Short Link N stimulates intervertebral disc repair in a novel long-term organ culture model that includes the bony vertebrae

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INTRODUCTION: Degenerative disc disease remains poorly understood, yet it is a disorder that is very common and can lead to a dysfunctional spine. Continued catabolism of the disc matrix, accompanied by impaired synthesis of aggrecan and collagen leads to disc degeneration. Although the intervertebral disc (IVD) has limited endogenous repair activity, induced repair may be possible by the intradiscal injection of growth factors to stimulate the production of matrix molecules. Link N (DHLSDNYTLDHDRAIH) is a naturally occurring bioactive 16 amino acid peptide released by proteolysis from its parent protein, link protein. Many studies using disc cells or a rabbit model of disc degeneration have shown that it has proteoglycan and collagen anabolic effect. In a recent publication, we found that cells from the AF can release an enzyme that can cleave Link N generating a one-eight bioactive amino acid peptide termed short Link N (sLink N). Separately, we developed a novel organ culture model which possesses vertebrae and can be kept in culture alive for more than 6 months. The aim of this study was to evaluate the effect of sLink N on matrix restoration in this model.

METHODS: Preparation of vIVDs: Tails of 22- to 28-month-old steers were obtained from the local abattoir within 4 hours of slaughter and isolated using the PrimeGrowth Media kit (developed by Intervertech and licensed to Wisent Bioproducts) as previously described. Briefly, the largest 4 IVDs were prepared for organ culture by parallel cuts through the adjacent vertebral bodies at 1 cm from the endplates using an IsoMet®1000 precision sectioning saw (Buehler, Germany). After extraction the vIVDs were incubated for 1 h in PrimeGrowth Isolation Medium (Cat# 319-511-EL) followed by three times wash in PrimeGrowth Neutralization Medium (Cat# 319-512-CL). After the isolation step, the vIVDs were cultured for seven days in PrimeGrowth Culture Medium (Cat# 319-510-CL) with medium replaced every three days.

Induced degeneration and treatment: After 7 days of preconditioning in culture, degeneration was induced in IVDs by a single injection of 50 μg trypsin into the nucleus pulposus (NP). Seven days after induced-degeneration, the trypsin-treated discs were injected with sLink N (100 μg/disc, n=6 discs/group). Four of the trypsin-treated degenerate discs were injected with PBS alone to serve as a control for degeneration while four discs served as non-degeneration controls. At 4 weeks post treatment vIVDs were processed for biochemical analyses. Proteoglycan (predominantly aggrecan) synthesis in the NP was monitored as sulfated glycosaminoglycan using the 1,9-dimethylmethylene blue dye-binding assay, and Western blotting was performed to determine the expression of aggrecan and type II collagen in the tissue.

RESULTS: After 4 weeks of culture, the proteoglycan content measured as glycosaminoglycans (GAGs) significantly increased compared to the degeneration control when degenerate discs were treated with sLink N (Figure 1). Histological analysis revealed that the newly synthesized proteoglycan was able to restore tissue content even in areas remote from the cells. The quantity of extractable type II collagen and aggrecan was also increased when the degenerate discs were treated with sLink N.

DISCUSSION: We have shown for the first time that sLink N can restore IVD proteoglycan content in a novel organ culture model. The level of increased proteoglycan content in the degenerate disc after injection of sLink N was similar to that observed with Link N supplementation which suggests that although AF cells can cleave Link N, the peptide generated is bioactive. Treatment with sLink N can also enhance the production of collagen, which is another important molecule in the disc. Furthermore, we show that the culture of intact motion segments for long periods of time developed recently by our group can be used for testing sLink N and possibly other therapeutics in a well-controlled environment.

SIGNIFICANCE: These results support the concept that biological repair of disc degeneration is feasible, and that the administration of sLink N has therapeutic potential in early stages of the disease.

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Figure 1. Proteoglycan intensity and content in vIVDs. A, Safranin O staining of control, degenerative (DG), and sLink (DG+sLN) treated vIVDs (scale bar, 100 μm). B, GAG content was measured in the NP of control, DG, and DG+sLN treated vIVDs. The results are represented as mean ± SD of six discs from different bovine tails (** p ≤ 0.01).


