Roles of Impact and IL-1β in Post-Traumatic Osteoarthritis using a Canine in vitro Model

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Introduction

Post-traumatic osteoarthritis (PTOA) is a common sequela to traumatic joint injury. Biomechanical insult to articular cartilage incites pro-inflammatory responses of varying degrees depending on several variables, including impact level. Often joint injury results in an increase joint inflammation, which can illicit an increase in tissues production of degradative and inflammatory proteins. This increase in joint inflammation can exacerbate the the degradation of the cartilage tissue after an impact injury. Therefore, understanding how the combination of cartilage injury and tissue inflammation affects the cartilage tissue metabolism can give insight into how PTOA develops clinically.

Objective

To evaluate the effects of tissue injury and IL-1β treatment, alone and in combination, on articular cartilage tissue structure, viability, and metabolism.

Methods

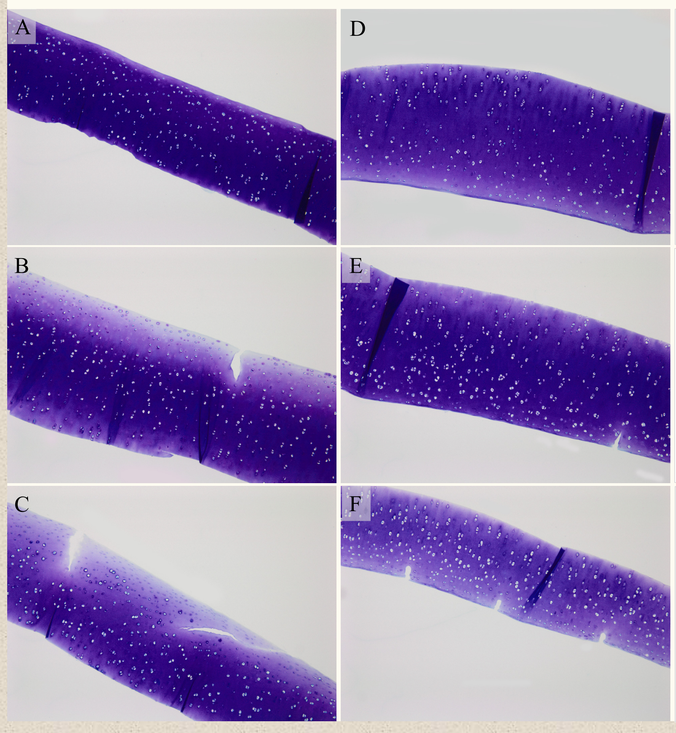
With IACUC approval, full-thickness articular cartilage explants (n=48) were harvested from humeral heads of 8 dogs. One explant per dog was assigned to 1 of 6 groups:

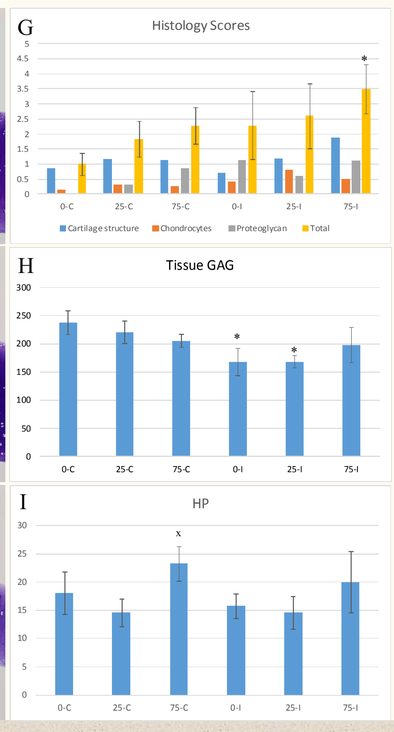
- 0-C: Control (no impact or IL-1 β)
- 0-I: 0.1 ng/ml rcIL-1 β (no impact)
- 25-C: Single unconfined impact to 25% strain at 100 mm/sec
- 75-C: Single unconfined impact to 75% strain at 100 mm/sec
- 25-I: 25% strain impact + IL-1
- 75-I: 75% strain impact + IL-1

