A Multi-Vector Approach to Treating Post-Traumatic Osteoarthritis using scAAV2 IL-1ra/IGF-I in an Equine Preclinical Model

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INTRODUCTION: Osteoarthritis (OA) is a prevalent degenerative joint disease estimated to affect over 14% of the adult population in the United States (around 35-40 million people) [1]. Significant disease modification strategies have remained elusive; treatment in most cases is palliative and does little to address pathogenic changes in the joint [2]. Given the multi-faceted nature of OA (an inflammatory, catabolic environment), we sought to investigate the efficacy of a combined scAAV-based gene therapy approach to treating post-traumatic osteoarthritis (PTOA) in a highly translational equine preclinical model utilizing IL-1ra to mitigate inflammation and IGF-I to promote anabolic cartilage repair. We hypothesized that intra-articular administration of scAAVIL-1ra and scAAVIGF-I alone or in combination would result in the production of therapeutic levels of both proteins up to four months in an equine model of PTOA.

METHODS: PTOA was established in equine middle carpal joints via surgical induction of an osteochondral chip fragment in twenty-four horses (genders mixed) in accordance with Institutional Animal Care and Use Committee protocol #1517. 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 $5x10^{11}$ viral genomes (vg) of scAAV2IL-1ra, a combination of $5x10^{11}$ vg scAAV2IL-1ra and scAAV2IGF-I each ($1x10^{12}$ vg total), or an equal volume of saline via intra-articular injection. Each treatment group had N=8 horses, with the exception of the saline treatment group as one horse was removed from the study due to colic post-surgery, giving N=7. Synovial fluid aspiration and lameness exams were conducted every 2 weeks over the 16-week study period, with radiographs made every 50 days and MRI and gross examination of the joints on day 112. 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 $P \leq 0.05$. Data is displayed as mean +/- standard error.

RESULTS: IL-1ra in the synovial fluid of treated joints increased significantly in both the scAAV2IL-1ra and scAAV2IL-1ra/scAAV2IGF-I combination treated groups at all time points after treatment administration (respectively peaking at 300x and 218x increases over the saline control group, Figure 1A). IGF-I in the synovial fluid of scAAV2IL-1ra/scAAV2IGF-I treated joints increased marginally over the study period (peaking at 4x over the saline control group, Figure 1B). The scAAV2IL-1ra treated group demonstrated the least PTOA progression in nearly all outcome measures, including improvement in lameness over the study period, radiographic and MRI scoring (Figure 2) of the joints, and gross synovium inflammation scoring of the joints after necropsy. The scAAV2IL-1ra/scAAV2IGF-I treated group demonstrated greater PTOA progression in all outcome measures as compared to the saline control group.

DISCUSSION: The scAAV2IL-1ra/scAAV2IGF-I treated group demonstrated elevated levels of both therapeutic proteins in the synovial fluid, confirming that use of multiple scAAV vectors can induce expression of two proteins simultaneously in equine joints. However, IL-1ra levels were consistently higher in the scAAV2IL-1ra treated group than the scAAV2IL-1ra/scAAV2IGF-I treated group, indicating that the use of multiple scAAV vectors may decrease induced levels of individual therapeutic proteins. Lower expression levels in the scAAV2IL-1ra/scAAV2IGF-I treated group may also be caused by increased levels of serum neutralizing antibodies due to higher vector dosage [3]. While the scAAV2IL-1ra treatment was able to delay PTOA progression, greater progression was observed in the combination treated group, which could potentially be caused by vector-dose associated inflammation noted in similar studies [4]. It is also likely that the modest increase in IGF-I expression seen in this study was not sufficient to induce anabolic changes in the PTOA-induced joints, given that other *in vivo* studies utilizing rAAV-induced IGF-I expression to treat osteochondral defects have observed pro-anabolic changes [5, 6]. This study was limited by the relatively small sample size, which, may have limited the ability to detect even stronger treatment effects. In summary, simultaneous co-transduction of therapeutic proteins is possible in equine joints, however increased viral dosage due to the use of multiple vectors may inhibit therapeutic efficacy. More efficient viral vectors are needed to decrease viral load, with dual transgene expression from a single vector poised to potentially overcome this limitation.

SIGNIFICANCE/CLINICAL RELEVANCE: Current drug therapies to treat PTOA for both humans and horses usually result in mild to moderate symptom relief and do relatively little to modify disease progression caused by matrix breakdown and collagen degradation [2]. This study was designed to determine the viability of utilizing two scAAV vectors to promote simultaneous expression of an anti-inflammatory cytokine and an anabolic factor in equine joints in order to simultaneously provide anti-inflammatory symptom relief and modify disease progression by attenuating cartilage breakdown.

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