Effect of Link-N on Disc Repair in a Rabbit Model of Intervertebral Disc Degeneration

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INTRODUCTION

Intervertebral disc (IVD) degeneration is associated with proteolytic degradation of proteoglycan aggregates present within the extracellular matrix of the disc. Link-N peptide is the N-terminal peptide of link protein, which stabilizes the proteoglycan aggregates. It is generated in vivo by proteolytic degradation during tissue turnover. We have previously shown that this peptide can stimulate the synthesis of proteoglycans and collagens by IVD cells in vitro. However, to date, there have been no reports on the effect of Link-N on the IVD in vivo. The purpose of the present study was to determine the effect of intradiscally administration of Link-N peptide on disc cell survival and extracellular matrix synthesis using a rabbit annular needle puncture model of IVD degeneration.

MATERIALS AND METHODS

Twelve New Zealand white rabbits (~ 3.5 kg; 5-6 months old) received an annular puncture with an 18-gauge needle on 2 non-contiguous discs (L2-L3 and L4-L5). The disc (L3-L4) between the punctured discs and that above (L5-L6) was left intact as internal controls. Two weeks after the initial puncture, the anterior surfaces of the previously punctured discs (L2-L3 and L4-L5) were injected with either saline (10µl/disc) or Link-N (100µg in 10µl saline/disc) into the center of the NP. Disc height was radiographically monitored biweekly. After 12 weeks post-injection, all the rabbits were euthanized and the IVDs from both experimental groups were removed from each lumbar spine for biochemical analysis. The nucleus pulposus (NP) was separated from the annulus fibrosus (AF), the specimens weighed (wet weight), the content of DNA measured using PicoGreen, and the total contents of sulfated glycosaminoglycans (GAG) measured by the 1,9-dimethylmethylene blue (DMMB) assay.

RESULTS

Following needle puncture that initiates disc degeneration, the disc height index (DHI) decreased by about 25%. By 6 weeks after Link-N injection, the mean percent DHI of injected discs in the Link-N group was higher than in the saline group. This difference in mean percent DHI was maintained during the rest of the follow-up. Puncturing the IVD also led to a decrease in proteoglycan content in both the NP and the AF after 12 weeks in saline-treated discs. Treatment with Link-N stimulated proteoglycan synthesis (GAG) in both the NP and AF by about 20%. Link-N did not cause an increase in the DNA content of the discs.

DISCUSSION AND CONCLUSION

Previous studies have shown that the Link-N can act as a growth factor and stimulate the synthesis of proteoglycans and collagens in human articular cartilage as well as bovine IVD in vitro. Here, we show that Link-N can stimulate proteoglycan production in vivo when administered to degenerate disc. This stimulation occurs in both the NP and AF of the disc and in the absence of any effect on cell division. The changes observed with Link-N on proteoglycan synthesis are similar to those reported after injection of osteogenic protein-1 (OP-1). Thus, Link-N appears to be equally effective at stimulating repair of the IVD in vivo. One major advantage of Link-N over OP-1 for therapeutic use is the large saving in cost, Link-N being about 400 times cheaper than OP-1.