Localization and Doxycycline Control of In Vivo Transgene Expression in Injured Joints

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INTRODUCTION: Sustained intra-articular (IA) delivery of therapeutic agents that can promote cartilage repair is of high potential clinical importance. IA injection of aden-ovector-associated virus (AAV) has been used in clinical trials for delivery of bioactive substances. Methods to localize and control in vivo transgene expression are important to enhance the therapeutic efficiency and safety of IA gene therapy. This has been achieved in intact joints; however, as intact/undamaged articular cartilage is unlikely to require treatment, AAV administration strategies in the setting of joint injury will provide invaluable information to improve gene therapy strategies for cartilage repair. This study tests the hypotheses that 1) in vivo AAV transgene expression localizes to damaged cartilage when AAV is administered post-injury, 2) AAV injection pre-injury leads to the creation of a soft tissue reservoir that persistently expresses transgene products at high levels, and 3) in vivo transgene signal can be externally controlled after joint injury using oral doxycycline.

METHODS: Animal experiments were performed following IACUC-approved protocols. Longitudinal in vivo study was performed using thirty male Sprague-Dawley rats with surgically created unilateral joint injuries of high clinical relevance: osteochondral defect (OCD) and anterior cruciate ligament transection (ACLT). To characterize the localization and persistence of transgene expression, post-injury AAV injection and pre-injury AAV injection time points were investigated. For the post-injury AAV injection group, six rats underwent OCD surgery, and six rats underwent ACLT surgery. OCD rats received a single IA injection of AAV2-CMV-Luciferase (Luc) to both injured and uninjured stifles 1 week post-injury. For the ACLT injury model, AAV2-CMV-Luc was injected after 3 weeks to allow for sufficient time for cartilage damage to occur. In the pre-injury AAV injection group, six rats received a single IA injection of AAV2-CMV-Luc to both stifles and underwent unilateral OCD surgery after 4 weeks. To characterize the controllability of transgene expression, 12 rats received a single injection of AAV2-tetracycline response element (TRE)-Luc to both injured and uninjured stifles 1 week after OCD or 3 weeks after ACLT surgery. Rats injected with AAV2-TRE-Luc were killed after 48 hours to allow for sufficient time for luciferase expression to occur. In the pre-injury AAV injection group, six rats received a single IA injection of AAV2-CMV-Luc to both stifles and underwent unilateral OCD surgery after 4 weeks. To characterize the controllability of transgene expression, 12 rats received a single injection of AAV2-tetracycline response element (TRE)-Luc to both injured and uninjured stifles 1 week after OCD or 3 weeks after ACLT surgery. The luciferase expression remained unchanged from the pre-surgery time points, for both injured and intact joints. No difference in the magnitude of the bioluminescence signal was detected between the joints (Fig. 2B, p > .05). Rats that received the inducible AAV2-TRE-Luc after joint injury showed gene expression upregulation with addition of Dox and downregulation following its removal from the drinking water (Fig. 3).

RESULTS: Longitudinal evaluation of in vivo luciferase expression revealed persistent transgene expression regardless of whether the AAV was injected pre- or post-injury (Figs. 1 and 2). When the joints were opened for post-injury AAV-injected rats (Fig. 1B), luciferase expression was found to be highly localized to the vicinity of the cartilage injury and associated repair tissue, whereas the signal was more diffusely expressed in IA soft tissues for the intact joint. Due to the more localized nature of the transgene expression in the injured joints, the magnitude of the bioluminescence signal was lower for the OCD and ACLT joints compared to the uninjured side (Fig. 1C, p < .001). Given this differential gene expression, we then investigated AAV injection pre-injury to further characterize the effect of injury. For the joints that received AAV before injury creation, luciferase expression remained unchanged from the pre-surgery time points, for both injured and intact joints. No difference in the magnitude of the bioluminescence signal was detected between the joints (Fig. 2B, p > .05). Rats that received the inducible AAV2-TRE-Luc after joint injury showed gene expression upregulation with addition of Dox and downregulation following its removal from the drinking water (Fig. 3).

DISCUSSION: These data show that AAV allows for sustained, stable, and regulated IA release of bioactive factors from injured joint tissues. In intact joints, AAV transduces and is mainly expressed by soft tissues, as shown in a previous study. However, if the AAV is injected after joint injury, as would likely be the case in a clinical setting, the transgene signal localizes to the vicinity of the injured cartilage and associated repair tissue. This highly localized gene expression is advantageous for gene therapy strategies that deliver anabolically growth factors that would aid in cartilage repair, especially genes that need to specifically target repair cells to be effective. The persistence of transgene expression following injury, as seen when AAV is injected prior to injury, provides a potential model to study the prophylactic delivery of protective factors to individuals that may be at high risk for cartilage degeneration. Finally, controllability of transgene expression in injured joints provides an added measure of safety, as well as the ability to alter the timing and duration of exposure to bioactive factors.

SIGNIFICANCE: These data support further study of the in vivo therapeutic potential of AAV for safe, localized, and controlled delivery of bioactive substances to injured joints. This can aid in cartilage repair, which may delay or prevent the onset of debilitating osteoarthritis.