Cationic Interleukin-1 Receptor Antagonist (catIL-1RA) for Post-Traumatic Osteoarthritis Pain Relief

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA) is driven by inflammatory mediators that contribute to both joint degeneration and pain[1]. Interleukin-1 (IL-1) is one of the potent pro-inflammatory cytokines elevated in synovial fluid within weeks to months after joint injury[2]. Interleukin-1 receptor antagonist (IL-1RA, or Anakinra) competitively blocks IL-1 signaling and is FDA-approved for rheumatoid arthritis. However, in osteoarthritis, its therapeutic impact is hindered by poor joint retention, rapid clearance with an intra-articular (IA) half-life of about 4 h, and limited cartilage penetration, leading to suboptimal therapeutic efficacy targeting chondrocytes[3]. Although blocking IL-1 receptors on chondrocytes and nociceptors has strong potential to reduce pain mediators and modulate pain signaling, clinical trials of Anakinra in OA have shown only transient and inconsistent relief, and its effectiveness in chronic OA pain remains largely unresolved, with limited preclinical investigation[4,5]. To address these challenges, we recently engineered a recombinant cationic fusion protein, catIL-1RA, which combines IL-1RA to a cartilage targeting cationic peptide carrier (CPC) for enhanced cartilage matrix penetration and sustained chondrocyte targeting[6]. This enables catIL-1RA to bind IL-1 receptors on chondrocytes, suppress IL-1-induced pain mediators, and potentially reduce nociceptor activation and peripheral sensitization **(Fig. 1A)**. Here, we evaluated the analgesic efficacy, retention, and downstream effects of catIL-1RA in a PTOA rat model (medial meniscus transection, MMT), compared with saline and Anakinra administration.

METHODS: catIL-1RA was produced as previously described[7]. The IL-1RA domain was fused at its C-terminus to a cationic CPC sequence, R(RRRNN)$_3$RRRR, via a short hybrid linker (EAAAKGGGGS) **(Fig. 1A)**. All surgical and *in vivo* procedures were approved by the IACUC. Sham or medial meniscus transection (MMT) surgeries were performed on the right knees of eighteen 10-week-old male Lewis rats. Hind limb weight bearing was measured twice weekly using an incapacitance meter (IITC) from 1-3 weeks post-surgery. At 3 weeks, rats received an IA injection of 0.17 mg Anakinra, catIL-1RA, or saline into the right knee, and weight bearing was assessed at 4h, 8h, 12h, 1d, 2d, 3d, 4d, 5d, 7d, 10d, and 14d post-administration. Weight Bearing (WB%) was calculated as: [Right Leg Weight Bearing / (Right + Left Leg Weight Bearing)] × 100%. Each data point represents the mean of 12 measurements, with observers blinded to treatment. Serum of all groups was collected until 3 days post-IA administration, and IL-1RA concentrations were quantified by ELISA. Two weeks post-administration, right knees were collected for cartilage and synovium histology, and ipsilateral L4–L5 DRGs were harvested for bulk RNA sequencing. Data are shown as mean ± SEM. Weight-bearing data were analyzed by two-way ANOVA with Tukey's post hoc test; serum IL-1RA levels were compared by unpaired t-test. Significance was set at $p < 0.05$ (* vs. Sham; $ vs. MMT or MMT-saline; # vs. MMT-Anakinra).

RESULTS: During the first three weeks post-surgery, sham rats (N = 5) maintained balanced weight bearing (WB% ~ 50%) while MMT rats (N = 13) stabilized around 45% after 2 weeks **(Fig. 1B-i)**. After IA injection with saline, sham rats **(black dots, Fig. 1B-ii)** maintained baseline ~50% WB% throughout the study. MMT rats were divided into treatment groups receiving saline, Anakinra, or catIL-1RA (N = 4–5 per group). At 4 hours post-injection, Anakinra-treated MMT rats **(blue triangles, Fig. 1B-ii)** transiently returned to sham-like weight bearing, and gradually diminished afterward. This effect maintained a non-significant trend toward improved weight bearing for a week compared to MMT-saline controls. In contrast, catIL-1RA **(purple inverted triangles, Fig. 1B-ii)** treatment produced delayed but sustained pain relief, with significant improvement on days 2–3 and a trend toward reduced pain from 8 hours through 10 days post-injection. Serum IL-1RA levels indicated that catIL-1Ra had a longer joint retention, consistent with its prolonged analgesic effect **(Fig. 1C)**. Bulk RNA-seq analysis showed broad transcriptional changes relative to MMT-saline controls: 519 genes were downregulated and 338 upregulated in the MMT-Anakinra group, while 822 were downregulated and 696 upregulated in the MMT-catIL-1RA group **(Fig. 1D)**. Several pain- and neuroimmune-related genes showed consistent regulation across both treatments. Nerve growth–related genes *Tuba1c* and *Vegfc*, and immune pathway genes *Cxcr4*, *S100a8*, and *S100a9*, which were upregulated in MMT-saline compared to sham, were downregulated with both treatments. IL-1 pathway–associated genes (*Il1rn*, *Il1r2*) were also downregulated in treatment groups. The neuron survival gene *Pik3r1*, suppressed in MMT rats, was restored by both treatments.

