

Localized Gene Therapy for Prolonged Anti-Inflammatory Treatment to Prevent or Delay Post-Traumatic Osteoarthritis in an Equine Model

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INTRODUCTION: Osteoarthritis (OA) is characterized by the breakdown of articular cartilage and joint tissues, leading to pain and impaired mobility. Aside from its debilitating physical effects OA also creates a massive healthcare burden, ranking as the 15th highest cause of years lived with disability [1]. Significant disease modification strategies have remained elusive; treatment in most cases is palliative and continues to result in significant side effects [2]. Given the long-term debilitating progression of OA, we sought to investigate the efficacy of an anti-inflammatory scAAV2IL-1ra-based approach to prevent or delay post-traumatic osteoarthritis (PTOA) progression in a highly translational equine model over a 1-year period. We hypothesized that intra-articular administration of scAAVIL-1ra would result in elevated IL-1ra levels for up to a year in an equine model of PTOA.

METHODS: PTOA was established in equine middle carpal joints via surgical induction of an osteochondral chip fragment in sixteen horses (genders mixed) in accordance with Institutional Animal Care and Use Committee protocol #1520. Power analysis for this experimental design was performed using outcome parameters of articular cartilage erosion and synovial cellular hyperplasia. Utilizing an effect size of 0.9 and standard deviation of 0.5, a power of 0.922 could be achieved with an N of 8 animals per group. 14 days after PTOA induction affected joints received either 5x10¹¹ viral genomes (vg) of scAAV2IL-1ra or an equal volume of saline via intra-articular injection. The scAAV2IL-1ra treatment group had N=8 horses while the saline treatment group had N=7, as one horse was removed from the study due to colic post-surgery. Lameness exams were conducted every 2 weeks over the 1-year study period, while synovial fluid aspiration was performed on days 0, 14, 28, 70, 98, 217, 252 and 365. Radiographs of the joints were made pre-surgery as well as days 14, 70, 126, 182, 224, 298, and 365. MRI and gross examination of the joints took place at the end of the study on day 365. A linear mixed effects model was utilized to analyze outcome measures, with fixed effects including treatment/OA status of each joint and day of collection (if applicable) plus all interactions. Random effects included subject and subject*OA when applicable (to capture effects of the joint over repeated measures). An estimated marginal means test with a Tukey adjustment was used to estimate and test comparisons of interest. Significance was established at P≤0.05. Data is displayed as mean +/- standard error.

RESULTS SECTION: Levels of IL-1ra in the synovial fluid were significantly and highly elevated in the scAAV2IL-1ra treated joints at all time points after treatment administration over the 365-day study period (Figure 1). IL-1ra levels in the treated group peaked at day 28 with an average concentration of ~8,600 ng/mL, a roughly 25,000-fold increase as compared to the saline control group. IL-1ra levels continued to remain highly elevated over the entire study period, averaging ~30 ng/mL on day 365, a 90-fold increase compared to the saline control group. Outcome measures revealed that the scAAV2IL-1ra treatment successfully delayed some facets of PTOA. The scAAV2IL-1ra treated group showed a greater improvement in lameness over the study period (Figure 2), lower radiographic OA scoring through the midpoint of the study (Figure 3), and higher GAG content at the end of the study. Some outcome measures at the end of study, including radiographic and MRI scoring and synovium inflammation scoring were similar between treatment groups, indicating the long-term safety of this therapy.

DISCUSSION: The scAAV2IL-1ra treatment successfully elevated synovial fluid IL-1ra levels over the 365 study period, indicating that scAAV-mediated gene delivery is a viable long-term treatment for OA/PTOA. Six of the eight horses treated with scAAV2IL-1ra had elevated levels of IL-1ra on day 365 (50 weeks post-treatment administration), while IL-1ra levels in two of the horses dropped below the limit of detection by day 98. This may reflect inter-patient variability or the presence of pre-existing neutralizing antibodies in some patients, a vital consideration for future gene therapeutics (follow-up analysis in progress). Prior to this study, the translational equine PTOA model had not been utilized for a significantly long time frame, with studies typically ending two to four months post-PTOA induction. Nearly identical scores in many outcome measures between treated and control groups at the endpoint of the study (day 365) may be due to attenuation of PTOA in this model or may suggest that scAAV2IL-1ra alone is not sufficient to prevent PTOA progression long-term. In summary, this study suggests that scAAV-mediate gene delivery is a viable long-term treatment for PTOA, although expression of anabolic factors in conjunction with an anti-inflammatory mediator may be necessary to delay or attenuate damage to articular cartilage.

SIGNIFICANCE/CLINICAL RELEVANCE: Despite palliative treatments OA remains a significant hindrance to those affected, with no long-term disease modifying treatment options available. This study was designed to evaluate both longevity and efficacy of a scAAV2IL-1ra treatment for PTOA, with the ultimate goal of developing a single-administration, disease modifying treatment.

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