ATP Treatment promotes Collagen Deposition and Gene Expression in Intervertebral Disc Cells

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Disclosures:

Introduction: The intervertebral disc (IVD) is formed by two anatomically distinct structures: the nucleus pulposus (NP), which is rich in proteoglycan and resists compression; and the annulus fibrosus (AF), which is rich in collagen and allows bending and torsion of the disc. The proteoglycan gel and the collagen network are the main components of the IVD extracellular matrix (ECM), which is a structure that undergoes constant remodeling. The energy demanding processes of biosynthesis, accumulation and breakdown of ECM constituents by IVD cells determine the quality and integrity of the ECM and thus, the disc’s biomechanical response. Our recent studies have demonstrated that mechanical loading promotes ATP production and release from IVD cells [1,2]. ATP serves not only as an intracellular energy source but also as an extracellular signaling molecule that drives a variety of cellular activities including ECM synthesis [3] via purinergic pathways. Thus, the mechanosensitive ATP release found in our previous study may be involved in the process of mechanotransduction of IVD cells. Our hypothesis was that extracellular ATP may affect the ECM synthesis of IVD cells. Therefore, the objective of this study was to investigate the effect of extracellular ATP on the collagen production and gene expression in IVD cells.

Methods: NP and AF tissue was harvested from the spine of young pigs within 4 hours after sacrifice. Cells were isolated and seeded in 2% agarose gels containing 1 x 10⁷ cells/ml. Three-dimensional agarose culture was chosen because of its minimal binding interaction with cells and capability to maintain cellular phenotype. Agarose constructs were incubated at 37°C, 5% CO₂ in DMEM supplemented with 10% fetal bovine serum and antibiotics. To examine the effect of ATP on collagen deposition, the agarose constructs were treated for three weeks with 20 μM and 100 μM ATP (NP: n = 9; AF: n = 5 for Control and each treatment group). The ATP concentrations were chosen based on our previous study that found high extracellular ATP concentration in NP tissue (165.3±40.8 μM) [4]. After three weeks, a hydroxyproline assay was performed as an indirect method to measure collagen levels in each sample. The collagen content of each sample was normalized by its DNA content and each treatment group was normalized by its respective Control group. A comparison of collagen accumulation between different treatment groups of the same cell type was performed by one-way ANOVA following by post hoc SNK test. T-tests were performed to compare collagen levels between AF and NP cells of the same group of treatment. To examine the effect of ATP on gene expression, the agarose constructs were treated with 100 μM ATP for 16 hours. mRNA levels of aggrecan and collagen type II were measured using real-time PCR and t-tests were performed to compare gene expression levels between the Control and treated groups.

Results: For NP cells, the 100 μM ATP group exhibited significantly higher collagen content than the Control and 20 μM groups while no significant difference between the Control and the 20 μM ATP groups was found. For AF cells, the 100 μM ATP group showed a significantly higher collagen level than the 20 μM ATP and Control groups. In addition, the 20 μM ATP group exhibited significantly higher collagen content than the Control group(Figure 2). Comparison of the responses of NP and AF cells to the same ATP concentration showed a significantly higher collagen deposition by NP cells under all ATP treatment conditions. Aggrecan and collagen type II showed upregulation of gene expression in NP and AF cells after 16 hours of 100 μM ATP treatment (Figure 3).
**Figure 1.** Collagen deposited by NP cells treated with ATP at different concentrations for three weeks

**Figure 2.** Collagen deposited by AF cells treated with ATP at different concentrations for three weeks

**Figure 3.** Aggrecan and collagen type II expression of NP and AF cells treated with 100 μM for 16 hours

**Discussion:** The results of this study support our hypothesis that extracellular ATP influences ECM biosynthesis of IVD cells. Since mechanical loading can promote ATP release from IVD cells, extracellular ATP may play an important role in the mechanobiological responses of IVD cells. Our previous finding of high extracellular ATP in NP tissue [4] explains that NP cells are less sensitive to the ATP treatment than AF cells and thus, a significant increase in collagen levels was only seen in the 100 μM ATP group of NP cells. Furthermore, higher collagen deposition in the NP treatment groups compared to their AF counterpart group suggests that NP cells are more metabolically active than AF cells. Moreover, the increase in collagen deposition by the ATP treatment is supported by the upregulation of collagen type II gene expression in both cell types. In addition, the upregulation of aggrecan expression supports the stimulation of proteoglycan accumulation reported in our previous study [5]. Furthermore, since ATP is often hydrolyzed into adenosine and ADP which can also mediate cellular function, the biological responses of IVD cells to ATP derivatives (i.e., adenosine and ADP) need to be further investigated.

**Significance:** Insufficient maintenance of the ECM may promote disc degeneration, which is associated to low back pain and affects millions of people in the US. The results of the present study suggested that extracellular ATP may play an important role...
in the mechanotransduction of IVD cells, which regulates ECM biosynthesis.

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