Effect of Link N Peptide on the Expression of Type I and Type II Collagens in Human Intervertebral Disc Cells

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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 (DHLSDNYTLDHRAIH) is the N-terminal peptide of link protein which stabilizes the proteoglycan aggregates. It is generated in vivo by proteolytic degradation during tissue turnover [1]. It has previously been shown that this peptide can stimulate the synthesis of collagens by articular cartilage [2] and bovine IVD [3] cells in vitro. Being a synthetic peptide, Link N has considerable financial benefits for clinical use over recombinant growth factors because it is extremely cheap to produce. The purpose of the present study was to determine the effect of Link N on the expression of types I and II collagen and investigate the cellular mechanisms of Link N signal transduction in human IVD cells.

MATERIALS AND METHODS
Human lumbar IVDs, from a 54-year-old donor (5 discs) without spinal pathology, were obtained through organ donations via Transplant Quebec within 24h after death. The procedure was approved by local Research Ethics Committee. Nucleus pulposus (NP) and anulus fibrosus (AF) tissues were initially digested in Ham F-12 medium containing 5% fetal bovine serum (FBS), 150 mg/ml gentamicin, 100 µg/ml penicillin, and 100 U/ml streptomycin (medium A), supplemented with 0.2% pronase (Sigma-Aldrich) for 60 min at 37⁰C with gentle agitation. The tissues were then washed 3 times with PBS and digested overnight in medium A containing collagenase type II (Sigma-Aldrich) at 0.01% for the NP and 0.04% for the AF as previously described [4]. Cells were grown in DMEM-high glucose medium containing 10% FBS, 100 µg/ml penicillin, and 100 U/ml streptomycin. Cells were incubated in serum-free DMEM in the absence (control) or the presence of 100 ng/ml of Link N peptide. Cells were then lysed and proteins separated on 10% acrylamide gels (SDS-PAGE), and transferred to nitrocellulose membranes. Proteins were detected by Western blot using specific antibodies against type I and II collagen (Abcam), p38, phosphorylated-p38 (p-p38), JNK, phosphorylated-JNK (p-JNK), ERK, phosphorylated-ERK (p-ERK), AKT, and phosphorylated-AKT (Cell Signaling). GAPDH was used as housekeeping gene and served to normalize the results for the expression of collagens while the native proteins served to normalize the level of phosphorylation.

RESULTS
Link N stimulated the expression of type I collagen in the human NP but not in the human AF cells (Figure 1). Results also show that, in human, Link N stimulated the expression of type II collagen in a time-dependent manner in both NP and AF cells (Figure 2).

In parallel, Link N transiently inhibited the phosphorylation of p38 (p-p38), a protein of the MAP kinase family in NP but not in AF cells (Figure 3). However, Link N had no effect on the phosphorylation of JNK (p-JNK) and ERK (p-ERK), two other protein of the MAP kinase family (results not shown). Link N also had no effect on the phosphorylation of AKT (p-AKT), a member of the protein kinase B family that plays important roles in mammalian cellular signaling (results not shown).

Figure 1: Effect of Link N on the expression of type I collagen (COL1) in human IVD cells.

Figure 2: Effect of Link N on the expression of type II collagen (COL2) in human IVD cells.

Figure 3: Effect of Link N on the phosphorylation of p38 (p-p38) in human IVD cells.

DISCUSSION
Link N is known to stimulate the expression of collagen in articular cartilage [2] and bovine IVD cells [3]. The present results suggest that Link N stimulates the expression of types I and II collagen in human intervertebral disc cells. More specifically, Link N stimulated the expression of type I in NP cells, but not in AF cells. Since Link N also decreased the phosphorylation of p38 in NP cells only, results suggest that p38 is a mediator of the effect of Link N on COL1 expression. p38 is a member of the MAPK family highlighted by three major cascades: p38, JNK, and ERK pathways [5]. Link N had no effect on these other pathways, suggesting a specific effect of Link N on the p38 cascade. On the other hand, Link N stimulated the expression of type II collagen in both NP and AF, suggesting that other mechanisms are implicated in the control of type II collagen expression in disc cells, without excluding p38 for the NP. In conclusion, the present study showed that Link N can modulate the expression of collagen in human IVD cells through MAPK.

REFERENCES

ACKNOWLEDGEMENT
This work was supported by grants from the Canadian Institute of Health Research (CIHR), the North American Spine Society (NASS), and the AO Foundation (Switzerland).