

Intra-articular anakinra injection mitigates peri-articular bone loss in a murine model of non-invasive anterior cruciate ligament rupture with delayed reconstruction

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Introduction: Post-traumatic osteoarthritis (PTOA) develops in up to 60% of patients within the first two decades after anterior cruciate ligament (ACL) injury. Immediately following ACL injury, intra-articular levels of pro-inflammatory cytokines (IL-1 β , IL-6, and TNF α) and proteases (MMP-1 and MMP-13) increase, and growing evidence suggests that biochemical, rather than biomechanical, factors may play a critical role in the development of PTOA. Despite this, few preclinical studies have evaluated the effect of intra-articular anti-inflammatory therapies on PTOA development. The primary aim of the present study is to evaluate the impact of intra-articular injection with either (1) anakinra (IL-1 receptor antagonist), (2) alpha-2-macroglobulin (A2M, protease inhibitor), (3) corticosteroid (triamcinolone acetone, Kenalog®), or (4) saline (control) on the development of PTOA in a murine model of non-invasive (“closed”) ACL rupture followed by delayed ACL reconstruction. The secondary aim is to evaluate the effect of the aforementioned injections on knee range of motion (ROM) and pain-related gait behaviors.

Methods: Following IACUC approval, forty 11-12-week-old male C57BL/6 mice underwent non-invasive ACL rupture (Croen et al., AJSM, 2023) followed by an immediate intra-articular injection with 4 microliters (μ L) of either (1) anakinra, (2) A2M, (3) corticosteroid, or (4) saline. At 7 days post-injury, all mice underwent delayed ACL reconstruction utilizing flexor digitorum longus (FDL) tendon autograft as previously described (Camp et al., JBJS, 2017). Mice were sacrificed at 28 days post-injury (21 days post-surgery). The primary outcome measure was quantification of proximal tibial epiphyseal and distal femoral epiphyseal bone volume using micro-computed tomography (μ CT) as a measure of early PTOA (Figure 1). The secondary outcomes included knee ROM and gait analysis.

Results: The anakinra group had less proximal tibial epiphyseal bone loss compared to the A2M ($p < 0.05$) and saline ($p < 0.05$) groups. The anakinra group also had less distal femoral epiphyseal bone loss compared to the A2M ($p < 0.01$), corticosteroid ($p < 0.05$), and saline ($p < 0.01$) groups (Figure 2). The corticosteroid group had decreased ROM compared to the A2M (average ROM 117 degrees versus 119 degrees, respectively, $p < 0.05$) and saline (average ROM 118 degrees versus 119 degrees, respectively, $p < 0.01$) groups, and the Anakinra group had decreased ROM compared to the saline group (average ROM 118 degrees versus 119 degrees, respectively, $p < 0.05$, Figure 3). There were no differences between groups with respect to time in swing phase, time in stance phase, stride length, or stride frequency on gait analysis. There was one joint infection in the corticosteroid group, but there were otherwise no infections or wound complications appreciated in any of the other groups.

Discussion: This study demonstrates that an intra-articular anakinra injection administered immediately following non-invasive ACL rupture attenuated bone loss in the peri-articular bone, perhaps indicating mitigation of PTOA development in this murine model of delayed ACL reconstruction. While many factors likely play a role in the development of PTOA after ACL rupture, this suggests that inhibition of pro-inflammatory cytokines, specifically IL-1, may help to mitigate PTOA. Corticosteroid injection was associated with increased stiffness (decreased ROM) at 28 days post-injury. However, while these differences were statistically different, the absolute differences between groups were only 1-2 degrees. Therefore, the differences in range of motion seen in this preclinical model are likely not clinically significant. Only male mice were utilized in this study, which is a limitation. Future studies are required to evaluate the effects of dose and timing of various pharmacologic approaches for the mitigation of PTOA. Our ongoing work will use histological analysis and transcriptional profiling to evaluate in greater depth the early degenerative changes in this clinically relevant model.

Significance/Clinical Relevance: IL-1 receptor antagonism with anakinra attenuates peri-articular bone loss after non-invasive ACL rupture with delayed ACL reconstruction in mice. This study identifies anakinra as a potential therapeutic agent that has translational potential to attenuate PTOA in human patients with ACL ruptures.

Figures:

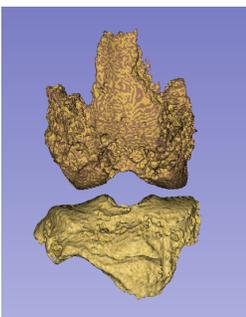


Figure 1. Proximal tibial and distal femoral epiphyseal bone volume segmentation. The proximal tibial epiphysis and distal femoral epiphysis are isolated, and a wrap solidify tool is used to create a three-dimensional rendering of the entire epiphysis in order to calculate proximal tibial and distal femoral epiphyseal bone volume.

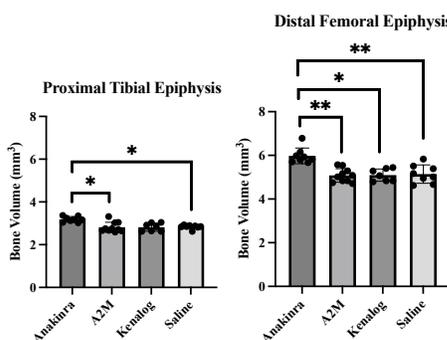


Figure 2. Proximal tibial epiphyseal (left) and distal femoral epiphyseal (right) bone volumes. A2M: alpha-2-macroglobulin. *Statistical significance at $p < 0.05$. **Statistical significance at $p < 0.01$.

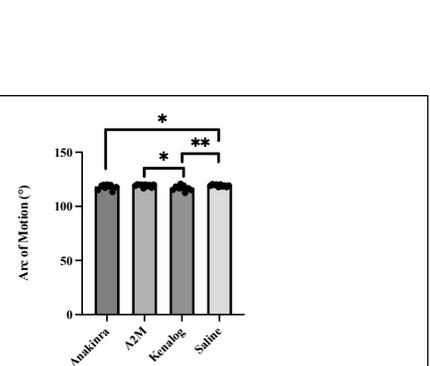


Figure 3. Knee range of motion (knee flexion minus knee extension). A2M: alpha-2-macroglobulin. *Statistical significance at $p < 0.05$. **Statistical significance at $p < 0.01$.