After impact explants were cultured for 21 days. Culture media was changed every 3 days and collected for biomarker analysis. Media from days 3, 6, and 9 of culture were tested NO, PGE2, MMP-1, -2, -3, -13, MMP activity, ADAMTS4 activity, MCP1, KC, IL8 and IL6. After 21 days of culture, explants were evaluated for chondrocyte viability, GAG and HP content, and evaluated histologically using the OARSI scoring system. Data were compared for statistically significant ($p<0.05$) differences using the paired T-test.

Results

Figure 1: Tissue Architecture and Extracellular Matrix





Representative photomicrographs (10X) of T-Blue stained tissues used for histological evaluation A) 0-C, B) 25-C, C) 75-C, D) 0-I, E) 25-I, F) 75-I.

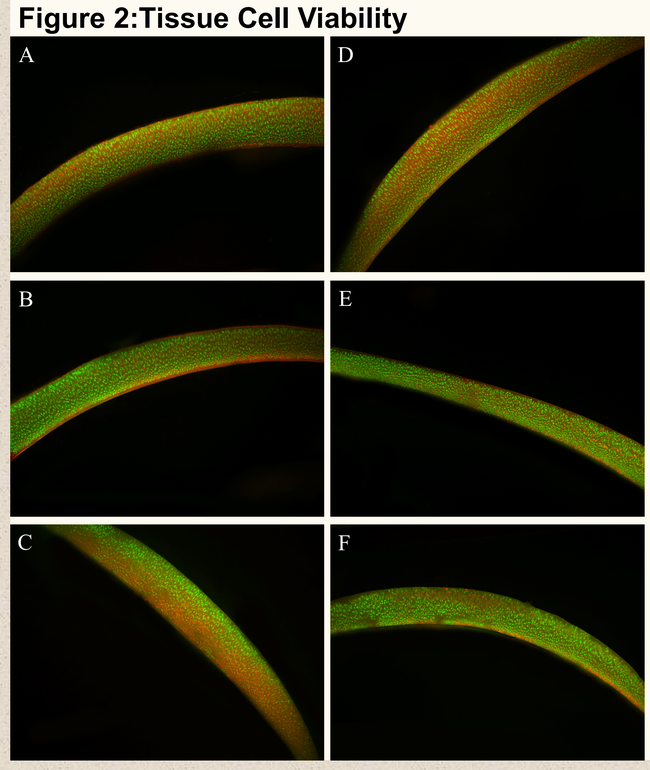
G) Histological scoring using the OARSI system found that the 75-I group had significant higher scores compared to the 0-C control group.

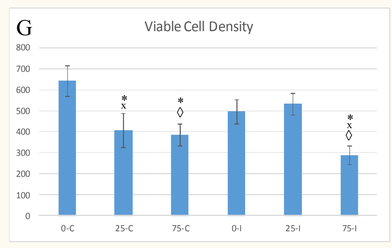
H) Tissue GAG (µg/mg dry wt) content was significantly lower in the 0-I and 25-I groups compared to the 0-C control group after 21 days of culture.

I) Tissue Collagen (µg/mg dry wt) content (HP) was significantly higher in the 75-C group compared to the 0-C control group.

(*) Significantly different than control (0-C) (X) Significant difference between higher and lower impact groups

