Analysis of the Metabolic Response of Meniscal Tissue to Injury and Inflammation in vitro

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Introduction: In the study of osteoarthritis (OA), it is important to consider the joint as an organ in which all articular tissues potentially contribute to the development and progression of disease. Meniscus is a tissue that plays a vital role in the maintenance of knee biomechanical function. However, injury to the tissue not only disrupts the biomechanical function of the joint, it can produce key inflammatory and degradative biomarkers associated with OA development. This study was designed to begin to characterize the metabolic responses of meniscal tissue to injury and inflammation. We hypothesize that impact injury and IL-1β stimulation of meniscal explants would result in significant increases in MMP activity and relevant cytokine production in culture compared to controls.

Methods: Tissue collection: All procedures were performed with ACUC approval. The lateral and medial menisci were aseptically collected from the knees of skeletally mature dogs (n=9) euthanatized for reasons unrelated to this study. Meniscal explants (4mm) were created from the middle of the meniscal body to exclude synovial tissue and include tissue from the red zone to the white zone of the meniscus. Meniscal explants were then assigned to one of six groups: 1) IL-1β (0.1ng/ml) treated (IL), 2) 25% strain (25), 3) 75% strain (75), 4) 25%+IL-1β (25IL), 5) 75%+IL-1β (75IL), or 6) 0%+no IL-1β control (NC).

Tissue impacting and culture: An Instron 8821S servo-hydraulic testing machine was used to apply a single impact load to the tissue. The meniscus explant was placed in a stainless steel well (4mm diameter) and a 3.9mm diameter flat punch attached to the ram was used to measure the thickness of the explant. The thickness measurement was used to calculate the parameters to apply a 25% or 75% strain impact at 100mm/sec based on previous work.

Tissue culture: Impact and control explants were cultured in 2ml of supplemented DMEM with or without 0.1ng/ml rIL-1β. Explants were cultured for 12 days at 37°C, and media were changed and collected for biomarker analysis every 3 days. On day 12, explants were collected for evaluation of extracellular matrix composition.

Tissue ECM Analysis: Explants were weighed to determine the wet weight, lyophilized, and weighed again to determine the dry weight of the tissue. Lyophilized tissues were then digested with papain, and the digests were tested for proteoglycan (GAG) and collagen (HP) content using the DMMB and Hydroxyproline assays, respectively.

Media Biomarker Analysis: Media were analyzed for MMP activity; ADAMTS 4 activity; GAG; nitric oxide (NO); prostaglandin E2 (PGE2); IL-6, IL-8, MCP-1, and KC; MMP-1, MMP-2, and MMP-3 concentration using commercially available assays.

Data Analysis: Data were analyzed for significance by the Student’s t-test with significance set at p≤ 0.05.

Results: NO: Media NO concentration was significantly (p<0.05) higher in the 25IL and 75IL group compared to the NC, 25, and 75 groups at all time points except the 75IL group was not significantly different than the 25 and 75 groups on day 6. The NO concentration in the 25IL group was significantly (p<0.05) higher than the 75IL group at days 3 and 6. NO concentration in the IL group was significantly
(p<0.05) higher than the NC, 25, 75 and 75IL groups for Days 6, 9 and 12. There was not a significant
(p>0.05) difference in the NO concentration between the NC, 25, and 75 groups at any time point.
PGE2: Media PGE2 concentration was significantly (p<0.05) higher in the 75IL groups compared to the
NC, IL, and 25 groups at all time points; the 75 group on days 3, 6 and 9; and the 25IL group on days 9
and 12. The 25IL group was significantly higher than the NC group at all time points, but not significantly
different than any other group. There was not a consistent significant (p>0.05) difference between any
other groups tested in this study.
MMP concentration: There was not a consistent significant (p>0.05) difference in the concentrations of
MMP-1, 2, or 3 under any treatment and at any time point tested.
MMP activity (Figure 1): General MMP activity was significantly (p<0.05) higher in the IL and 25IL groups
compared to the all other groups, but not each other, at all time points. The MMP activity in the 75IL
group was significantly higher than the 75 group at all time points. The MMP activity in the 25 group was
significantly higher than the 75 group on day 3. There were no other significant differences observed
between the test groups in this study.
ADAMTS4 activity: The level of ADAMTS4 was relatively low in all samples, and there were not any
consistent significant (p>0.05) differences observed between groups in this study.
GAG and Collagen concentration: GAG concentration in the media and the tissue was not significantly
(p>0.05) affected by cytokine or impact treatment at any time point when compared to the negative
controls. Collagen content in the tissue was not significantly (p>0.05) affected by cytokine or impact
treatment when compared to the negative controls
Cytokine/Chemokine concentration (Figure 2): Application of impact did not significantly effect MCP-1
production. Treatment with IL-1β resulted in a significant increase in MCP-1 concentration on days 9
and 12 compared to untreated samples. The 25 group did not significantly increase production of the
cytokines tested compared to the NC group. The 75 group significantly increased the production of IL-6,
IL-8, and KC compared to the NC and 25 groups at various time points from days 3 to 9. The 25IL and
75IL groups significantly increased the concentration of IL-6, IL-8, KC, and MCP-1 compared to the NC, IL,
25, and 75 groups at many of the time points tested. These data indicate that a combination of impact
and IL-1β exposure stimulates a significant increase in sustained cytokine production by the tissue.
Discussion: After meniscal injury, there is typically an increase in joint inflammation associated with the
attempted healing process of the affected tissues. These data indicate that meniscal tissue is a
potentially potent source of inflammatory mediators when injured. Further, that an inflammatory
cytokine known to be involved in the disease processes of OA can exacerbate the response by the tissue
after injury. Additionally, even though the concentration of MMPs tested in in this study did not increase
significantly, the level of MMP activity did increase with both injury and cytokine treatment, indicating a
loss of regulation of MMP activity by the tissue. Therefore, injury to the meniscus not only has the
potential to destabilize the biomechanical function of the knee, a known factor in the initiation of OA,
but can also contribute to the increase in joint inflammation and degradative enzyme concentration
often associated with OA pathogenesis and progression. Take together; these findings highlight the
critical importance of menisci in the biological pathways of joint health and disease in addition to their
well-established biomechanical functions in the knee.
Significance: The data from this study indicates that after injury, the meniscus can increase the
production of inflammatory mediators and degradative enzyme activity, which is exacerbated by
concurrent stimulation with IL-1β. Therefore, the loss of biomechanical integrity to the knee is not the only way that the meniscus contributes to the development of OA after injury.

Figure 1: Media MMP Activity. NC-neg control; IL-0.1ng/ml IL-1β; 25-25% strain; 75-75% strain; 25IL-25%+IL-1β; 75IL-75%+IL-1β. *=significantly higher than NC group

Figure 2: Media MCP-1, KC, IL-8 and IL-6 concentrations. NC-neg control; IL-0.1ng/ml IL-1β; 25-25% strain; 75-75% strain; 25IL-25%+IL-1β; 75IL-75%+IL-1β. *=significantly higher than NC group