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.

Results

Figure 1: Tissue Architecture and Extracellular Matrix

Representative photomicrographs (10X) of 10% alc 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 significantly 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 (HPI) was significantly higher in the 75-C group compared to the 0-C control group.

Figure 2: Tissue Cell Viability

A) Tissue stained with calcien AM (green) and EthD-1 (red) A) 0-C, B) 25-C, C) 75- C, D) 0-I, E) 25-I, F) 75-I.

G) Viable cell density was determined by dividing the number of 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-I and 75-I groups compared to the 0-C control. However, the combination of impact and inflammatory (IL-1β) significantly (p<0.05) decreased viable cell density only in the 75-I group compared to the 0-C control.

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.

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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 increase MMP activity, MCP1, KC, IL8 and IL6. 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.

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 one of 8 treatment groups:

- 0-C: Control (no impact or IL-1 β)
- 0-I: 0.1 ng/ml rIL-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-S: 25% strain impact + IL-1
- 75-S: 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.

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