Substance P Stimulates Production of Inflammatory Cytokines in Human Disc Cells
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Background:
Substance P (SP), classically described as a neurotransmitter, acts as an inflammatory regulator in other tissue types but its role within the disc has not been characterized. SP has been identified within sensory neurons growing into degenerative discs and postulated to serve as the link between disc degeneration, inflammation and nociception although there has been little evidence to directly support this.

The objective of this experiment was 1) To determine the effect of SP on the expression pattern of inflammatory mediators in cervical nucleus pulposus (NP) and annulus fibrosus (AF) cells; 2) To evaluate whether SP and its receptors (NK1R-F and NK1R-T) are expressed in cervical disc cells.

Methods:
AF and NP cells from grade 3 and 4 degenerated human cervical discs from 7 individuals were expanded in monolayer, maintained in alginate bead culture in the presence or absence of $10^{-6}$ M and $10^{-4}$ M SP for two days. After treatment, the cells were recovered using dissolving buffer 0.055M NaCitrate, pH 6.8. RNA was isolated using RNeasy Mini Columns and transcribed into cDNA. Quantitative RT-PCR was performed to evaluate expression of inflammatory mediators, SP and its receptors. Cytokine patterns in conditioned media were determined by RayBio Biotin label-based human antibody array.

Results:
Disc cells treated with SP at $10^{-4}$M demonstrated significant upregulation of IL-1ß (2.3 fold in NP, p=0.038 and 2.3 fold in AF, p=0.006), IL-6 (2.9 fold in NP, p=0.005 and 2.9 fold in AF, p=0.002) and IL-8 (2.2 fold in NP, p=0.015 and 2.8 fold in AF, p=0.005) (Figure 1).

Treatment with SP at $10^{-4}$M demonstrated significant upregulation of RANTES in the AF only (1.8 fold, p=0.001) (Figure 2). Treatment with SP at $10^{-4}$M demonstrated significant upregulation of TNF-a in the AF only (2.3 fold, p=0.039). Treatment of disc cells with SP at $10^{-6}$M did not cause significant upregulation of any cytokine or chemokine.

Conclusion:
We have shown, for the first time, the effect of SP on expression of inflammatory mediators in disc cells. We found that SP upregulates inflammatory mediators in human NP and AF cells. Furthermore, this study demonstrates expression of SP and SP receptors by disc cells.

Significance:
The expression of SP receptors by disc cells suggests that SP acts in an autocrine or paracrine manner in IVD cells. In addition to providing a potential mechanism for transmission of painful stimuli to sensory nerves from degenerative disc cells, SP influences the inflammatory phenotype of disc cells; Substance P may provide a medium for “crosstalk” between disc cells and neurons in the intervertebral disc.

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Figure 1: Real-time RT-PCR analysis of interleukins IL-1ß, IL-6 and IL-8 in NP and AF cells. Relative gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and is represented as mean ± standard error.

Figure 2: Analysis of RANTES and TNF-a in NP and AF cells. Relative gene expression was normalized to GAPDH and is represented as mean ± standard error.

Figure 3: Photomicrograph of two representative cytokine antibody arrays. Each dot represents one of 42 distinct cytokines and growth factors. (P. Ctr= positive control; Ctr=control)