INTRODUCTION: Insulin-like growth factor-I (IGF-I) has been shown to be important for normal articular chondrocyte function. For example, treatment of cultured chondrocytes with recombinant insulin-like growth factor-I (IGF-I) increases matrix production and enhances chondrocyte proliferation and survival. While normally, the ability of cartilage tissues to heal is limited, we have previously shown that combining IGF-I with chondrocyte transplants in experimentally-induced full-thickness cartilage defects results in morphological and biochemical improvement in the repair tissue. However, IGF-I residence time in defects is transient and better repair may follow extended IGF-I persistence in the lesion. Gene therapy offers the prospect of extending the time IGF-I is released at the site of cartilage injury. Previously we have also shown that articular tissues can be successfully transduced in vitro using an adenoviral construct with the genetic sequence for IGF-I (AdIGF-I). Preliminary short-term in vivo analyses suggest that transplanted AdIGF-I modified chondrocytes improve healing of articular cartilage defects. The purpose of this study was to investigate the molecular, biochemical and biomechanical effects of AdIGF-I genetically modified chondrocytes on the repair and surrounding tissue compared to defects implanted with naïve chondrocytes.

MATERIALS AND METHODS: Sixteen horses underwent arthroscopic repair of a single 15 mm cartilage defect in each femoropatellar joint under an IACUC approved protocol. One joint was repaired with AdIGF-I-transduced chondrocytes (20 x 10⁶) and the opposite limb was implanted with the same number of control (uninfected) chondrocytes. Each horse therefore acted as its own control. Horses were sacrificed at the following time periods after surgery: 4 horses at four weeks, 4 horses at 9 weeks, and 8 horses at 8 months. At necropsy, the gross appearance of repair tissue was evaluated with the investigators unaware of treatment group. A composite healing score was based on color and surface properties, proportion of the defect filled, integration with surrounding normal cartilage, and integration with subchondral bone and height of repair tissue relative to the surrounding cartilage. The central one-third rectangular block of the repair tissue plus 5 mm of surrounding intact cartilage and underlying subchondral bone was fixed in 4% paraformaldehyde for histology. The remaining repair tissue was removed for RNA and DNA isolation. Histological, immunohistochemical and in situ hybridization were performed along with biochemical analysis for collagen Type II and proteoglycan content. Furthermore, Taqman PCR was performed to measure mRNA content of repair tissue to compare IGF-I, collagen type II and collagen type I. Biomechanical data was available for the horses necropsied at 8 months. 5 mm diameter discs were harvested from tissue within the defect (“lesion”), immediately adjacent to the defect (“peri-leision”), and at a distance from the repair site (“remote”). Dynamic and aggregate moduli were determined by confined compression tests. Proteoglycan (PG) content was measured by dimethyl-methylene blue (DMMB) dye binding assay. Histologic, immunohistochemical and in situ hybridization studies were performed, along with biochemical analysis of type II collagen. A paired t test was used to compare parametric data and a Wilcoxon Signed Rank was used to compare all nonparametric data.

RESULTS: Composite scores for defects that received AdIGF-treated chondrocytes were improved over defects that received naïve chondrocytes in the short-term (4 and 9 weeks) and long-term analysis (8 months, Figure 1). Type II collagen content was also increased in the AdIGF-treated group (Figure 2A) over the naïve-treated defects (Figure 2B) on immunohistochemical analysis as well as by biochemical analysis (cyanogen bromide cleavage, Figure 3). When analyzed by Taqman PCR analysis Collagen Type II expression and IGF-I expression in the AdIGF-treated group was elevated over control (p<0.05) at 4 weeks but no difference was detected between treated and controls at 9 weeks and 8 months (Figure 4 and 5). Proteoglycan content was tested only at 8 months. At this timepoint, no difference in PG content was observed between treatment groups as assessed by dimethyl-methylene blue dye binding assay. The biomechanical properties of the treated and control tissues did not differ between treatment groups. However, an interesting finding was that both PG content and mechanical properties improved with increasing distance away from the lesion. The dynamic modulus of the lesion tissue was 50% of the remote cartilage, and the peri-leision tissue was 80% of the remote tissue. The aggregate modulus of the lesion tissue was 12% of remote, while that of peri-leision tissue was 50% (Figure 6).

DISCUSSION: The present study evaluated the short and long-term effects of AdIGF-I-transduction of chondrocytes prior to implantation. Treatment of chondrocytes with AdIGF-I increased IGF-I expression in the repaired defect for 4 weeks after repair. This increase correlated with an increase in Collagen Type II expression as well as an increase in the percentage of Collagen Type II for up to 8 months. These findings suggest that chondrocyte-based gene transfer may be a promising strategy of improving cartilage repair. While the biomechanical studies showed no difference in the treated versus control defects at 8 months, an interesting observation was made regarding the peri-leision tissue directly adjacent to the repaired defect. The mechanical and biochemical properties of the perileision tissue appeared to be significantly and negatively affected by creating a defect. This finding suggests that this type of quantitative analysis of the peri-leision tissue may provide a good functional measure of the success of future strategies to improve cartilage defect repair.


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