A SYNTHETIC PEPTIDE OF LINK PROTEIN STIMULATES SYNTHESIS OF COLLAGENS II AND IX, AND PROTEOGLYCANS IN INTERVERTEBRAL DISC CELLS

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Introduction: Disc degeneration has been implicated as a major etiologic component of low back pain. Thus the interaction of proteolytic fragments of link protein and disc cells may be important in regulating collagen and proteoglycan metabolism in both normal and degenerated discs. Previous studies have shown that a synthetic peptide from link protein DHLDNYTLDHRAIH cleaved from the N-terminus by stromelysin can act as a growth factor and stimulate synthesis of proteoglycans and collagen in human articular cartilage in explant culture [1]. By examining isolated disc cells in pellet cultures and using immunochemical methods we show that the peptide can also stimulate the synthesis of collagens II and IX, and proteoglycans in intervertebral disc. We also demonstrate that type IX collagen was synthesized predominantly in the long form (with the NC4 domain) demonstrating its importance in maintaining matrix structure and function.

MATERIALS AND METHODS: Tail intervertebral discs from 2-3 year-old steers (Les Abattoirs Z. Billette Inc., Québec, Canada) were dissected from their adjacent vertebral bodies. Cells were separated from the annulus fibrosus (AF) and nucleus pulposus (NP) regions. The AF and NP cells were cultured at a density of 1x10⁶ cells/mL in 10 mL tubes as pellets after centrifugation at 500 x g for 10 min [2]. The cells were cultured at 37°C and 5% CO₂ in DMEM, 50 µg/mL ascorbic acid (prepared fresh), 5 µg/mL transferrin, 5 ng/mL sodium selenite, 1 mg/mL bovine serum albumin, with or without the addition of 10 ng/mL or 100 ng/mL of link peptide. Media were changed every second day and stored until analysis. Pellets were maintained for 4, 8, 12, 16 or 20 days in serum-free medium. The amino peptide of link protein (DHLSDNYTLDHRAIH) was synthesized at Shriners Hospital (Montréal, Canada) and was added to the culture medium from day 2. Cultured pellets were treated with α-chymotrypsin and proteinase K as previously described [3-5], for DNA, proteoglycan and collagen analysis. DNA was measured by the Hoechst DNA assay [6]. Sulfated glycosaminoglycans (predominantly proteoglycan aggrecan) were analyzed using the DMMB dye binding assay [7]. Total type II collagen was measured by the COL2-3/4m assay, which measures an intra-chain epitope in the triple helical domain [3], and the production of the carboxy-terminal neoepitope generated by the cleavage of native human type II collagen by collagenease was measured by the COL2-3/4Cshort, in α-chymotrypsin extracts [4]. Type IX collagen was measured by the NC4 and COL2 assays [5]. All experiments were performed in triplicates and the results were normalized to DNA. Results were considered statistically significant at p<0.05 by Student’s t-test.

RESULTS: The N-terminal peptide from link protein was found to stimulate proteoglycan (Figure 1), type II collagen and type IX collagen biosynthesis by both NP and AF cells. The synthetic peptide inhibited however the cleavage of type II collagen by collagenase. These results confirmed that this novel peptide is a potent stimulator of matrix synthesis for not only articular cartilage but also intervertebral disc, and showed that the long form of type IX collagen was predominantly stimulated.

DISCUSSION: Our results demonstrate that in normal bovine pellet cultures, a single peptide of link protein can stimulate the synthesis of collagens II and IX, and proteoglycans. Thus, degradation products of link protein generated by matrix metalloproteinases can “feed-back” and may have a regulatory role in maintaining the integrity of the intervertebral disc.

REFERENCES

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48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0822

Figure 1. Stimulation of proteoglycan synthesis by link peptide. Proteoglycan content of the annulus fibrosus (A) and nucleus pulposus (B) in pellet cultures of bovine IVD cultured up to 20 days. * p<0.05 and ** p<0.001.