

Assessment of cellular response to synovial joint fluid in osteoarthritic and healthy dogs



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Introduction

Osteoarthritis (OA) is a debilitating disease that impairs movement in both dogs and humans. However, the biological factors that drive the development and progression of OA are still poorly understood. Therefore, this study was designed to determine how cells from cartilage, meniscus and synovial tissue respond to synovial fluid obtained from the stifle of knees of dogs with and without OA. Once the differential response of the cells are identified, the factors within the synovial fluid that stimulates those changes can then be identified and studied in future studies.

Objective

To compare cellular response to healthy and osteoarthritic synovial fluid by measuring expression of inflammatory and proteoglycan matrix biomarkers.

Hypothesis

Cells from cartilage, meniscus, and synovium will show significant increases in degradative enzyme and inflammatory gene expression, and significant decreases in extracellular matrix production gene expression, when cultured with OA synovial fluid in comparison to normal synovial fluid and untreated cells.

Methods



All procedures were performed with ACUC approval. Synovial fluid was collected from 5 healthy dogs euthanized for reasons unrelated to this study and 6 dogs undergoing a surgical procedure for OA of the stifle at the MU Veterinary Health Center.



Primary cell cultures of chondrocytes (CAR), synoviocytes (SYN), and meniscal fibrochondrocytes (MEN) were created from healthy dogs euthanized for reasons unrelated to this study. Cells were cultured on 24 well plates in 0.5 ml of media containing 10% normal (NOR) or OA synovial fluid. Control cells were not exposed to synovial fluid (NEG). Cells were cultured for one or three days.



Total RNA was extracted and gene expression relative to GAPDH was determined by real time RT-PCR for Aggrecan, COL I, COL II, COL 6, COMP, Lubricin, HAS-1, HAS-2, INOS, COX-2, IL-1 β , TGF- β , TIMP-1, TIMP-2, TIMP-3, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, ADAMTS4, and ADAMTS 5.

Significant differences between groups was determined using a t-test or Rank Sum test with significance set at $p < 0.05$.

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Results:

(* significantly greater than Normal of same time point. (†) significantly greater than OA of same time point. (◇) significantly greater than NEG of same time point



CARTILAGE: On day 1, MMP-1 (Fig. B), TIMP-2 (Fig. A), and TIMP-3 (Fig. D) expression were significantly higher in NOR compared to OA. On day 3, TIMP-2 and TIMP-3 expression were significantly higher in NOR compared to OA.

In the NEG group, COX-2 (Fig. C) expression was significantly lower than OA on day 1, and both SF groups on day 3. The expression of ADAMTS5 and MMP-13 (Day 1); MMP-2, TIMP-1, and COL2 (Day 3); and TIMP-2, AGG, COMP, and TGF-b (Day 1 and 3) was significantly higher in the NEG compared to SF groups (Fig. A, E, F, data not shown).

MENISCUS: On day 1, TIMP-2 (Fig. G) and AGG (Fig. H) expression were significantly higher in NOR compared to OA, and ADAMTS5 (Fig. L) and IL-1b (Fig. J) expression were significantly higher in OA compared to NOR. On day 3, IL-1b expression was significantly higher in NOR compared to OA (Fig. J).

In the NEG group, AGG (Fig. H) expression was significantly lower than SF groups on day 1 and higher on day 3, and COL1 (Fig. I) expression was significantly higher on Day 1 and lower on Day 3. MMP-13 (Day 1); TIMP-1, COL6, INOS, and COX2 (Day 3); and ADAMTS5, MMP-2, TIMP-2, COL2, COMP, and TGF-b (Day 1 and 3) expression were significantly higher in the NEG group compared to SF groups (Fig. G, K, L, data not shown).

SYNOVIAL TISSUE: HAS-2 (Day 1) and TIMP-2 (Day 3) expression was significantly higher in NOR compared to OA (Fig. M and R). MMP-3 expression was significantly higher in OA compared to NOR at Day 3 (Fig. Q).

In the NEG group, ADAMTS5, MMP-13, MMP-2, TIMP-1, COL1, INOS, and TGF-b expression were significantly higher in NEG compared to SF treated group at Day 3 (Fig. N, O, P, data not shown).

Conclusions

- Synovial fluid downregulated gene expression at Day 1 and Day 3 for CAR and MEN and at Day 3 for SYN compared to untreated cells.
- Normal SF stimulated TIMP-2 expression in CAR, MEN, and SYN; MMP-1 and TIMP-3 expression in CAR, and HAS-2 expression SYN compared to OA SF.
- OA SF stimulated ADAMTS5 and IL-1b expression in MEN and MMP-3 expression in SYN compared to Normal SF.