Acute Mechanical Injury To The Human Intervertebral Disc Initiates Events Implicated In Disc Degeneration

Bashar G. Alkhatib, Bachelor's of Science, Derek H. Rosenzweig, PhD, Emerson Krock, Bachelor's of Science, Rahul Gawri, M.B.B.S., Lorne Beckman, Thomas Steffen, MD, PhD, MBA, Michael Weber, MD, PhD, Jean Ouellet, MD, Lisbet Haglund, PhD.

1McGill University, Montreal, QC, Canada, 2McGill University Health Centre, Montreal, QC, Canada, 3McGill Scoliosis and Spine Centre, Montreal, QC, Canada.

Disclosures:

Introduction:
Low back pain is a prevalent chronic disorder among individuals world-wide. Disc degeneration is a major cause of low back pain in adults. One hypothesized mechanism for the initiation of disc degeneration is mechanical overload of the intervertebral disc. Previous studies have demonstrated that complex mechanical loading of lumbar motion segments causes an inhibition of disc cell metabolism (1). Furthermore, mechanical wedge-loading of bovine caudal discs causes induction of catabolic genes such as IL-1ß and members of the metalloproteinase and ADAMTS family of proteases as well as a loss of aggrecan and increased cell death (2). Taken together, these are thought to be initiating events for degeneration of intervertebral discs. The aim of this study is to investigate cellular responses and matrix changes in human intervertebral discs immediately after acute mechanical injury.

Methods:
Lumbar spine segments were harvested from consenting donors via the Transplant Quebec organ donation program in Montreal, Quebec. This was performed in accordance with the institutional review board guidelines of the Montreal General Hospital. Intervertebral discs were mechanically loaded using an MTS material testing system at both 5% strain (low strain), representing a minimally loaded control, and 30% strain (high strain), representing acute mechanical injury. 30% strain consistently cracked cartilage endplates allowing visual confirmation of acute trauma. Discs were immediately cultured in low-serum containing media after loading and media was collected at days 3, 7, 10 and 14. Cultured discs were harvested at days 7 and 14 and analyzed. Proteoglycan release was evaluated in media from all culture days and on Guanidium hydrochloride extracts of tissue from the loaded discs. Live/Dead analysis was performed on 4mm tissue biopsies from 7 different regions of the discs and confocal microscopy was used to quantify live and dead cells. Furthermore, media from cultured discs was also incubated with the PC12 cell line to study the potential for neurite sprouting. Neuronal growth factor (NGF) levels were measured using a commercially available ELISA assay. Commercially available cytokine arrays were used to investigate protein levels of cytokines previously implicated in disc degeneration. Tissue biopsies were analysed for cell viability and proteoglycan content, discs were placed in 80% methanol in preparation for cryosectioning and histological analysis. Sections were stained with Safranin-O, hematoxylin, and Fast Green.

Results:
Considerable cell death was observed in discs loaded with a single impact load of the high strain 7 and 14 days after the load event (NP 40% viable and AF 50% viable). Discs exposed to a single impact load of low strain maintained a cell viability of 85% in the NP and the AF. Investigation of GAG levels revealed that high strain induced a considerable release GAG from the tissue into the culture media as compared to low strained samples. This was confirmed by histological analysis where a loss of Safranin-O staining was apparent in the disc tissue exposed to the high strain. Furthermore, conditioned media from the high strain group caused neurite outgrowth in PC12 cells comparable to when PC12 cells were exposed to media containing NGF. NGF ELISA confirmed 2.5 times elevated levels of NGF protein in culture media from the high strain group compared to the low strain group. Cytokine arrays revealed elevated levels of IL-5, IL-6, IL-7, IL-8, MCP-2, GROα, and MIG,in media from the high strain group which are all inflammatory cytokines associated with disc degeneration. However, decreased protein levels of IL-1α, MCP-1, TGF- β1, and TNF-α, were observed in culture media from the high strain group.

Discussion:
Cell death and cytokine production combined with loss of GAG indicate that acute mechanical injury initiated degradative processes that could lead to disc degeneration. Acute injury also stimulated cells which remained viable after the event to produce elevated levels of inflammatory cytokines. Once the matrix has been degraded, elevated levels of NGF could lead to neuronal in-growth, through the depleted AF possibly into the NP, and cause pain often associated with disc degeneration.

**Significance:**
Our results demonstrate that a single load event has the ability to initiate degradation and induce the production of NGF which may lead to nerve in-growth and chronic pain. Further investigation may yield insights to novel diagnostics and therapies for degenerative disc disease.

**Acknowledgments:**
Funding for this work was provided by AO Spine and CIHR.

**References:**
Figure 1. A. Live/Dead cell viability assay of disc tissue loaded with 5% and 30% strain. Stained cells were visualized using confocal microscopy.

B. DMMB analysis of conditioned media from 5% and 30% strained discs at days 3, 7, 10, and 14. Amount of GAG is reported in mg/mL.
Figure 2. A. Cytokine array blot of 30% strained disc conditioned media as compared to 5% strained disc conditioned media. All values are reported as a fold difference above or below 5% strained values.

B. NGF ELISA of 5% vs. 30% strained conditioned media at days 3 and 7. All values are reported in picograms per ml.