Introduction

Osteoarthritis (OA) is the most common form of arthritis affecting nearly 27 million people in the United States. OA is caused by the loss of articular cartilage due to increased production of proteolytic enzymes such as matrix metalloproteinases (MMPs) and aggrecanases. We have previously reported the development and validation of a sensitive and quantitative ELISA to measure AGGS-aggrecan (1), a predominant aggrecan fragment generated in the joint by specific aggrecanase activity and released into the synovial fluid (SF). In this study, we investigated the time-dependent release of AGGS-aggrecan in a rat meniscal tear (MT) model of joint injury following surgically induced joint instability. Furthermore, we evaluated the effect of oral administration of an Aggrecanase Specific Inhibitor (ASI) in the rat MT model; the same Aggrecanase Specific Inhibitor has previously been shown by us to result in histological chondroprotection and disease modification in this rat model tested at doses similar to the current study (2). The present findings validate the AGGS-aggrecan ELISA as a biomarker of joint injury and supports the development of aggrecanase inhibitor as a potential therapeutic for joint injury.

Methods

Rat Meniscal Tear Model: Male Lewis rats weighing approximately 300 g were purchased from Harlan, Inc. They were subjected to medial meniscal tear surgery in the right knees to induce joint instability as described (3). Left, non-operated knees served as contra lateral controls. Synovial fluid from both knees of surgery and naive (non-surgery) animals were collected at different time points post surgery. Serum was also collected for use in a urea assay to correct for dilution of the SF lavage samples. In the ASI treatment study, animals were dosed with ASI at 30 mg/Kg or 100 mg/Kg BID or received vehicle treatment starting the day before surgery.

ASI: Aggrecanase Specific Inhibitor is a potent ADAMTS4/5 inhibitor that was designed and prepared at Wyeth. It is selective over several MMPs tested and TACE.

AGGS-aggrecan ELISA: The 96-well format sandwich ELISA used monoclonal anti-aggrecan antibody AHP0022 (Invitrogen) as capture and AGGS-aggrecan specific monoclonal antibody BC-3 (Abcam) conjugated to HRP for detection. SF samples were deglycosylated and analyzed by ELISA alongside rat aggrecan standards (cleaved with rADAMTS4, and deglycosylated) for quantitation of AGGS-aggrecan.

Results

In the rat meniscal tear (MT) model of joint instability, AGGS-aggrecan in SF from the surgery knees increased approximately 4-fold at 4 days and 1 week time points following surgery compared to non-surgery contra-lateral knees or to knees of naive animals (Fig. 1). The increase dropped to 2-fold after 2 weeks, but elevated AGGS-aggrecan was maintained for at least 8 weeks. A one-way ANOVA analysis indicated the difference in the release of AGGS-aggrecan from surgery vs. contra lateral/naive knees was statistically significant.

We then tested the effect of the ASI in the rat MT model at 1 week following surgery (Fig. 2A). Analysis of the SF from the surgery knees showed a statistically significant reduction of AGGS-aggrecan in the ASI-dosed groups when compared to the vehicle-treated controls in a dose-dependent fashion. Inhibition was approximately 19% at 30 mg/Kg ASI, and 34% at 100 mg/Kg (Fig. 2B).

Discussion

The results presented herein using a rat model indicate that there is a substantial increase in aggrecanase activity immediately after joint injury, providing a window of opportunity for pharmacologic intervention. Moreover, we demonstrate a dose-dependent and statistically significant reduction of AGGS-aggrecan release from the joint by systemic administration of ASI. The rat MT model closely mimics the pattern of AGGS-aggrecan release in humans immediately following acute joint injury (4). Thus, MT-induced osteoarthritis in rats serves as a relevant animal model representing joint injury in humans, and should prove to be valuable for assessing the efficacy of potential therapeutics.

References


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