Comparison of the Basal and Cytokine Stimulated Production of Biomarkers by Cartilage, Synovium, and Meniscal Tissue In Vitro

Alex Cook, Aaron M. Stoker, MS, PhD, James L. Cook, DVM, PhD. University of Missouri Columbia, Columbia, MO, USA.

Disclosures: A. Cook: None. A.M. Stoker: None. J.L. Cook: None.

Introduction: In the study of osteoarthritis (OA), it is important to consider the joint as an organ in which all articular tissues contribute to development and progression of OA. Cartilage, synovium, and menisci are tissues in the knee that, when injured or insulted, can produce key factors that participate in the pathogenesis and pathobiology of clinical OA. This study was designed to characterize the biological responses of these articular tissues after exposure to known OA-related inflammatory mediators. By analyzing and comparing specific biomarkers released during tissue culture, we sought to understand the contributions of each tissue type to biomarkers involved in development and progression of OA. We hypothesized that cartilage, meniscus, and synovial tissues would be associated with unique in vitro basal and IL-1β stimulated biomarker profiles.

Methods: Tissue collection: All procedures were performed with IACUC approval. Tissues were collected aseptically from skeletally mature dogs euthanatized for reasons unrelated to this study. The lateral and medial menisci were collected from the knees of 6 dogs, articular cartilage was obtained from the humeral head of 13 dogs, and synovial tissue was collected from the knees of 13 dogs. A biopsy punch was used to create 4mm Meniscal (MEN) explants, 6mm articular cartilage (CART), and 4mm Synovial (SYN) explants for culture.

Tissue culture: Tissues were cultured in 2mls of DMEM with (inflammation) or without (basal) 50ng/ml rcll-1β. The explants from each dog were the placed into the following groups: Inflammation (1) CART 50 (2) SYN 50 and (3) MEN 50 or Basal (4) CART N, (5) SYN N, and (6) MEN N. Culture media were collected and replenished every 3-4 days for biomarker analysis. Tissues were cultured up to 21 days at 37°C. At the end of culture, explants were collected for evaluation of extracellular matrix composition.

Media Biomarker Analysis: Media were analyzed for MMP activity, ADAMTS 4 activity, 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.

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

Results: MMP and ADAMTS4 Activity: The basal level of MMP activity was significantly (ps≤0.001-0.043) higher in the SYN group compared to the MEN and CART groups, and the MEN group was significantly (p=0.004-0.028) higher than the CART group. With IL-1β stimulation the MEN and SYN groups had significantly (ps≤0.001-0.017) higher MMP activity compared to the CART group. The basal level of ADAMT4 activity in the SYN group was significantly (ps≤0.001-0.020) higher than the CART and MEN groups. There was not a consistent difference between MEN and CART basal ADAMT4 activity. None of the groups had a consistent, significant increase in ADAMT4 activity with IL-1β stimulation.

MMP Concentration: SYN and MEN tissues produced significantly (ps≤0.001-0.032) higher levels of MMP-1, 3, and 9 compared to cartilage under basal and IL-1β stimulated conditions. SYN tissues produced significantly (ps≤0.001-0.043) higher levels of MMP-2 compared to MEN and CART tissues under basal and IL-1β treatment conditions. There was not a consistent, significant difference between CART and MEN for MMP-2 production. There was not a consistent difference between SYN and MEN groups for MMP-3 production under basal conditions, but SYN produced significantly (ps≤0.001-0.026) higher levels of MMP-3 compared to MEN after IL-1β treatment. The SYN group produced significantly (p=0.029-0.930) higher levels of MMP-9 compared to the MEN groups under basal and IL-1β treatment conditions at most time points. Under basal conditions there was not a significant difference in the production of MMP-13 between CART, SYN, and MEN tissues. With IL-1β stimulation CART and MEN tissues produced significantly (ps≤0.001-0.033) higher levels of MMP-13 compared to the SYN group.

Cytokine Concentration: The MEN group produced significantly (ps≤0.001-0.043) higher levels of IL-6, IL-8, and KC under basal and IL-1β treatment conditions compared to the CART group. The SYN group produced significantly (ps≤0.001-0.041) higher IL-6, KC, and MCP-1 under basal and IL-1β treatment conditions compared to the CART group. The production of IL-8 by the SYN group was significantly (p=0.002-0.004) higher than the CART group under basal conditions, but not after IL-1β stimulation. While the production of IL-8 was not significantly different between the MEN and SYN group under basal conditions, the MEN group produced significantly (ps≤0.001-0.002) higher levels of IL-8 after IL-1β stimulation. The production of IL-6 was significantly (ps≤0.001-0.043) higher in the SYN groups at early time points of culture compared to the MEN group, but significantly (ps≤0.001-0.020) higher in the MEN group compared to the SYN group at later time points of culture under basal and IL-1 β treatment conditions. There was not a significant difference between the MEN and SYN group for KC production under basal conditions, but was significantly (ps≤0.001-0.036) higher in the MEN group after IL-1β treatment. The basal production of MCP-1 by meniscal tissue was significantly (ps≤0.001-0.043) higher than the CART group under basal conditions, but not after IL-1β treatment. The basal production of MCP-1 by the SYN group was significantly (ps≤0.001-0.043) higher than the MEN group for the duration of
culture, but only through 9 days of culture after IL-1β treatment. PGE2: The basal production of PGE2 by the SYN and MEN groups was significantly (p≤0.001-0.004) higher than the CART group. With IL-1β stimulation, the SYN group produced significantly (p≤0.001-0.006) higher levels of PGE2 compared to the MEN and CART groups at most time points tested. There was not a consistent, significant difference between CART and MEN groups after IL-1β stimulation.

NO: Under IL-1β treatment conditions the MEN and CART group consistently produced significantly (p≤0.001-0.034) higher levels of NO compared to the SYN group. The basal production of NO by MEN was significantly (p≤0.001-0.002) higher than the SYN group. The MEN group produced significantly (p≤0.001-0.048) higher levels of NO compared to the CART group under basal and IL-1β conditions.

Discussion: These data indicate that articular cartilage, synovium and meniscus each have unique basal metabolic activities as well as unique responses to IL-1β stimulation. Articular cartilage and meniscus appear to be primarily responsible for production of IL-8, NO, and MMP-13, while synovium may primarily be responsible for production of PGE2, MMP-2, and MMP-3. Understanding the dynamics of these tissue responses, will aid in developing and validating clinically relevant diagnostic and therapeutic strategies for the broad spectrum of insults and injuries affecting diarthrodial joints.

Significance: These data indicate that articular cartilage, synovium and meniscus each have unique basal metabolic activities as well as unique responses to IL-1β stimulation. Understanding the dynamics of these tissue responses, will aid in developing and validating clinically relevant diagnostic and therapeutic strategies for the broad spectrum of insults and injuries affecting diarthrodial joints.

Acknowledgments:

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ORS 2014 Annual Meeting
Poster No: 0462