Effect Of Removal Of Nucleus Pulposus Cells On The Annulus Fibrosis Of The Intervertebral Disc

Chitra Dahia¹,², Atiq Durrani¹, Eric Mahoney¹,², Christopher Wylie²
¹Orthopaedic Surgery, Cincinnati Children’s Hospital and Medical Center, Cincinnati, OH; ²Developmental Biology, Cincinnati Children’s Hospital and Medical Center, Cincinnati, OH

Introduction: Backpain is one of the major health issues not only in America but also around the globe. Intervertebral disc (IVD) degeneration is one of the most common causes of backpain. IVD has mainly two components; the outer rings of fibrous cartilage form the annulus fibrosus (AF) while the inner gelatinous material with cells is known as the nucleus pulposus (NP). The relationship between the nucleus pulposus and the annulus fibrosis in normal differentiation and maintenance of the IVD is not clearly understood. We studied the effect of removal of NP cells on the AF in mouse lumbar intervertebral discs.

Materials and Methods: The nucleus pulposus cells were surgically aspirated from L2-L3 and L3-4 discs of 2-week old male mice (during the period of rapid postnatal vertebral growth), using a 27-gauge syringe. L4-5 disc in the same mouse was sham-operated (needle insertion without aspiration) as control. The effects on the IVD were assayed 2-8 weeks after the surgery. The lumbar vertebrae was collected from the mice at different ages and fixed and decalcified using 5% TCA. 8 μm cryosections were collected in the coronal plane and histological analysis was carried out using H&E staining. Cell proliferation and cell death was determined by phospho Histone H3 (PH3) and active caspase-3 staining respectively.

Results: 5-weeks following removal of the NP cells there was significant collapse of the IVDs from which the NP had been removed, compared with those of sham-operated controls. By 7-weeks, fibrocartilage cells derived from the AF were invading the disc space, which became completely filled by 8 weeks. Growth of the disc was reduced in both cranio-caudal and transverse diameters by 30%, compared to control discs. Mitotic cells were absent in the AF indicating that AF cells stopped proliferating in the absence of NP cells. A large number of activated caspase-3 positive cells were found in AF cells 8 weeks after removal of NP cells, compared to controls. However, 2-weeks after the removal of NP cells none of the cells stained positive for activated caspase-3.

Discussion: Removal of NP cells leads to invasion of the disc space by fibrosis AF cells which is very similar to that observed in the human degenerated discs. It was also observed that with time there was increased cell death of the AF cells, and decrease in disc growth. This suggests that if there is an early intervention in the disc degeneration, there could be possible therapeutic rescue of the discs. These data suggest a requirement for the NP cells in normal differentiation and growth of the AF cells. The significance of the study lies in the observation that there appears to be a window of time in which the cells of the AF are alive despite removal of NP cells. A potential exists for intervention during this time to prevent the AF from degeneration.

Acknowledgements: Children’s Hospital Research Foundation.

Histological analysis of the sham operated (Fig. A) and NP cells ablated (Fig. B) IVD 8 weeks after the experiment. No cell death was observed in the sham operated IVD (Fig. C) which following removal of NP cells, activated caspase-3 staining was observed in the fibrosed IVD (Fig. D).