Inhibition of Interleukin-1 Prevents Post-Traumatic Arthritis Following Articular Fracture in the Mouse Knee

Poster No. 0711 • ORS 2012 Annual Meeting

INTRODUCTION: Post-traumatic arthritis (PTA) is an accelerated form of osteoarthritis (OA) that commonly follows joint trauma, such as articular fracture.1,2 While the mechanisms involved in joint degeneration are not fully understood, recent studies suggest that pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) play a role in the pathogenesis of PTA.1 For example, the C57BL/6 mouse strain develops PTA and demonstrates elevated levels of IL-1 and TNF-α in serum and synovial fluid acutely following fracture.4 Conversely, a specialized inbred mouse strain, the MRL/MpJ “super-healer,” is protected from PTA and demonstrates reduced levels of IL-1 and TNF-α in both serum and synovial fluid acutely following fracture.4 We hypothesized that exogenous inhibition of systemic or local pro-inflammatory cytokines following articular fracture will reduce arthritic changes in the joint. Our objective was to examine the effects of inhibiting IL-1 using IL-1 receptor antagonist (IL-1Ra) after fracture using two different delivery mechanisms: osmotic pump (continuous systemic) and intra-articular injection (acute local).

METHODS: All procedures were performed in accordance with an IACUC approved protocol. Male C57BL/6 mice (n=48) were subjected to a closed intra-articular fracture of the tibial plateau after anesthetization using an established protocol.5 A 3.2mm indenter displacement limit was used to create moderate intra-articular fractures.6 Inhibition of IL-1 was achieved using human IL-1Ra, commercially known as anakinra or Kineret® (Amgen; Thousand Oaks, CA). Serum levels of human IL-1Ra were monitored during treatment from serial post-fracture blood collections. Immediately following fracture, one group of mice received a continuous systemic infusion of either IL-1Ra (n=12) or saline (n=11) for 4 weeks with a subcutaneous osmotic pump (Alzet; Cupertino, CA) eluted at 0.25 µl/hr, an IL-1Ra dose of 1.0 mg/day. A second group received a single intra-articular injection (6µl, 30G ½” needle) of either 1.0 mg IL-1Ra (n=9) or saline (n=8) immediately following fracture. A group with fracture only and no treatment was also included (n=8), as well as non-fracture controls (n=3). After fracture and treatment, all mice were permitted full weight bearing activity and unlimited range of motion. At 8 weeks post-fracture, all mice were sacrificed. Immediately after sacrifice, both the left (fractured) and contralateral right (non-fractured) limbs were harvested. Histology sections (8µm thick) of all limbs were taken in the coronal plane, and stained with safranin-o and fast green or hematoxylin and eosin (H&E). The degree of cartilage degeneration was assessed using a modified Mankin score by at least 3 blinded observers.7,8 Each quadrat—lateral tibia (LT), lateral femur (LF), medial tibia (MT) and medial femur (MF)—was evaluated separately for both stains. Serum and synovial fluid were also collected at sacrifice for future analysis.

Statistical analysis of cartilage degeneration was performed using repeated measures ANOVA (Fisher LSD post-hoc) reported at the 95% confidence interval. Statistical analysis of synovitis was performed using non-parametric analyses (Wilcoxon matched pairs, Kruskal-Wallis ANOVA) reported at the 95% confidence interval.

RESULTS: Histological Assessment of Cartilage: Mice who received a single intra-articular injection of IL-1Ra demonstrated significantly reduced Mankin scores in the fractured knee (22.7±8.4, p<0.05) compared to all other treatment groups—fracture with no treatment (43.2±10.0), local saline (33.6±15.2), systemic IL-1Ra (49.2±21.3), and systemic saline (33.8±11.3)—with the exception of non-fracture controls (20.2±7.1, Figure 1A). Furthermore, the local IL-1Ra group qualitatively displayed preserved articular cartilage compared to the other treatment groups (Figure 1B). Histological Assessment of Synovium: Mice receiving local IL-1Ra demonstrated reduced synovial inflammation in the medial femur and medial tibia that showed no statistical difference to the contralateral non-fractured knee (p>0.05, Figure 2A). All other treatment groups, with the exception of non-fracture controls, demonstrated significantly elevated synovial inflammation in the fractured knee compared to the contralateral non-fractured knee (p<0.05) in all quadrants. Furthermore, the local IL-1Ra group qualitatively displayed reduced inflammatory changes in the synovium compared to other treatment groups receiving fractures (Figure 2B).

DISCUSSION: Acute treatment of moderate intra-articular fractures with a single injection of IL-1Ra significantly reduced arthritic changes in the articular cartilage when compared to untreated fractures or joints treated with continuous infusion of IL-1Ra or saline (Figure 1). Moreover, fractures receiving local IL-1Ra prevented inflammatory changes in the synovium similar to the contralateral, non-fractured knee (Figure 2A). The observation that local administration of IL-1Ra immediately following fracture prevented arthritic changes suggests a critical role of intra-articular and synovial inflammation in the development of PTA. While the role of anti-cytokine therapy in PTA remains to be firmly established, its role in immunogenic mouse models of arthritis is relatively well-characterized.9-10 Our results corroborate evidence suggesting that intra-articular IL-1Ra provides benefit in humans following acute knee injuries,11 but differ from evidence that local IL-1Ra provides little benefit in patients with chronic OA.12

SIGNIFICANCE: This study may not only provide a novel method of treating acute joint injury to augment surgical stabilization, but also provides evidence for conducting larger clinical trials of anti-cytokine therapy (which are commercially available) for acute joint trauma.


ACKNOWLEDGEMENTS: Steve Johnson for his technical support. Arthritis Foundation Grant 5244; NIH Grants AR50245, AG15768, AR48852, and AR48182.