Effect Of Link N Treatment On Pain Related To Inter-vertebral Disc Degeneration

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Introduction: Low back pain associated with intervertebral disc (IVD) degeneration is an insidious disorder that by age 70 affects about 60% of the population. Previous studies have shown that discogenic back pain was due to the invasion of nociceptive nerve fibers into the aneural inner annulus fibrosus (AF) and nucleus pulposus (NP) of the IVD during degeneration [1]. The neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have been identified in the human IVD and have been implicated in the mechanisms associated with nerve in growth and nociception in degeneration of the IVD. Pro-inflammatory cytokines IL1β and TNFα have been shown to stimulate gene expression of NGF, BDNF and tachykinin gene (TAC1) in human NP and AF cells. We previously showed that Link N can stimulate extracellular matrix biosynthesis and is a potential stimulator of IVD repair in vivo and in vitro. We also showed that Link N could regenerate damaged AF and NP cells. The aim of the current study is to determine the effect of Link N on NGF, BDNF and TAC1 and their neurotrophic tyrosine kinase receptors TRK1, TRK2 and TAC1R gene expression in human AF and NP cells from normal and degenerated discs and to determine the Link N effect on phosphorylation/activation of these receptors and also to measure the release of pain neurotransmitter, substance P in human AF and NP cells as well as in injured bovine IVDs.

Methods: Human AF and NP cells isolated from normal IVDs were cultured in monolayers and stimulated with TNFα (100ng/mL) and IL1β (10ng/mL) in the presence or absence of Link N (1μg/mL) for 48 hours. Human AF and NP cells isolated from IVDs of different degenerative grades were cultured with or without Link N (1μg/mL). Total RNA was isolated and gene expression was measured using RT PCR. Release of substance P in to the culture media was measured after cells were stimulated by TNFα (100ng/mL) and IL1β (10ng/mL) in the presence or absence of Link N (1μg/mL) at different time points (1h, 2h, 4h, and 24h). Coccygeal IVDs from the tails of adult bovine steers (20 to 25 months) were used for disc isolation. Four discs with cartilage endplates were isolated and treated (control, capsaicin (1.5μg/mL), Punctured by 16 G needle, Link N (10μg/mL) treated) after preconditioning for 24 hours in complete DMEM. Disc culture media was collected at different time points for analysis. Substance P in the media was concentrated by Solid Phase extraction and was assayed by ELISA.

Results: Link N significantly inhibited substance P release from punctured bovine discs after 4h of treatment (76pg/mL) when compared to the untreated punctured disc (92pg/mL) (Figure 1). Link N also significantly inhibited TNFα induced gene expression of NGF in human AF cells (Figure 2) after 48h of treatment. Furthermore, Link N suppressed TNFα and IL1β induced mRNA levels of BDNF and TAC1 in both AF and NP cells.

Discussion: These results indicate that Link N appear to exert additional roles such as inhibition of pain neurotransmitter release besides biologically induced human disc repair [2] and suppression of calcification [3]. Our recent studies showed that Link N in humans acts via the BMP Smad signaling pathway. Earlier it was reported that BMP signaling suppresses peripheral innervation [4]. This suggests that Link N has the potential to inhibit pain induced by neuronal innervation caused by disc degeneration.

Significance: Disc degeneration is often associated with low back pain, Link N represents a potential economical growth factor with beneficial effects on disc repair. It would be of clinical significance to see if Link N has any potential in reducing the pain caused by neuronal invasion during disc degeneration.

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Figure 1: EFFECT OF LINK N TREATMENT ON SUBSTANCE P RELEASE FROM PUNCTURED INTERVERTEBRAL DISCS ISOLATED FROM ADULT BOVINE TAILS:
Coccygeal IVDs from the tails of adult bovine steers (20 to 25 months) were used for disc isolation. Three discs with cartilage endplates were isolated and were preconditioning for 24 hours in complete DMEM. After 24 hours the discs were punctured by 16 G needle with or without Link N (10μg/mL) injection. Non-punctured untreated was taken as control. Disc culture media were collected at different time points for analysis. Substance P in the media was concentrated by Solid Phase extraction and was assayed by ELISA. Data represents an average of 4 independent experiments using 4 different disc isolations (MEAN ± SE, *p ≤ 0.05).
Figure 2: EFFECT OF LINK N ON TNF α STIMULATED NGF GENE EXPRESSION IN HUMAN ANNULUS FIBROSUS CELLS: Human AF cells isolated from normol IVDs were cultured in monolayers and stimulated with TNFα (100ng/mL) in the presence or absence of Link N (1μg/mL) for 48 hours. Untreated cells and cells treated with Link N (1μg/mL) alone were used as controls. Total RNAs were isolated and gene expression was measured by RT PCR method using SYBR green approach. The data presented in the histogram is the average of 3 independent experiments, mRNA levels were normalized to 18s and were presented as fold change from untreated control (MEAN ± SE, p ≤ 0.05 is considered significant).