**INTRODUCTION:** The intervertebral disc (IVD) has been shown to express cytokines under pathological conditions, such as IVD herniation or with degeneration. Cytokines play a variable role in the pathogenesis of IVD degeneration and other spinal disorders. In particular, increases in both protein and mRNA levels of interleukin-1 (IL-1) and its major regulator, tumor necrosis factor (TNF-α), have been observed in degenerated or herniated IVD tissues and are spontaneously expressed by these tissues in culture [1-7].

Signaling by p38 Mitogen Activated Protein Kinase (MAPK) is critical to the catabolic and anti-anabolic action of many cytokines, including IL-1 and TNF-α, in a rabbit model of IVD degeneration [8]. p38 MAPK is involved in both the induction and action of inflammatory cytokines; this kinase is implicated in MMP production, inducible nitric oxide synthase (iNOS) induction/NO synthesis, cyclooxygenase-2 (COX2) induction/prostaglandin E2 synthesis, and transcription of other factors that comprise the "inflammatory response" [9].

p38 MAPK inhibition increased the gene expression of matrix proteins and anabolic factors and decreased the gene expression and production of factors associated with inflammation, pain and disc matrix catabolism [10]. The purpose of this study is to evaluate the *in vitro* effects of p38 MAPK inhibitor (p38i) on proteoglycan synthesis by human IVD cells and to determine the *in vivo* effects of p38i administered intradiscally on gene expression of catabolic factors in this rabbit IVD degeneration model.

**MATERIALS AND METHODS:**

**Cell Preparation:** Human nucleus pulposus (NP) and anulus fibrosus (AF) cells were isolated from four cadaveric IVD tissues (grades 2-3). The tissues were cultured in 2% alginate for 7 days in DMEM/F12/10% FBS/ascorbate. NP and AF cells were then cultured with p38i (10 nM, 100 nM; provided from Advanced Technology and Regenerative Medicine LLC, MA). PG Synthesis: Radiolabeling with ³⁵S-sulfate (20 µCi/ml, 4 hours) and a rapid filtration assay [11] were used. Measurement of matrix metalloproteinase-3 (MMP-3) in the control media: MMP-3 levels in the media, collected on day 3, were quantified by ELISA (MMP-3; Invitrogen, CA).

The Rabbit Anular Puncture Model and p38i Injection: Eight adolescent New Zealand white rabbits (weighing 3.5-4.5 kg) were used in this study with institutional animal care committee approval. Under general anesthesia, the anulus in two non-contiguous discs (L2/3, 3/4 and L4/5) was punctured with an 18G needle to induce disc degeneration using the left retroperitoneal approach. Four weeks later, rabbits were assigned to four treatment groups. Phosphate buffered saline (PBS) (10 µl) or p38i (10 µM in 10 µl PBS) was injected into the center of the NP of previously punctured discs using an open procedure. The discs of both L2/3and L4/5 were injected with p38i as the experimental group and the disc of L3/4 injected with PBS was used as the control disc. The rabbits were monitored for up to 1 or 4 weeks after the injections.

**Quantitative PCR (q-PCR):** Total RNA was isolated from AF and NP cells and q-PCR performed using the gene-specific primers for IL-1β, TNF-α, IL-6 and ADAMTS 4, -5. Standards were made by cloning the PCR products into the Pdrive vector using a PCR cloning kit (Qiagen, CA). The results of this study demonstrate that p38i at very low concentration up-regulated proteoglycan synthesis and suppressed the expression of MMP-3 in primary human degenerative IVD cells cultured in alginate beads at very low concentration. Moreover, the injection of p38i in the rabbit anular puncture model showed a significant suppressive effect on the mRNA expression of pro-inflammatory cytokines and ADAMTS 4, -5 in the *in vivo* rabbit anular puncture model.

The inhibition of p38 MAPK in human and rabbit degenerative IVD tissues may have a positive effect on the anabolic/catabolic balance of degenerative IVD tissues. This approach to block p38 MAPK could provide a therapeutic strategy to intervertebral disc degeneration.

**RESULTS:**

**In vitro study (human IVD cells)**

**PG synthesis:** p38i significantly up-regulated PG synthesis in NP cells (10 nM; +36%, 100 nM; +43%). In AF cells, p38i did not affect PG synthesis.

**MMP-3 Level in the media:** The concentration MMP-3 in conditioned media was significantly suppressed in NP and AF cells (NP, 10, 100 nM; 23%; AF, 100 nM; -40%) by p38i (p<0.05). (Fig. 1)

**Cytokine expression in rabbit anular puncture model:**

- Cytokine expression: In both tissues, the injection of p38i significantly, and almost completely, inhibited the mRNA expression of IL-1β at the 1 and 4 week time point (p<0.01). Similarly, the mRNA expression of TNF-α was significantly inhibited by the injection of p38i in both tissues; the effect was more apparent in the NP tissue. p38i significantly inhibited the mRNA expression of IL-6 in both tissues (p<0.01 (Fig. 2 left)).

**Expression of ADAMTS 4, -5:** The injection of p38i significantly inhibited the mRNA expression of ADAMTS 4 in both tissues (p<0.01), ADAMTS 5 in the p38i group had significantly lower mRNA expression in both tissues. The effect of p38i on the expression of ADAMTS 4, -5 was more apparent in the AF than the NP (Fig. 2, right).

**DISCUSSION:**

The results of this study demonstrate that p38i at very low concentration up-regulated proteoglycan synthesis and suppressed the expression of MMP-3 in primary human degenerative IVD cells cultured in alginate beads at very low concentration. Moreover, the injection of p38i in the rabbit anular puncture model showed a significant suppressive effect on the mRNA expression of pro-inflammatory cytokines and ADAMTS 4, -5 in the *in vivo* rabbit anular puncture model.

The inhibition of p38 MAPK in human and rabbit degenerative IVD tissues may have a positive effect on the anabolic/catabolic balance of degenerative IVD tissues. This approach to block p38 MAPK could provide a therapeutic strategy to intervertebral disc degeneration.

*Department of Orthopedic Surgery, Rush Medical College, Chicago, IL.
**Department of Orthopedic Surgery, Kobe University, Kobe, Japan

**REFERENCES:**


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