INTRODUCTION

Although intervertebral disc degeneration is a leading cause of spinal disorders, few approaches are available for its prevention and treatment, mandating investigation of novel approaches such as gene therapy. Intervertebral disc degeneration is characterized in part by changes in nucleus pulposus resulting in loss of proteoglycans and water content. Thompson and colleagues showed that addition of TGF-β1 to canine disc tissue in culture could stimulate in-vitro proteoglycan synthesis (1). However, sustained delivery of growth factors to an intervertebral disc in-vivo would be difficult to achieve clinically with present technology. An alternate possibility is to genetically modify the disc cells such that the cells express the desired growth factors endogenously. The objective of the current study was to determine the feasibility of direct, adenovirus-mediated transfer of a therapeutic gene to rabbit nucleus pulposus cells in-vivo. We hypothesized that direct adenovirus-mediated gene transfer can deliver the human TGF-β1 encoding gene to rabbit nucleus pulposus cells in-vivo, resulting in increased TGF-β1 production and increased proteoglycan synthesis.

METHODS

We used an adenovirus construct (Ad/CMV-hTGFβ1) containing the human TGF-β1 encoding gene. The anterior aspects of the L2-3, L3-4, and L4-5 intervertebral discs of 5 rabbits were exposed using a retroperitoneal approach under general anesthesia. In 3 rabbits (n = 9 discs), 20 μl of saline containing Ad/CMV-hTGFβ1 (6x10⁶ PFU) were injected into the nucleus pulposus. In the fourth and fifth rabbits (n = 3 discs each), 20 μl of saline with or without Ad/CMV-luciferase (6x10⁶ PFU) were injected, respectively. For each rabbit, the L1-2 intervertebral disc was used as the intact control. One week after injection, the rabbits were sacrificed and nucleus pulposus tissue was harvested and cultured in Neuman-Tytell serumless medium for 48 hours, after which the medium was extracted for ELISA to detect TGF-β1 production. New medium containing 35S-sulfate (10 μCi/ml) was added to the cultures, and 48 hours later, the medium together with nucleus pulposus tissue was harvested and combined with an equal volume of solution containing 8M guanidine hydrochloride (GuHCl), 20 mM EDTA, and a mixture of proteinase inhibitors, and was incubated at 60°C for 48 hours. For quantitative evaluation of the 35S-labeled proteoglycans, aliquots of the extracts were eluted on Sephadex G-25M PD-10 columns, and the radioactivity of the newly synthesized proteoglycans was measured by a scintillation counter. All of the data were normalized by wet tissue weight, and statistical analysis was performed (one-way ANOVA with Fisher’s Protected LSD post-hoc test). Two discs from the Ad/CMV-hTGFβ1 group were excluded from the data analysis because of technical problems during surgery.

RESULTS

The disc tissue from the rabbits injected with the hTGF-β1/adenovirus construct exhibited significantly elevated production of active TGF-β1 (p<0.05) compared to the intact control discs and discs injected with luciferase/adenovirus construct and saline only solution (Figure 1). Notably, the observed increase in TGF-β1 production was achieved without procedures to activate latent TGF-β. The discs of the Ad/CMV-hTGFβ1 group also exhibited significantly increased proteoglycan synthesis (p<0.05) compared to the other discs (Figure 1).

DISCUSSION

The current study has demonstrated that human TGF-β1 encoding genes can be transferred to rabbit nucleus pulposus cells in-vivo, resulting in increased production of active TGF-β1 as well as increased proteoglycan synthesis. Previously, we reported successful transfer of the lacZ marker gene to rabbit nucleus pulposus cells in-vivo, with long-term in-vivo transgene expression (up to 24 weeks) using the direct, adenovirus-mediated gene transfer technique (2). This supports the possibility that long term gene expression might be achievable with the Ad/CMV-hTGFβ1 construct as well. The ability to genetically modify the intervertebral disc and hence influence its internal biological environment raises the possibility that gene transfer might also enhance disc mechanical properties. Adenovirus-mediated gene transfer therefore appears to have excellent potential for clinical applications such as the prevention and treatment of degenerative disc disease.