DISCUSSION: Our findings indicate that intra-articularly injected Anakinra provides transient pain relief at 4 h post-administration, which diminishes thereafter. In contrast, catIL-1RA, with enhanced joint retention and chondrocyte targeting, produces a delayed but significant and sustained analgesic effect at days 2–3. Both treatments regulated similar pain-related genes, supporting their analgesic activity. Ongoing studies are assessing joint histopathology, including synovial pathology, nerve innervation, and macrophage infiltration to further elucidate treatment mechanisms.

SIGNIFICANCE: Cartilage-penetrating catIL-1Ra and Anakinra modulate IL-1 driven pain mediators, highlighting their potential as intra-articular analgesics for chronic OA.

REFERENCES: [1]Miller+, *Cytokine* 2014; [2]Irie+ *Knee* 2003; [3]Chevalier+ *A&R* 2009; [4]Kraus+ *OAC* 2012; [5]Allen+ *JOR* 2010; [6]Boyer+ *ORS* 2025

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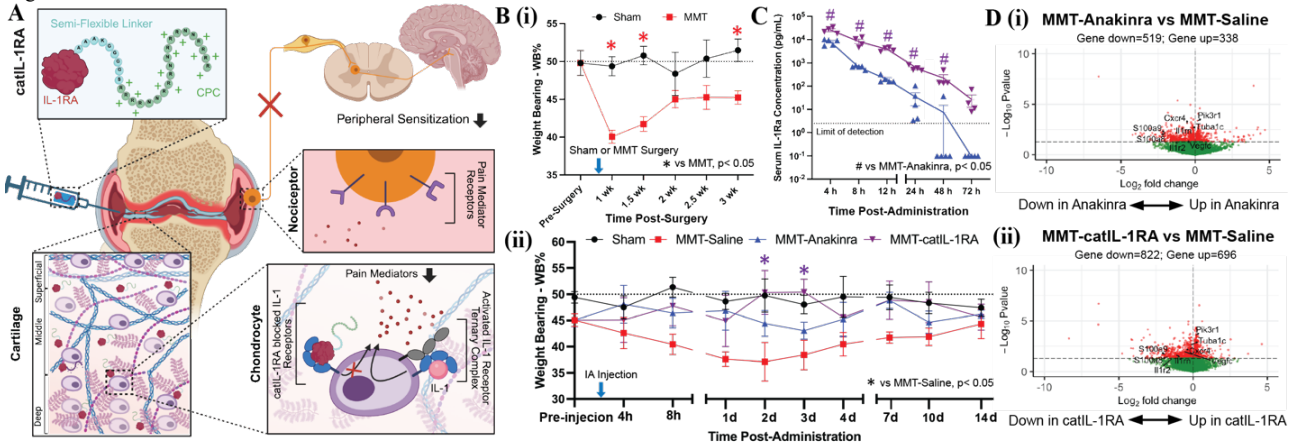

Figure. 1A. Schematic illustration of catIL-1Ra for OA pain relief. catIL-1Ra is composed of IL-1Ra fused to a cationic peptide carrier (CPC) via a semi-flexible linker (top left). After IA injection, it penetrates cartilage by electrostatic interactions with negatively charged glycosaminoglycans (bottom left), binds IL-1 receptors on chondrocytes to suppress IL-1-induced pain mediators (bottom right), and consequently reduces nociceptor activation and peripheral sensitization (top right). **B.** Hind limb weight-bearing performance of **(i)** sham and MMT rats over 3 weeks post-surgery, **(ii)** saline, catIL-1RA, or Anakinra treatment over 2 weeks post-administration. **C.** Serum IL-1RA concentrations measured over 3 days post-administration. **D.** Bulk RNA sequencing of ipsilateral L4-5 DRG at 2 weeks post-administration. Volcano plots show differential gene expression of **(i)** MMT-Anakinra and **(ii)** MMT-catIL-1RA rats compared with MMT-saline (cutoff: False Discovery Rate < 0.05). All data are presented as mean ± SEM. Statistical significance: $ vs. MMT or MMT-saline; # vs. MMT-Anakinra.