GROWTH FACTOR TREATMENT IN DEGENERATED INTERVERTEBRAL DISCS

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Introduction

New approaches to treating disc degeneration seek to restore healthy structure and function to the disc by replenishing the appropriate cell and matrix contents. Growth factors have a promising role in this approach for their capacities to regulate cell division and function. Recent studies have reported increases in cell proliferation and matrix synthesis when normal discs were exposed to various in vitro or in vivo growth factors [1,2]. However, the response of cells within degenerated discs remains untested. The purpose of this study is to evaluate the response in vivo of degenerated intervertebral discs to a single injection of growth factor. Our hypothesis is that direct injection of each of the factors chosen will result in chondrocyte migration, differentiation and/or proliferation.

Methods

A previously reported model of intervertebral disc degeneration in the caudal discs of mice was used (approved by the university Committee on Animal Research) [3]. The model uses static compression applied to the 10th caudal disc in the tail to induce degeneration. Loads were applied by first inserting perpendicular pins through adjacent vertebral bodies. Calibrated, elastic loops were placed around opposing pins to apply 1.3 MPa compressive stress to the disc. After one week of loading, the elastics were removed and the mice were allowed unrestricted activity for three weeks. This procedure reproducibly results in a degenerated phenotype.

The degenerated disc of each mouse was injected with one of the following growth factor solutions: 8 mg/ml bFGF (n=3), 3.5 mg/ml GDF-5 (n=5), 1 µg/ml IGF-1 (n=2), 100 ng/ml TGF-β (n=2). A fifth group of mice with degenerated discs was used as uninjected controls (n=3). Injections were made using a 33-gauge needle inserted directly into the nucleus pulposus. However, for each solution used, 8 µl was injected over a period of 10 minutes to allow time for the fluid to diffuse into the tissue. Preliminary experiments using FITC-labeled dextran (Molecular Probes) in solution verified that the injection protocol successfully delivers solutions to the center of the disc.

One week after injection, the mice were sacrificed and the entire motion segment containing the treated disc was dissected. The tissue was fixed, decalcified, and paraffin embedded. Sections were stained by the BHQ method for analysis of morphology. Immunostaining for proliferating cell nuclear antigen (PCNA) and hematoxylin counterstaining were performed to measure the fraction of proliferating cells and the cell density in the annulus. Immunostaining for proliferating cell nuclear antigen (PCNA) and hematoxylin counterstaining were performed to measure the fraction of proliferating cells and the cell density in the annulus. Simple PCI software (Compix, Inc.) was used for image analysis and cell quantification. In situ hybridization was done to identify cells expressing aggrecan and Type II collagen mRNA.

Results

Figure 1 shows the morphology for a portion of the nucleus and annulus from a normal mouse disc. (Dark lines in the images are folds in the tissue and should be ignored.) Static compression of the disc leads to decreased cell numbers in the nucleus and annulus and disorganization of the nuclear and annular matrix (Figure 2). No changes to the cellularity or morphology of the nucleus were observed in any of the growth factor treated discs. However, for each factor tested, clusters of cells were observed in portions of the annulus. Figures 3 and 4 show multiple cells in clusters throughout the inner and middle annulus in GDF-5 and IGF-1 treated discs, respectively. Similar results were observed for TGF-β and bFGF treated discs. These cells are chondrocyte-like in appearance and express both aggrecan and Type II collagen mRNA.

The mean annular cell density (cells/mm²) was 1090 ± 470 for uninjected controls, 1490 ± 650 for GDF-5 treated discs, 1680 ± 560 for bFGF treated discs, 2170 ± 700 for IGF-1 treated discs, and 1870 ± 670 for TGF-β treated discs. A statistically significant difference (P<0.05) was noted only for IGF-1 treatment versus control following a one-way ANOVA and Tukey test for pair wise comparisons. Differences between other groups were not statistically significant. The mean ratio of PCNA-positive cells to total cells in the annulus was 59 ± 14% for control, 77 ± 10% for GDF-5 treated discs, 63 ± 23% for bFGF treated discs, 57 ±16% for IGF-1 treated discs, and 67 ± 16% for TGF-β treated discs. One-way ANOVA indicates that the differences are not statistically significant (P=0.10).

Discussion

This study demonstrates that annular cells in degenerated intervertebral discs are responsive to growth factors in vivo. Compared with controls, we observed an increase in isogenic groups of chondrocytes in the inner annulus of growth factor treated discs. Measures of annular cell density suggest a trend of higher cell densities in the treated groups although only the IGF-1 group was significantly different from the control. The percentage of proliferating cells in the annulus was higher in GDF-5, bFGF, and TGF-β treated discs compared to control. The differences were not significant, however, due to high variability between animals.

The growth factors chosen for this study have been shown to increase chondrogenesis and matrix synthesis in cartilaginous tissues. Our objective in using them for disc repair is to provide a stimulus for chondrocyte migration and proliferation and proteoglycan synthesis in the nucleus pulposus and inner annulus of degenerated discs. Following this we hypothesize a rise in the nuclear swelling pressure and restoration of disc structure and function.

The effects observed in this study were limited to cell populations in the annulus. To effect changes to the nucleus, longer timepoints or sustained delivery of growth factors may be required. A key benefit of studying the effects of growth factors in our mouse model of degeneration is that it allows us to screen a wide range of potential agents in an environment that mimics human degeneration. We intend to use the model to identify agents of potential benefit to humans and to define a beneficial outcome from growth factor treatment.

References


Acknowledgments

BioSTAR Project; NuVasive, Inc.; Biopharm GmbH, Orquest, Inc.; Jennie Chin; Ellen Liebenburg.