CDK9 INHIBITION ATTENUATES INFLAMMATORY RESPONSE AND APOPTOSIS IN CARTILAGE EXPLANTS TO PRESERVE MATRIX INTEGRITY IN A SINGLE IMPACT MECHANICAL INJURY MODEL

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Purpose: Joint injury is associated with the development of post-traumatic osteoarthritis. Mechanical and physical damage to cartilage and surrounding joint tissues induces expression of pro-inflammatory cytokines (IL-1, IL-6, TNF) and catabolic enzymes (MMPs, ADAMTSs), which promote cartilage matrix degradation and chondrocyte apoptosis. Recent advances have established that the rate-limiting step for the transcriptional activation of primary response genes is controlled by the elongation factor cyclin-dependent kinase 9 (CDK9). Regardless of the sources, diverse inflammatory signals ultimately converge onto a regulatory bottleneck, at which CDK9 is recruited to target genes and phosphorylates RNA Polymerase II to stimulate transcription of full-length mRNAs. Therefore, targeting CDK9 may be an effective strategy to globally and effectively suppress inflammatory response. Our objective is to determine whether the pharmacological CDK9 inhibitor Flavopiridol can suppress the inflammatory response and prevent apoptosis in mechanically injured cartilage explants, thereby preserving their mechanical property.

Methods: Cartilage explants (6mm x 3mm) were harvested from the femoral condyles of bovine stifle joints. After 24hrs, explants (n = 6/condition) were mechanically injured by 30% compression (100%/sec), and cultured +/-300 nM Flavopiridol, a pharmacological CDK9 inhibitor. mRNA expression of inflammatory cytokines and catabolic enzymes was determined by qPCR normalized to 18s rRNA. Apoptosis was determined by DeadEnd Fluorometric TUNEL system (Promega). Glycosaminoglycan (GAG) release was determined by dimethylmethylene blue (DMMB) assay. After 4 weeks of culture, mechanical properties (instantaneous and relaxation moduli) were estimated from a sample of the explant. One-way ANOVA, w/LSD correction determined significance (* P<0.05).

Results: After a single impact load, injured explants increased expression of pro-inflammatory cytokine IL-6, and catabolic enzymes ADAMTS4, MMP1. Their induction was markedly reduced by the CDK9 inhibitor Flavopiridol (Fig.1). In uninjured controls, baseline expression of IL-6, ADAMTS4 and MMP1 and anabolic genes such as Col2a1 and aggrecan were not significantly affected by Flavopiridol (not shown). Mechanical injury increased apoptosis in chondrocytes, and this was reduced by Flavopiridol (Fig 2). Mechanical injury enhanced GAG release, but this was reduced to baseline by Flavopiridol (Fig 3). Lastly, the mechanical property of the explants was lowered in the injured group when compared to the control, but this was reversed by Flavopiridol (Fig 4). Collectively, these results indicate that CDK9 inhibition by Flavopiridol prevents inflammation-induced apoptosis and protects cartilage from the deleterious effects of mechanical injury.

Conclusions: CDK9 inhibition by Flavopiridol significantly reduced the inflammatory and apoptotic response to mechanical injuries in cartilage explants. CDK9 inhibition prevented degradation of GAG and preserved mechanical properties after explant injury. Thus CDK9 is a novel target for preventing the initiation of matrix degradation after mechanical injuries. Further studies are needed to test if Flavopiridol prevents the onset of post-traumatic osteoarthritis.

Figure 1: Stiffness distribution of cartilage at the micro-scale measured using an indentation rate of 1 μm/s

Figure 2: Histogram of the cartilage dynamic elastic modulus at the nano-scale measured using an indentation rate of 1 μm/s

Figure 1: CDK9 inhibition by Flavopiridol suppresses induction of IL-6, ADAMTS4 and MMP1 in injured cartilage explants.

Figure 2: CDK9 inhibition by Flavopiridol reduces injury-induced apoptosis in chondrocytes.
We hypothesized that ACL-injured cases would demonstrate significant changes that occur in the tibial articular cartilage following ACL injury. Additionally, tibial orientation has been shown to change after injury. Changes in articular cartilage thickness have been shown to be associated with the early onset of post-traumatic osteoarthritis, regardless of surgical or non-surgical intervention. Changes in cartilage thickness have been shown to occur on the femoral condyles and trochlea at one and two-year follow-up after ACL injury; however, little is known regarding changes that occur in the tibial articular cartilage following ACL injury. Additionally, tibial orientation has been shown to change after injury. We hypothesized that ACL-injured cases would demonstrate significant side-to-side differences in cartilage thickness, and controls would show no within subject differences. We also hypothesized that males and females would demonstrate differences in the location and magnitude of cartilage thickness change, and the magnitude of change would be explained by the orientation of the tibia relative to the femur during MRI data acquisition.

**Purpose:** Anterior cruciate ligament injury has been associated with the early onset of post-traumatic osteoarthritis, regardless of surgical or non-surgical intervention. Changes in articular cartilage thickness have been shown to occur on the femoral condyles and trochlea at one and two-year follow-up after ACL injury; however, little is known regarding changes that occur in the tibial articular cartilage following ACL injury. Additionally, tibial orientation has been shown to change after injury. We hypothesized that ACL-injured cases would demonstrate significant side-to-side differences in cartilage thickness, and controls would show no within subject differences. We also hypothesized that males and females would demonstrate differences in the location and magnitude of cartilage thickness change, and the magnitude of change would be explained by the orientation of the tibia relative to the femur during MRI data acquisition.

**Methods:** An IRB approved case-control study was used to evaluate 54 males and 54 females (27 case-control pairs for each sex). ACL-injured cases had sustained a first time non-contact ACL injury during participation in an organized high school or college sport. Healthy, uninjured teammates of ACL-injured subjects who were matched on age and sex, were used as controls. Bilateral Phillips Achivia 3T MRI scans were obtained on all subjects (before surgery and median 15 days post injury for cases).

**Results:** Analyses that did not consider tibiofemoral orientations as covariates revealed significant side-to-side differences in tibial cartilage thickness in ACL-injured subjects. Cartilage thickness increase was observed in the anterior (Males = 0.25 mm; Females = 0.0 mm) and central (Males = 0.29 mm; Females = 0.14 mm) regions of the medial compartment. No significant differences in thickness were observed in the uninjured controls. Male ACL-injured subjects exhibited greater changes in cartilage thickness when compared to female ACL-injured subjects.

**Conclusions:** Changes to tibial articular cartilage thickness in ACL-injured subjects occur as a function of altered contact mechanics in the injured knee in the acute time period after injury. Specifically, differences in thickness were explained by the anterior position of the tibia relative to the femur, suggesting altered cartilage loading. Alterations in loading could lead to the degenerative changes associated with PTOA. Male and female case subjects exhibited different magnitudes of thickness change; suggesting that ACL injury may affect articular contact mechanics between sexes differently. Future studies should include sex-specific analyses, and implementation of a 3D bone based...