The Effects Of Link N And Mesenchymal Stem Cells On Intervertebral Disc Regeneration

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Disclosures:

Introduction: Link N is a naturally occurring peptide that can stimulate regeneration of the degenerate intervertebral disc (IVD).1,2 IVD repair can also potentially be enhanced by mesenchymal stem cell (MSC) supplementation to maximize extracellular matrix production.3,4 In a previous study we have shown that Link N can inhibit osteogenesis and increase chondrogenesis of MSCs in vitro.5,6 The aim of the present study is to determine the effects of injecting MSCs and Link N alone or together in an attempt to repair the degenerated discs.

Methods: The largest first 4 caudal discs with intact cartilaginous endplates were isolated from the tails of 24- to 30-month-old steers. After a 3 days preconditioning period, the discs were injected with 100 μg trypsin in 75 μL PBS, in order to induce degeneration. They were then cultured for another 4 days, followed by the injection of MSCs (105 cells), Link N (75 μg), or a combination of MSCs and Link N, in a final volume of 75 μL PBS (seven discs per group). Seven of the trypsin-treated degenerate discs were injected with PBS alone to serve as a degeneration control. Seven discs were cultured without any injection to serve as non-degenerate control group. After the discs were cultured for another 14 days, a 750 μm section was taken through the center of the discs using an in-house designed cutting tool. The section was fixed with formalin free fixative Accustain and was stained with safranin O and fast green. The extracellular matrix (ECM) proteins and proteoglycans were extracted from the nucleus pulposus (NP) of the discs by guanidinium chloride. Sulfated glycosaminoglycans (GAGs) were analyzed in tissue extracts (n = 7/group) by the dimethyl methylene blue (DMMB) dye-binding assay. The proteoglycan composition was also analyzed by agarose gel and stained with toluidine blue. The expression of aggrecan and type II collagen was analyzed by western blot. To track the MSCs after injection, MSCs were labeled with PKH67 and observed under confocal microscopy when the discs were dissected.

Results: After injection, MSCs were distributed throughout the discs and retained their morphology. The final GAG concentration in the discs with induced degeneration decreased significantly compared to non-degenerate control discs. When the trypsin degenerated discs were injected with Link N and MSCs alone or together, the GAG content increased significantly, and reached a similar level as the non-degenerate control discs (Figure 1). The expression of aggrecan, type II collagen, and total proteoglycan also increased when the degenerated discs were injected with MSCs and Link N, either alone or together. A strong safranin O staining was observed in NP sections of degenerated discs after treatment with Link N and/or MSCs similar to the staining found in the non-degenerate control discs (Figure 2).

Discussion: MSCs and Link N can both increase GAG production in degenerated discs, with Link N showing a greater effect than MSCs when administered separately. An additive effect was found when MSCs and Link N were administered together. However, there was no statistically significant difference among the different treatments. The injection of MSCs and Link N can also increase the expression of aggrecan, type II collagen and proteoglycan in the NP of degenerated discs. Thus, the administration of MSCs and Link N has therapeutic potential in intervertebral disc repair.

Significance: Degenerative disc disease begins in the central nucleus pulposus (NP) region and has been implicated as a major component of spine pathology. Currently, the two major clinical procedures for treating disc degeneration are disc excision and spinal fusion. Although these procedures offer relatively good short-term clinical results in relief of pain, in many instances they have been disappointing because of altered spinal mechanics leading to subsequent degeneration at adjacent disc levels. Biological repair of the degenerate disc would be the ideal treatment, and the current data suggest that mesenchymal stem cell (MSC) and Link N supplementation may aid the repair or at least retard the degeneration of the NP.

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**Figure 1.** Ga3 concentrations in discs with induced degeneration (DE), discs treated with Link N, MSCs, Link N and MSCs, and non-degenerate control discs (No treatment). The results are represented as mean ± SD of seven discs (*p<0.05*).

**Figure 2.** Safranin O and fast green staining of the nucleus pulposus from discs with different treatments. Discs with induced degeneration (A-F) were cultured for 14 days following injection with: A. Link N; B. MSCs; C. A combination of Link N and MSCs; D and E FBS; F. non-degenerate control. The scale bar means 50 µm.

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