Single intra-articular injection of adeno-associated virus results in stable and controllable in vivo transgene expression in normal rat knees

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SUMMARY

Objective: To test the hypothesis that in vivo transgene expression mediated by single intra-articular injection of adeno-associated virus serotype 2 (AAV2) persists within intra-articular tissues 1 year post-injection and can be externally controlled using an AAV2-based tetracycline-inducible gene regulation system containing the tetracycline response element (TRE) promoter.

Methods: Sprague Dawley rats received intra-articular injections of AAV2-cytomegalovirus (CMV)-enhanced green fluorescent protein (GFP) and AAV2-CMV-luciferase (Luc) into their right and left knees, respectively. Luciferase expression was evaluated over 1 year using bioluminescence imaging. After sacrifice, tissues were analyzed for GFP+ cells by fluorescent microscopy. To study external control of intra-articular AAV-transgene expression, another set of rats was co-injected with AAV2-TRE-Luc and AAV2-CMV-reverse-tetracycline-controlled transactivator (rtTA) into the right knee, and AAV2-CMV-Luc and AAV2-CMV-rtTA into the left knees. Rats received oral doxycycline (Dox), an analog of tetracycline, for 7 days. Luciferase expression was assessed by bioluminescence imaging.

Results: Luciferase expression was localized to the injected joint and persisted throughout the 1-year study period. Abundant GFP+ cells were observed within intra-articular soft tissues. Transgene expression in AAV2-TRE-Luc injected joints was upregulated by oral administration of Dox, and down-regulated following its removal, at 14 days and 13 months post-AAV injection.

Conclusions: This longitudinal in vivo study shows that sustained and stable AAV-mediated intra-articular transgene expression can be achieved through a single intra-articular injection and can be controlled using a tetracycline-controlled inducible AAV system in a normal rat knee model. Highly regulatable long-term intra-articular transgene expression is of potential clinical utility for development of treatment strategies for chronic intra-articular disease processes such as inflammatory and degenerative arthritis.

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Introduction

Prevention of articular cartilage degradation or treatment of its damage in arthritis remains challenging due to the limited self-repair potential of articular cartilage. Currently, no therapeutic methods exist for complete re-establishment of cartilage function. Delivery of therapeutic agents that could promote articular cartilage repair or prevent its further degradation once damaged is an attractive therapeutic option. Protein biologics can be delivered systemically, or locally by direct injection or through polymer based delivery systems. However, due to the short half-life of proteins, administration of supra-physiological doses and/or repeated delivery are often necessary, significantly increasing the cost of these approaches. An attractive alternative is to deliver the genetic information to cells within the joint and engineer them to produce the therapeutic protein in situ.

Naked DNA, retrovirus, adenovirus, and herpes virus-based vectors have been explored for gene transduction in vivo; however, most were rendered suboptimal due to safety, efficacy, and duration issues. Recombinant adeno-associated virus (AAV) derived from an endemic and non-pathogenic parovirus is emerging as a promising delivery vehicle for musculoskeletal tissues, with the advantages of sustained transgene expression, reduced potential for host immune response, and the capacity to transduce both dividing and non-dividing cells in vitro and in vivo. Different serotypes of AAV exist, each having different preferential targets. AAV5 has better transduction efficiency in rodent arthritic joints; however, AAV2 is currently used in human clinical trials for arthritis.