The Effects of Substance P Administration on the Intervertebral Disc in a Rat Organ Culture Model

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Introduction: In the intervertebral disc (IVD), substance P (SP), a nociceptive neurotransmitter, has been used as a specific marker to identify ingrowth of nerves, which may transmit painful stimuli in degenerative discs. The addition of SP to human IVD cells has also been demonstrated to upregulate inflammatory mediators in a cell culture model. Previous models investigating disc degeneration, such as the needle puncture model, cause physical disruption of the disc, which does not replicate degenerative disc disease. The rat organ culture model allows for analysis of the disc/endplate unit without disruption. The effects of SP on the IVD are unknown in this model, and its addition may upregulate the expression of inflammatory mediators responsible for disc degeneration, and create a degenerative phenotype.

Methods: Six adult Wistar rats were sacrificed and lumbar discs (n=6/animal) were harvested under sterile conditions. The endplates were dissected and maintained in organ culture media with antibiotics for 8 days. The culture media was removed and replaced with serum free media for an additional 48 hours. Experimental discs were cultured in media containing antibiotics and Substance P (100µM). Previous experiments have demonstrated this dosage to not be cytotoxic to disc cells. At the time of harvest, the 6 lumbar discs from each animal were pooled together to create one sample for annulus fibrosus (AF) and nucleus pulposus (NP) tissue. The conditioned medium from the organ culture was probed for cytokine production using a rat cytokine antibody array and chemiluminescence was detected. Real-time RT-PCR analysis was completed for confirmation of cytokine expression from NP tissue. Significance was assumed at p<0.05.
**Results:** After treatment with SP, the conditioned medium demonstrated an increase in the production of several pro-inflammatory cytokines and growth factors (Interleukin-6 (IL-6), CXCL5, vascular endothelial growth factor (VEGF)) compared to controls (p=0.09, 0.04, 0.03, respectively). There was decreased production of Agrin, CD86, and Fractalkine after treatment with SP compared to controls (p=0.006, 0.049, 0.019, respectively). On real-time RT-PCR, the NP tissue demonstrated increased expression of IL-6 and matrix metalloproteinase-3 (MMP-3) compared to controls (p=0.001, 0.059 respectively). Cellular hypertrophy and matrix degradation of nucleus pulposus (NP) tissue and lower cell numbers were seen in AF tissue in discs treated with SP (Figure 1).

**Discussion:** Treatment of the IVD with Substance P leads to upregulation of inflammatory cytokines and growth factors, decreased expression of proteoglycan, and created phenotypic changes of degeneration in an organ culture model. These findings demonstrate new evidence that Substance P may be an important regulator of disc degeneration.

**Significance:** This research will advance the field of spine surgery because it identifies a potent local cytokine that significantly increases intervertebral disc degeneration. The effect of SP on leading to disc degeneration will have two major impacts. The first being it allows for the development of a more physiologic model for disc degeneration. The currently used needle puncture model causes disc degeneration through non-physiologic mechanical means. Furthermore, identifying a chemical that leads to physiologic disc degeneration will also stimulate further studies to determine if medications can target SP and prevent disc degeneration.