Preventing Chondrocyte Death after Closed Intra-articular Fracture in a Rat Model

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INTRODUCTION: Intra-articular fractures can result in post-traumatic osteoarthritis (PTOA), which is the cause of chronic pain, disability, and decreased quality of life for millions of people.[1] Chondrocyte death initiated at the time of injury to articular cartilage has been associated with the development of PTOA.[2] An increase in inflammatory proteins and signaling pathways within an injured joint likely drives chondrocyte death.[3–6] However, after high-energy blunt mechanism intra-articular fractures, the response of articular chondrocytes to anti-inflammatory treatment is unknown. The objective of this study was to determine the effect of anti-inflammatory treatment on chondrocyte viability after closed intra-articular knee fractures in a rat model. We hypothesized that anti-inflammatory treatment would mitigate negative chondrocyte responses in the early period after intra-articular fracture.

METHODS: This study was approved by the Institutional Animal Care and Use Committee. Using Sprague-Dawley rats, a closed intra-articular fracture of the distal femur or tibial plateau was created, as described previously.[7] Fractures were confirmed using fluoroscopy and micro-computed tomography (micro-CT) (Figure 1). After injury, 36 rats were assigned to one of three groups (N=12 per group): Group 1 received an intra-articular injection of saline (control group); Group 2 received an intra-articular injection of the corticosteroid dexamethasone; and Group 3 received an injection of the caspase inhibitor kZ-VAD-FMK. Injections were performed immediately after injury. Intra-articular location of the injection was confirmed using fluoroscopy. Rats were sacrificed at one of three time points: immediately after injury, 3 days after injury, or 6 days after injury. After sacrifice, chondrocyte viability was assessed via histomorphometric analysis of cartilage 1 millimeter from the fracture edge, and included terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and immunohistochemistry for anti-caspase 3 (N=5 control, 8 dexamethasone treatment, 6 k-ZVAD-FMK treatment; specimens with inadequate fracture visualization on histology were excluded). Articular cartilage was also harvested at the 3 day timepoint for RNAseq analysis (N=3 per group), which was used to identify differentially expressed genes (DEGs) associated with chondrocyte death. Pathway enrichment analysis was performed to compare cellular activity associated with chondrocyte death between groups. Statistical analysis for histomorphometry consisted of two factor analysis of variance (ANOVA) for time and treatment group. For RNAseq analysis, false discovery rates of extracted RNA were calculated. A p value of less than 0.05 was considered significant.

RESULTS: The difference in the proportion of TUNEL- and anti-caspase 3-positive chondrocytes was not significantly different among the three groups at any timepoint. There were no significant changes with time. Inflammatory pathways were downregulated in the dexamethasone group compared to the saline group. Dexamethasone was also associated with a decrease in pathways associated with cellular stress, including reactive oxygen species (ROS), reactive nitrogen species (RNS), angiogenesis, and lysosome activity (Figure 2). Other pathways associated with the progression of arthritis were downregulated in the dexamethasone group, including the serine/threonine kinase BRAF and platelet derived growth factor (PDGF) pathways (Figure 2). Genes associated with chondrocyte death were downregulated in the dexamethasone group compared to the saline group, including *completement 7* (*C7*), *Pdgfb, Panx3* and *Lmod2* (Figure 3). Genes associated with chondrocyte death were also downregulated in the kZ-VAD-FMK group compared to the saline group, including *Lmod2* and *Cavin4*.

DISCUSSION: In a rat model of a closed intra-articular fracture that simulates a high-energy mechanism of injury, intra-articular injection of the corticosteroid dexamethasone was associated with a downregulation of multiple pathways and genes that may contribute to the pathogenesis of PTOA. The caspase inhibitor kZ-VAD-FMK was associated with a downregulation in genes that may drive chondrocyte death. The association between inflammation and chondrocyte death has been established in other models of PTOA.[8] Intra-articular dexamethasone treatment has been shown to mitigate the progression of PTOA in animal models as well.[9] However, such other models have not compared treatments that may prevent chondrocyte death to a control in a model that mimics the common, high-energy causes of intra-articular fractures in humans, such as a motor vehicle collision or a pedestrian being struck. Further research is necessary to delineate the effect of both treatments in preventing longer-term progression of PTOA. Future translation of our results may include intra-articular injection of affordable and commonly used anti-inflammatory medications into an acutely injured joint (for example, dexamethasone into an ankle joint damaged by a pilon fracture).

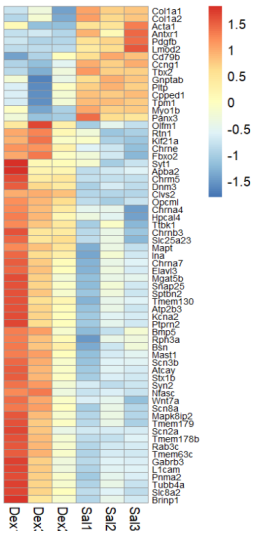

Figure 3 Heat map for differentially expressed genes between saline (control) and dexamethasone groups.

SIGNIFICANCE/CLINICAL RELEVANCE: PTOA resulting from intra-articular fractures causes pain and disability for many people. Intra-articular injection of anti-inflammatory medication at the time of injury may downregulate expression of genes and pathways associated with the development of PTOA.

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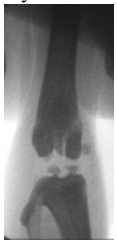

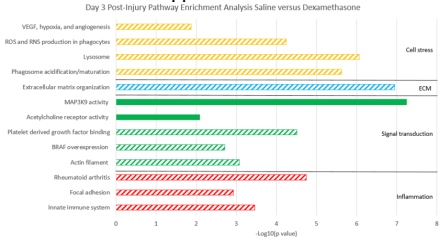

Figure 1 (left) Intra-articular distal femur fracture
Figure 2 (right) Day 3 post-injury pathway enrichment analysis for saline (control) versus dexamethasone groups. Striped bars indicate downregulated pathways and solid bars indicate upregulated pathways.