Response of Meniscal Tissue to 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 one such tissue that, when injured or insulted, can produce key factors in the biochemical pathways leading to OA. This study was designed to begin to characterize the biochemical responses of meniscal tissue associated with the inflammatory mediators involved in OA. By analyzing the biomarkers released to the culture media over a 21 day period, we sought to evaluate the potential contribution of menisci to the development and progression of OA. We hypothesize that 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: The lateral and medial menisci were aseptically collected from the knees of skeletally mature dogs (n=6) euthanatized for reasons unrelated to this study. Meniscal explants (4mm) were created using a biopsy punch and placed in supplemented DMEM culture media. Meniscal explants were then randomly assigned to either a cytokine-treated group (POS) or a non-cytokine-treated group (NEG).

Tissue culture: Explants were cultured in 2ml of supplemented DMEM culture medium with or without 50ng/ml rcIL-1β treatment. Explants were cultured for 21 days at 37°C, and media were collected and replenished for biomarker analysis every 3 days. At the end of the culture period, explants were collected for evaluation of extracellular matrix composition.

Mediation Biomarker Analysis: Media were analyzed for MMP activity, ADAMTS 4 activity, GAG concentration, NO concentration, PGE2 concentration, IL-6, IL-8, MCP-1, KC, MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13 using commercially available assays.

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 analyzed for proteoglycan (GAG) and collagen content using the DMMB and Hydroxyproline assays, respectively.

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

Results: PGE2 and NO: Media NO concentration was significantly (p<0.05) higher in the POS group compared to the NEG group at all time points tested. Media PGE2 concentration was significantly (p<0.05) higher in the POS group compared to the NEG group for Days 3 through 6 and Days 15 through 21. Days 9 and 12 did not show significantly (p<0.05) higher levels of PGE2 in the media.

MMP concentration: The concentrations of MMP-3 and MMP-13 were significantly (p<0.05) higher in the POS group compared to the NEG group at all time points. The production of MMP-2 significantly (p<0.05) lower in the POS group compared to the NEG group on days 3 through 15 of culture. There was not a consistent significant (p>0.05) difference between POS and NEG groups for the concentration of MMP-1 and MMP-9 at any time point tested in this study.

ADAMTS4 and MMP activity: ADAMTS4 activity was significantly higher in the POS group compared to the NEG group on days 6 though 18, but there was not a significant difference between the two groups on Day 3 or Day 21 of culture. General MMP activity was significantly (p<0.05) higher in the POS group compared to the NEG group for the duration of culture.

GAG concentration: GAG concentration in the media was not significantly (p>0.05) affected by cytokine treatment at any time point when compared to the negative controls.

Cytokine/Chemokine concentration: The concentration of IL-6, IL-8, KC, and MCP-1 were all significantly (p<0.05) higher in the POS group compared to the NEG group for the duration of culture.

Discussion: These data indicate that meniscal tissue is a potentially potent source of both inflammatory and degradative mediators when insulted with a pro-inflammatory cytokine known to be involved in the disease processes of OA. After IL-1β exposure, meniscal explants were capable of degradative enzyme synthesis and activity, (elevated MMP-2, -3, -13 concentrations and ADAMTS4 and MMP activity), pro-inflammatory and tissue repair responses (increases in IL-6, IL-8, KC, and MCP-1 concentrations), and participation in the inflammatory cascades (increased production of NO and PGE2) involved in OA. 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 highlight the potential contribution of meniscal tissue to the biological pathways of joint health and disease in addition to the well established biomechanical functions in the knee.

Acknowledgments:
References: