DISTINCT RESPONSES OF ANNULUS FIBROSIS AND NUCLEUS PULPOSUS CELLS TO GROWTH FACTORS

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Introduction
Intervertebral disc degeneration and associated disorders are a leading source of morbidity. Some evidence indicates that disc degeneration can begin in the nucleous pulposus, with a progressive decrease in proteoglycan content leading to dehydration of the nucleous pulposus and loss of biomechanical function. One possible approach to the management of disc disease would be to introduce therapeutic amounts of growth factors into the disc. Growth factors tested in animal models are widely reported to enhance tissue repair and factors such as TGF-β1 and IGF-I strongly stimulate matrix synthesis. In addition, TGF-β1 regulates expression of many components of the IGF growth factor system. These data have lead to attempts to combine multiple growth factors in an effort to maximize matrix synthesis by disc cells.

Methods
Intervertebral (IV) disk cells were isolated from the IA, OA, and NP of 5 adult rabbit discs (L1-L6) by enzymatic digestion (1600 u/ml hyaluronidase, 0.5 mg/ml collagenase/pronase E). The cells were suspended in alginate beads at a concentration of 1 x 10^5 cells/ml. Single beads (~25,000 cells/ bead) were cultured in 96-, or 48-well dishes in DMEM with 10% calf serum and were treated daily with recombinant human IGF-I at 10 ng/ml, or recombinant human TGF-β1 at 2 ng/ml (R&D Systems) or a combination of the two. 20 uCi/ml[^3]H-proline was used to measure collagen synthesis and 50 uCi/ml[^35]S04 was used to measure proteoglycan synthesis. Radiolabels were added on day 7 and the cultures incubated overnight before harvest. Medium and alginate bead were extracted in 4 M guanidine and the extracts fractionated on G-50 columns. Total incorporated[^3]H-proline and[^35]S04 (medium + alginate bead) was determined in a dual channel liquid scintillation counter. The results were analyzed by two way analysis of variance (ANOVA) using the Tukey test for multiple comparisons. Means and standard deviations represent 16-18 cultures for each treatment group.

Results
Radiolabel incorporation data are shown in the Figure.[^3]H-Proline incorporation (A) in NP cells was significantly lower than for OA or IA cells (p<.05). NP cells did not respond significantly to either TGF-β1 or IGF-I. IGF-I also failed to stimulate OA or IA cells, however, TGF-β1 significantly suppressed incorporation below control levels in OA and IA cells both in the presence and absence of IGF-I (p<.05). NP cells were also significantly less active than OA and IA cells in terms of[^35]S04 incorporation (p<.05). However, in contrast to the[^3]H-proline results,[^35]S04 incorporation for all three cell types was responsive to both IGF-I and TGF-β1 (p>.05) and the combination of the two factors stimulated the highest[^35]S04 incorporation levels.

Conclusions
Our results show that matrix synthesis activity depended on the cell source as well as the growth factor treatment and the method used to measure matrix synthesis. Matrix synthesis activities were significantly lower for NP cells than for OA and IA cells. Moreover, NP cells were insensitive to the suppressive effects of TGF-β1 on[^3]H-proline incorporation that characterized OA and IA cells. In contrast, the effects of TGF-β1 on[^35]S04 incorporation were primarily stimulatory and combining TGF-β1 with IGF-I stimulated synthesis to higher levels than did either factor alone. These mixed findings suggest that combined growth factor therapy for IV disc degeneration should be approached with caution since treatments that benefit one sub-population of disc cells may be harmful to others.

References

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