Figure 2:Tissue Cell Viability



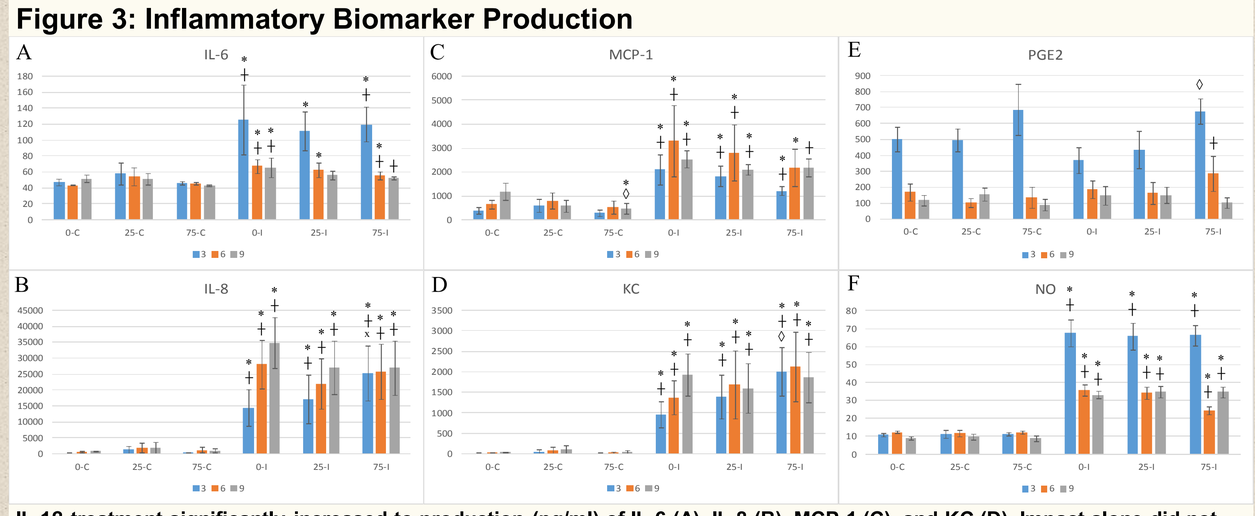


Representative photographs (4X) of tissue stained with calcien AM (green) and ETHD (Red) A) 0-C, B) 25-C, C) 75-C, D) 0-I, E) 25-I, F) 75-I.

G) Viable cell density (cells/mm^2)was determined by dividing the number of live, green staining, cells by the total area of the tissue. Impact alone resulted in a significant decrease in viable cell density in both the 25-C and 75-C groups compared to the 0-C control. However, the combination of impact and inflammation (IL-1β) significantly ($p<0.05$) decreased viable cell density only in the 75-I group compared to the 0-C control.

(*) Significantly different than control (0-C) (X) Significant difference between higher and lower impact groups (◊) Significant difference between 75% and 0 % groups

Figure 3: Inflammatory Biomarker Production



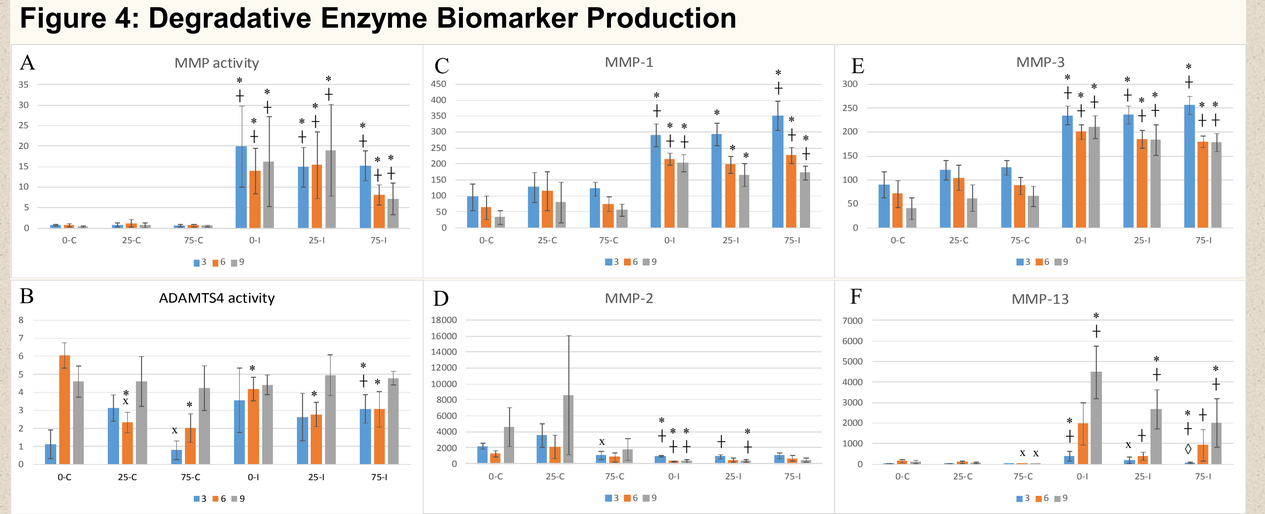
IL-1β treatment significantly increased to production (pg/ml) of IL-6 (A), IL-8 (B), MCP-1 (C), and KC (D). Impact alone did not significantly increase the production of these cytokines. Further, the combination of impact and IL-1β did not increase production over IL-1β treatment alone except for KC production on day 3 in the 75-I group.

