 Substance P Stimulates Cytokine Production in Human Disc Cells via Activation of P-38/ERK1/2 Pathways

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Disclosures:

Introduction: Substance P (SP), an eleven amino acid protein known to transmit nociceptive stimuli within nervous tissue[1], also acts as an inflammatory regulator in other tissue types[2,3]. Its role within the intervertebral disc (IVD) has only recently been characterized, and we have recently demonstrated expression of SP and its receptors in human IVD cells and have shown that treatment with SP caused upregulation of inflammatory mediators[4]. In this experiment, the objectives were 1) To confirm expression of the stereotypic SP receptor (NK1R) by human IVD cells; 2) To evaluate whether blockade of NK1R would attenuate its pro-inflammatory effect on IVD cells; 3) To evaluate the activation of p38 mitogen associate protein kinase (p38-MARK) and extracellular signal regulated kinase (ERK1/2) signaling pathways after treatment with SP; 4) To investigate the role of activated pathways in SP-induced chemokine synthesis.

Methods: Human tissue samples were obtained following surgery, under a protocol approved by the Institutional Review Board of the Thomas Jefferson University (Protocol #08D.525). Samples of degenerate nucleus pulposis (NP) and annulus fibrosis (AF) were obtained from patients, diagnosed by magnetic resonance imaging, undergoing anterior lumbar interbody fusion (ALIF) for degenerative disc disease. After harvest in the operating room, the tissue samples were placed into specimen cups with 0.9% saline and transferred on ice to the laboratory, within 30 minutes of harvest to minimize sample degradation. NP and AF were isolated and washed with cold PBS and extracted with lysis buffer for Western blot analysis. The extracted protein was transferred to a membrane, blocked with nonfat dry milk in PBS and incubated overnight with anti-Human NK1R monoclonal antibody. The binding of the secondary antibody was detected by enhanced chemiluminescence and results normalized to GAPDH as detected with a monoclonal GAPDH antibody.

AF and NP cells were expanded in monolayer, and then suspended in alginate beads. Before treatment with SP, alginate beads were treated with culture medium first containing a high affinity NK1R antagonist (L-760735) at different concentrations (1.0, 10.0, 50µM) for 45 minutes then with medium containing both NK1R antagonist and SP at two concentrations (1µM and 100µM SP) for 42 hours. After treatment, the cells were recovered using dissolving buffer. RNA was isolated using RNeasy Mini Columns and transcribed into cDNA. Quantitative RT-PCR was performed to evaluate expression of IL-1β, IL-6 and IL-8. Real-time quantitative RT-PCR, Western blotting, with human NP and AF cells were performed to examine levels of the phosphorylated p38, P-ERK1/2 and P-NF-κB p65. The cells were pretreated with specific inhibitors for p38 (SB203580), ERK1/2 (PD98059), NF-κB (SM7368) and SP NK1 receptor antagonist (L760735) and then stimulated with 100 µM SP.

Results: Western blot demonstrated the expression of NK1R by human NP and AF cells, and our data confirms earlier qRT-PCR experiments demonstrating the expression of NK1R isoforms in human IVD tissue. By treating the disc cells with the NK1R antagonist, we were able to suppress the expression of interleukins IL-1β, IL-6 and IL-8 in a dose-dependent manner. After treatment with SP there was an increase in P-p38 and P-p65 but not in P-NFκB p65 protein levels. Activation was maximal at 3 to 30 minutes and then decreased rapidly. There was no change in the level of total p-38, ERK1/2, and p65 during the treatment period. Inhibition of p38 and ERK1/2 activation reduced SP-induced IL-6 production in human disc cells.

Discussion: We have confirmed expression of NK1R, the stereotypic SP receptor, by human intervertebral disc cells by Western
blot. We used L-760735, a high affinity NK1R antagonist, to show that the stereotypic receptor NK1R is responsible for the pro-inflammatory effect of SP on disc cells and that this effect can be blocked by preventing binding of SP to NK1R. We have demonstrated that SP and L-760735 both act in a dose-dependent manner.

Significance: This study shows for first time that SP mediates signaling in disc cells through NK1R and that SP activates the pro-inflammatory p38 and ERK1/2 pathways. Blocking this pathway is possible with an NK1R antagonist, which attenuates the pro-inflammatory effect of SP on disc cells.

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