**GENE THERAPY WITH LMP-1 CAUSES UPRGULATION OF BMP-2, BMP-7, AND AGGREGAN MESSENGER-RNA IN RABBIT INTERVERTEBRAL DISCS IN VIVO**

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**INTRODUCTION:**
Intervertebral disc degeneration is associated with the loss of disc nucleus proteoglycan content and a reduction in the rate of newly synthesized proteoglycans. (1,2) Aggrecan is a high molecular weight proteoglycan that plays a critical role in disc function by increasing nucleus pulposus hydration. A decrease in aggrecan mRNA level has been noted in the nucleus pulposus of degenerated discs. (2) Thus a potential method of preventing or reversing disc degeneration is to increase proteoglycan synthesis by disc cells by means of an anabolic molecule. In vitro studies have shown that both BMP-2 and BMP-7 can stimulate sulfated-proteoglycan synthesis, especially aggrecan. (3,4) In recent in vitro studies, LIM Mineralization Protein-1 (LMP-1) has been shown to stimulate both BMP-2 and BMP-7 from disc cells. (5) LMP-1 is a highly conserved intracellular regulatory protein that is important in bone formation. Recently, evidence has been increasing that LMP-1 plays an important role in cartilage matrix anabolism. Overexpressing LMP-1 in disc cells in vitro with an adenovirus carrying the human LMP-1 cDNA increases proteoglycan synthesis through a BMP-2 and BMP-7 mediated mechanism. (5) These in vitro results led us to ask whether overexpressing LMP-1 in vivo can stimulate the synthesis of the regulatory proteins BMP-2 and BMP-7 and the major proteoglycan aggrecan. This is particularly important since there is very little data on the effect of stimulatory molecules in the disc in vivo and since this type of information is a critical step prior to clinical investigation.

**METHODS:**
Experiment 1: In this preliminary experiment, four New Zealand White rabbits were used. The anterior lumbar discs L2/3, L3/4, L4/5, and L5/6 were exposed through a left retroperitoneal approach. Replication deficient type 5 adenovirus with the CMV promoter driving either the marker (control) or experimental gene was used. (5) The control adenovirus carried the GFP marker gene (AdGFP). The experiment adenovirus carried the human LMP-1 gene (AdLMP-1). Either the AdGFP or AdLMP-1 virus at 10^7 plaque-forming units (pfu) was injected into each of the exposed discs in alternating fashion between AdGFP or AdLMP-1. The adenovirus was delivered in 10 microliters of phosphate buffered saline through a 30G Hamilton syringe. After 3 weeks, the nucleus pulposus tissues from the injected lumbar discs were harvested. Disc tissues from two rabbits were pooled into control and experimental groups to obtain sufficient mRNA for further analysis. Reverse transcription and real-time PCR were used to quantify the mRNA levels of total LMP-1 (rabbit and human), BMP-2, BMP-7, and aggrecan. All data are expressed as percent increase over the control (AdGFP group).

Experiment 2: In this experiment, different doses of the AdLMP-1 virus were tested in an attempt to establish a dose response relationship. AdLMP-1 at three different doses (10^6, 10^7, 10^8 pfu) and AdGFP at a single dose (10^7 pfu) (control) were tested. In this experiment, all the discs in one animal were injected with a single dose of virus. Two rabbits were used for each viral dose resulting in a total of eight rabbits. One of the AdGFP injected rabbits died after surgery from unknown causes. The surviving rabbits were then euthanized three weeks later and the mRNA from the discs was harvested. Treated discs from within each rabbit were pooled. Reverse transcription and real-time PCR were used to quantify the mRNA levels of total LMP-1, overexpressed LMP-1 (human), BMP-2, BMP-7, and aggrecan. All data are expressed as percent increase over the control (AdGFP group).

**DISCUSSION:**
The results show that overexpression of human LMP-1 by in vivo gene therapy with an adenoviral vector is capable of upregulating BMP-2, BMP-7, and aggrecan mRNA. These findings confirm the predictions of our previous short term monolayer culture experiments and represent a major step towards long term in vivo experiments to alter the course of disc degeneration. This is the first report of endogenous expression of LMP-1 in cartilaginous tissue. This suggests a physiologic role of LMP-1 in the nucleus pulposus as a regulator of BMPs that in turn control matrix synthesis by disc cells. The findings in this study are significant steps in the search for a method of increasing proteoglycan synthesis in the intervertebral disc in vivo.

**REFERENCES:**

**RESULTS:**
Experiment 1 demonstrated a measurable level of LMP-1 mRNA in the AdGFP injected discs (control) which indicated the presence of endogenous rabbit LMP-1 mRNA in nucleus pulposus tissue. This was used to calculate the increase in total LMP-1 mRNA in the AdLMP-1 injected discs. The control discs also expressed endogenous level of BMP-7 and aggrecan mRNA. Discs injected with AdLMP-1 expressed 830% higher levels of total LMP-1 mRNA than the discs injected with AdGFP. BMP-7 mRNA level was increased by 1100% over control. Aggrecan mRNA level was increased by 66% over control.

Experiment 2 demonstrated endogenous levels of BMP-2, BMP-7, LMP-1, and aggrecan mRNA. A correlation between increasing AdLMP-1 dose and total LMP-1 mRNA was seen (Figure 1). Overexpressed LMP-1 (human) mRNA was expressed in a similar pattern to the data seen in figure 1, and no expression was seen in the control group. The BMP-2 and BMP-7 mRNA levels were increased maximally at a dose of 10^7 pfu per disc of AdLMP-1 (Figure 2). AdLMP-1 at a dose of 10^6 pfu per disc led to the highest increase in aggrecan mRNA, 50% over control (Figure 3).

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