PHLPP Inhibitors Prevent Cartilage Extracellular Matrix Breakdown in a Model of Post-Traumatic Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a chronic and common disease affecting 1 in 4 adults. Late-stage OA is characterized by articular cartilage deterioration, osteophytes, and other joint changes. In early stages of the disease, declines in mechanical and structural qualities (e.g., stiffness) of the extracellular matrix precede cartilage loss. Interventions that prevent loss of articular cartilage integrity and/or regenerate cartilage hold the best promise for disease modification. Phlpp1 is a protein phosphatase that slows chondrocyte proliferation and extracellular matrix production. Previous studies showed that genetic deletion of Phlpp1 and small molecule inhibitors of Phlpp1/2 prevent cartilage loss and pain-related behaviors associated with PTOA [1, 2]. We recently showed that genetic deletion of Phlpp1 does not alter structural properties, including elastic modulus, in mice [3]. Here we determined how PHLPP inhibitors affected articular cartilage stiffness after injury.

METHODS: PTOA was induced in 12-week-old male C57Bl/6 mice with surgery that destabilized the medial meniscus (DMM). A single intra-articular injection of the PHLPP inhibitor NSC117079 or saline was administered 4 weeks after DMM. Mice were sacrificed 4 and 6 weeks post-DMM surgery. Atomic Force Microscopy (AFM) micro-indentation was used to measure elastic modulus of femoral articular cartilage during disease progression as described [3]. Activity assays were performed at baseline (BL), time of injection, and 4 or 6 weeks after injury to monitor activity levels.

RESULTS: Six weeks after DMM surgery, the elastic modulus of the articular cartilage in the injured limb injected with saline was lower compared to contralateral limb. In contrast, the elastic modulus of femoral articular cartilage in injured joints that received intra-articular injections of NSC117079 was not significantly different from measurements on the contralateral femur (Figure 1). Mice treated with NSC117079 also had better activity levels at 6 weeks post-DMM compared to baseline, indicating that the PHLPP inhibitor relieves pain-related behaviors in addition to cartilage structure (Figure 2). As seen in other studies of this earlier timepoint, histological changes (quantified with the OARSI scoring method) were not detected any at 6 weeks post DMM surgery.

DISCUSSION: DMM surgery induces structural changes in the elastic modulus of murine articular cartilage within two to six weeks of injury [4]. Here we showed that the PHLPP inhibitor NSC117079 prevented loss of elastic modulus in the articular cartilage when administered two weeks after injury. These results are consistent with earlier reports showing that a single injection of NSC117079 prevented histological signs of cartilage degradation and pain associated behaviors for five weeks after injection and 12 weeks after injury [1].

SIGNIFICANCE: PHLPP inhibitors can prevent early stages of PTOA-associated cartilage degradation. Preservation of articular cartilage structure and integrity immediately after injury will slow disease progression to preserve joint function and patient quality of life.

REFERENCES:

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4. Li, Q., et al., *Mediation of Cartilage Matrix Degeneration and Fibrillation by Decorin in Post-traumatic Osteoarthritis.* Arthritis Rheumatol, 2020. **72**(8): p. 1266-1277.

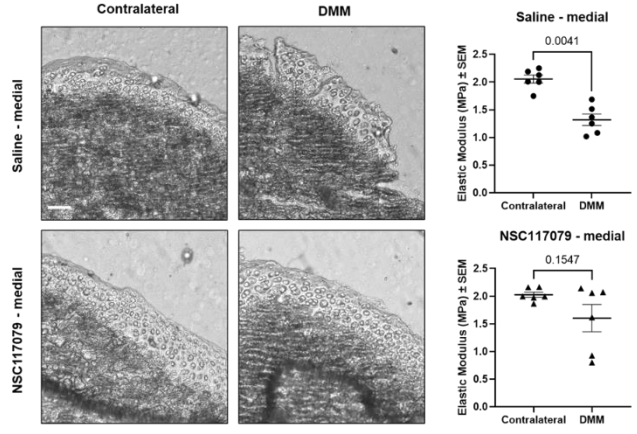

Figure 1: Images taken during AFM showing subchondral bone (dark) and articular cartilage (light) of distal femora in the 4 study groups. Elastic modulus measurements show differences between contralateral and injured limbs after saline or PHLPP inhibitor injection.

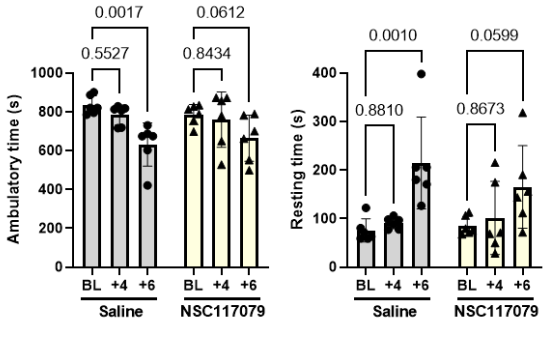

Figure 2: Activity assay data showing changes in ambulatory time and resting time from baseline pre-DMM (BL), and at 4- and 6-weeks post-injury.