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Basic Science

Intact glycosaminoglycans from intervertebral disc-derived notochordal cell-conditioned media inhibit neurite growth while maintaining neuronal cell viability

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Abstract

BACKGROUND CONTEXT: Painful human intervertebral discs (IVDs) exhibit nerve growth deep into the IVD. Current treatments for discogenic back pain do not address the underlying mechanisms propagating pain and are often highly invasive or only offer temporary symptom relief. The notochord produces factors during development that pattern the spine and inhibit the growth of dorsal root ganglion (DRG) axons into the IVD. We hypothesize that notochordal cell (NC)-conditioned medium (NCCM) includes soluble factors capable of inhibiting neurite growth and may represent a future therapeutic target.

PURPOSE: To test if NCCM can inhibit neurite growth and determine if NC-derived glycosaminoglycans (GAGs) are necessary candidates for this inhibition.

STUDY DESIGN: Human neuroblastoma (SH-SY5Y) cells and rat DRG cells were treated with NCCM in two-dimensional culture in vitro, and digestion and mechanistic studies determined if specific GAGs were responsible for inhibitory effects.

METHODS: Notochordal cell-conditioned medium was generated from porcine nucleus pulposus tissue that was cultured in Dulbecco's modified eagle's medium for 4 days. A dose study was performed using SH-SY5Y cells that were seeded in basal medium for 24 hours and neurite outgrowth and cell viability were assessed after treatment with basal media or NCCM (10% and 100%) for 48 hours. Glycosaminoglycans from NCCM were characterized using multiple digestions and liquid chromatography mass spectroscopy (LC-MS). Neurite growth was assessed on both SH-SY5Y and DRG cells after treatment with NCCM with and without GAG digestion.

RESULTS: Notochordal cell-conditioned medium significantly inhibited the neurite outgrowth from SH-SY5Y cells compared with basal controls without dose or cytotoxic effects; % of neurite expressing cells were 39.0±2.9%, 27.3±3.6%, and 30.2±2.7% and mean neurite length was 60.3±3.5, 50.8±2.4, 53.2±3.7 μm for basal, 10% NCCM, and 100% NCCM, respectively. Digestions and LC-MS determined that chondroitin-6-sulfate was the major GAG chain in NCCM. Neurite growth from SH-SY5Y and DRG cells was not inhibited when cells were treated with NCCM with digested chondroitin sulfate (CS).

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CONCLUSIONS: Soluble factors derived from NCCM were capable of inhibiting neurite outgrowth in multiple neural cell types without any negative effects on cell viability. Cleavage of GAGs via digestion was necessary to reverse the neurite inhibition capacity of NCCM. We conclude that intact GAGs such as CS secreted from NCs are potential candidates that could be useful to reduce neurite growth in painful IVDs.

Keywords: Notochordal cells; Intervertebral disc; Neuronal cells; Glycosaminoglycans; Chondroitin sulfate; Neurite outgrowth

Introduction

Low back pain is the leading global cause of disability causing substantial socioeconomic burden, and intervertebral disc (IVD) disease is commonly implicated in its pathogenesis [1,2]. Although the etiology of discogenic back pain is not completely understood, back pain patients have demonstrated increased nerve growth into diseased IVDs [3]. Analgesics provide only short-term pain relief and current biological strategies to treat painful IVDs focus largely on repair and regeneration of the IVD rather than targeting the source of pain itself. There is a need to develop therapies that focus on the mechanisms associated with the induction and propagation of discogenic back pain, and addressing neurovascular invasion is a natural target [4].

The healthy immature IVD is largely avascular and aneural and rich in proteoglycans. The gelatinous nucleus pulposus (NP) is surrounded circumferentially by the fibrous annulus fibrosus and contained cranially and caudally by the cartilage end plates, providing the IVD with the ability to withstand high mechanical forces and maintain motion [5]. Aging and degeneration of IVD results in increased matrix degradation, proinflammatory cytokine expression, decreased water content, and inferior mechanical properties [6]. These degenerative changes, including fissures, may provide a permissive microenvironment for neurovascular growth and sensitization of nerve fibers in the IVD [7]. Small unmyelinated nociceptive neurons expressing the neuropeptide Substance P and axonal elongation marker GAP43 have been demonstrated to grow into the painful human IVD [3,8]. These nerves also express the high affinity receptor for nerve growth factor (NGF), tyrosine kinase A, and accompany microvascular blood vessels that express NGF [9]. The likely sources of neoinnervation and neovascularization are defects in the annulus fibrosus or vertebral end plates [9]. Neurovascularization has been identified in posterior radial and transdiscal tears of human cadaveric IVDs [10], and in such tears, a decrease in the stress profile along the defect and also focal depletion of proteoglycans were observed, providing a path for nerves and blood vessels to grow into the IVD [7]. Defects in the vertebral end plate are also associated with neoinnervation, where nerve growth has shown to be the greatest in fibrovascular end plate marrow defects compared with annular tears or other end plate pathologies [11].

The healthy IVD produces factors with the ability to inhibit growth of nerves and blood vessels into the IVD, yet expression of such factors decreases with age and their absence may be associated with promoting neurovascular growth in diseased states [7,12–14]. Disc cells express vascular endothelial growth factor, NGF, and brain-derived neurotrophic factor, and expression of these nerve promoting factors increases with the severity of degeneration [15–18]. These growth factors are also upregulated by the proinflammatory cytokines TNFα and IL-1β in vitro, and as such, they may enhance the increase in expression observed with disease progression. The disc also produces neuropeptides (ie, Substance P) involved in pain perception that can upregulate proinflammatory cytokines themselves and increase with the severity of degeneration [19,20]. Human neuronal cells cocultured with human degenerate NP cells show increased neurite growth [21], whereas coculture with human NP cells isolated from the healthy nondegenerate IVDs had inhibitory effects on neurite growth. Human aggrecan from the healthy IVD can inhibit both neurite growth and endothelial migration in vitro, and taken together, these studies suggest that soluble factors from the healthy IVD could be harnessed to repel neurovascular growth in painful IVD [12,13].

Recapitulation of the processes that occur during the developmental patterning of the healthy immature notochordal cell (NC)-rich IVD may help inform therapeutic strategies to treat painful IVD degeneration [4]. The notochord patterns the IVD during development through secretion of diverse developmental ligands that give rise to the aneural and avascular structure. These include soluble neurovascular repulsive factors, such as semaphorin 3A and noggin [22,23]. Chondroitin sulfate (CS) proteoglycans expressed by the notochordal sheath are also associated with neuronal patterning and inhibition during the development of spine [24]. Understanding the processes that occur during development may inform treatments for discogenic back pain, and we believe that such natural processes should be emulated to enhance therapeutic strategies. A number of species such as pig and rabbit retain NCs from development through to adulthood and do not experience the disc disease common in humans [25,26]. As a consequence, NCs have received much attention with regard to the possible mechanisms associated with their loss and also their therapeutic potential [4,27–30]. It has previously been shown that