The production (pg/ml) of PGE2 (E) on day 3 of culture was a significantly increased in the 75-I group compared to the 0-I group, and on day 6 there was a significant increase in the 75-I group compared to the 75-C group. However, this increase in production was not maintained and was not noted in any other treatment group.

The production (nM) of NO (F) was significantly higher with IL-1β treatment, but not after impact. Further, the combination of impact and IL-1β did not increase production over IL-1β treatment alone

(*) Significantly different than control (0-C) (┼) Significant difference between groups with and without IL-1β treatment (X) Significant difference between higher and lower impact groups (◊) Significant difference between 75% and 0 % groups

Figure 4: Degradative Enzyme Biomarker Production



MMP (ng/ml) activity (A) was significantly higher in the IL-1β treated groups than the non-treated groups at all time points tested. Application of a single impact to the tissue did not result in a significant increase MMP activity.

ADAMTS4 (ng/ml) activity (B) was significantly increased in the 75-I group compared to the 0-C and 75-C groups on day 3, and 75-C was significantly decreased compared to 25-C. On day 6 the 0-C group had significantly higher than all other groups.

There was a significant increase in the production (pg/ml) of MMP-1 (C), MMP-3 (E), and MMP-13 (F) with IL-1β stimulation at all time points tested compared to untreated groups. MMP-2 (D) production was significantly decreased in the 0-I group compared to the 0-C group at all time points.

(*) Significantly different than control (0-C) (┼) Significant difference between groups with and without IL-1β treatment (X) Significant difference between higher and lower impact groups (◊) Significant difference between 75% and 0 % groups

Conclusions

- A single traumatic injury alone resulted in significant cell death and tissue disruption, but not significant metabolic changes in the proteins
- Inflammation alone stimulated a strong metabolic response by the tissue
- The combination of traumatic injury and inflammation resulted in more pathologic tissue changes, but not a higher metabolic response by the tissue.

Discussion

Traumatic joint injury often results in the development of OA in both veterinary and human patient populations. However, it is unclear why some patients develop OA and other do not after similar injuries. Further, the rate of OA progression is highly variable between patients. The data from this study indicates that a single traumatic impact to cartilage tissue does result in a significant changes to tissue architecture (Fig. 1) and loss of cell viability (Fig. 2). However, there was not a consistent detectable change in the tissues production of inflammatory (Fig. 3) and degradative biomarkers (Fig. 4) after impact. This indicates that additional stimuli may be required to progress the damage associated with a traumatic injury toward the development of osteoarthritis. Stimulation of the tissue with IL-1β resulted in a significant increase in the tissue production of inflammatory (Fig. 3) and degradative (Fig. 4) biomarkers, but the combination of tissue injury and IL-1β treatment did not result in a significant increase in production over IL-1β treatment alone. However, the combination of tissue injury and cytokine treatment did result in increased tissue degradation and cell loss, indicating that the combination of tissue injury and joint inflammation resulted in a faster progression of tissue degradation over either stimuli alone. Therefore, one of the factors that may contribute to development of OA in a patient after tissue injury is the level of inflammation in the joint after injury.

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