragments in Human Synovial Fluid, Serum and Urine

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Introduction: Proteolytic degradation of aggrecan in articular cartilage is a hallmark feature of osteoarthritis. Aggrecanases (ADAMTS-4 and ADAMTS-5), the primary proteases responsible for aggrecan degradation, cleave aggrecan at five sites within the core protein including the NITEGEVIL...LKG sequence within the interglobular domain (IGD). Aggrecanase-cleaved fragments of aggrecan are found in synovial fluid from patients with osteoarthritis, joint injury and inflammatory joint disease. Specific aggrecanase-generated aggrecan fragments in biological fluids may serve as potential biomarkers of the pharmacodynamic activity of aggrecanase inhibitors, and detection and quantification of these biomarkers may facilitate both the preclinical and clinical development of aggrecanase inhibitors. Assays currently available to measure aggrecanase-cleaved ARGS-containing fragments in biological fluids are limited to detection in synovial fluid, presumably due to assay sensitivity limitations in other biological fluids (serum, urine). In order to develop a biomarker assay for measuring ARGS fragments in more clinically-accessible human fluids (serum/urine), we improved the affinity of an anti-ARGS (BC3) antibody via antibody engineering and developed a sensitive sandwich ELISA that can measure this potential biomarker in human synovial fluid, serum and urine in the native state, without the need for deglycosylation.

Methods: Truncated human recombinant ADAMTS-4 (213-579) was purchased from Chemicon and used for digesting human aggrecan. Human synovial fluid was obtained from the knee joints of patients undergoing knee replacement surgery (St. Francis Hospital, Beech Grove, IN) with informed consent under an IRB approved protocol. Human serum and urine were obtained with informed consent from individuals enrolled in a methods study conducted by Lilly. Briefly, this study included 42 individuals that were identified as having no, mild or moderate OA (n=14 for each group). Categorization into groups was based on both clinical exams and radiographic analysis of the knee, hip and spine. Antibodies: The monoclonal α-HABR antibody directed against the hyaluronic acid binding region of aggrecan was purchased from Biosource and was conjugated with HRP. The anti-ARGS antibody (BC3) was obtained from Novus Biologicals, and the BC3-C2 antibody was developed at Lilly using CDR-saturation mutagenesis of a BC3 producing hybridoma supplied by Dr. Bruce Caterson (Hughes et al., Biochem J. 305: 799, 1995). BC3-C2/HABR sandwich ELISA: The BC3-C2 antibody was used to capture ARGS neoeptope containing fragments and the captured fragments were detected using the HRP-HABR antibody. A standard curve was generated with ADAMTS-4 digested truncated recombinant aggrecan GI-IGD-G2 (R&D Systems).

Results and Discussion: The optimized neoeptope antibody (BC3-C2) showed a 4-log improvement in affinity for the ARGS-containing peptide over the BC3 antibody and had no cross-reactivity with a spanning peptide. Using this antibody in combination with the anti-HABR antibody (that binds to the C2 domain of aggrecan) we developed a sensitive sandwich ELISA that specifically detects aggrecanase-digested aggrecan fragments, but not undigested aggrecan (Fig. 1). Deglycosylation of aggrecan was not necessary for detection in this assay. To test the potential clinical utility of this assay, we tested synovial fluid samples obtained from patients who underwent knee replacement surgery and human plasma and urine obtained from other sources. In contrast to results from previous ARGS ELISA assays, we were able to detect a specific ARGS peptide signal in human plasma and urine (Fig. 2). The specificity of detection was demonstrated via competition with a 22-mer peptide (ARGSVIL...LKG) containing the ARGS neoeptope and via the lack of competition with a 43-mer spanning peptide that does not have the neoeptope exposed (VQT...NITEGEARGSVIL...LKG).

To further demonstrate the utility of this ELISA in the clinical situation, we tested human serum and urine samples obtained from a methods study from individuals with no, mild or moderate OA. In serum, ~5000pg/ml ARGS-containing fragments were detected and there was a trend towards an increase in biomarker levels going from no OA to mild and moderate OA patients (Fig. 3). In addition, the sera from moderate OA patients showed a significantly higher level of the neoeptope compared to that with no OA. These data support the potential clinical utility of this assay for measuring ARGS biomarker levels, although there is a clear need to test additional patient samples to determine the diagnostic value of this biomarker. The ARGS-containing fragment was also detected in urine in all three patient groups (~0.6ng/mg creatinine), although there was not a statistically significant difference in levels between the groups (Fig. 4).

Conclusions: We have developed a novel ELISA that uses an optimized anti-ARGS antibody and have demonstrated for the first time, the ability to measure specific aggrecanase-cleaved fragments of aggrecan in human serum and urine. This assay has the potential to serve as a mechanistic drug activity biomarker assay for the clinical testing of aggrecanase inhibitors and other disease modifying osteoarthritic drugs.

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