A P38 Mitogen-Activated Protein Kinase Inhibitor, Scio-469, Inhibited the Production and Expression of Cytokines and Pain-Related Molecules by Human Intervertebral Disc Cells

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INTRODUCTION: The degradation of intervertebral disc (IVD) extracellular matrix components (i.e., aggrecan) is related to the increased activities of matrix-degrading enzymes produced by IVD cells and can lead to a loss of the shock absorber functionality of the IVD. The metabolism of IVD cells is controlled by cytokines and growth factors through endocrine, paracrine and autocrine mechanisms. Increased levels of cytokines [interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)] in the IVD have also been shown to correlate with symptoms of pain [1]. These cytokines stimulate nerve growth factor (NGF) production by disc cells and, therefore, possibly increase the perception of pain through the sensitization of NGF-sensitive neurons and extension of their axons [2].

A downstream signaling molecule, p38 mitogen-activated protein kinase (MAPK), participates in the signaling cascade controlling cellular responses to cytokines and stress. Blocking cytokine signaling pathways using a p38 MAPK inhibitor (p38i) may reduce the sensation of pain through the reduction of levels of NGF or other pain-related molecules. Recent studies have suggested that the application of p38i increased matrix production by inhibiting the production of cytokines, including IL-1 and TNF-α [3]. Therefore, the use of p38i may provide therapeutic effects both in counteracting matrix degradation and reducing pain.

Our objective in the present study was to evaluate the biologic effects of p38i on IVD cells and to see if it counteracts the effects of the pro-inflammatory cytokine, IL-1, on mRNA levels of cytokines (IL-1β, TNF-α, IL-6), proteases (ADAMTS4 and ADAMTS5), NGF, cyclo-oxygenase-2 (COX-2), as well as the production of nitric oxide (NO) and TNF-α.

MATERIALS AND METHODS: Cell Preparation: Human nucleus pulposus (NP) and anulus fibrosus (AF) cells isolated from cadaveric IVD tissues (3 separate donors, grades 2-3, 53, 51, 71 y.o., Ave. age, 58.3 y.o.) were cultured in 1.2% alginate for 7 days in DMEM/F12/10% FBS/ascorbate. Cells were serum-starved for 24 hrs and then treated for 24 hrs under one of the following conditions: 1) control (DMEM/F12); 2) IL-1β (5 ng/mL); 3) p38i (SCIO-469, 5 nM or 10 nM/mL; provided by Advanced Technologies and Regenerative Medicine, LLC, MA) or 4) IL-1β (5 ng/ mL) + p38i (5 and 10 nM/mL).

Total Nitrite and TNF-α production: Total nitrite and TNF-α levels in media were measured using the Nitric Oxide Metabolite Detection Kit (Cayman, MI) and TNF-α ELISA kit (Invitrogen, CA), respectively

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, ADAMTS4, ADAMTS5, NGF and COX-2. Standards were made by cloning the PCR products into the pDrive vector using a PCR cloning kit (Qiagen, CA). The copy numbers were calculated as molecules/ml and normalized by those for GAPDH.

RESULTS: Production of NO and TNF-α (**Fig.1**): IL-1β significantly stimulated the production of NO and TNF-α, while p38i significantly inhibited the production of both. Cytokine Expression (**Fig. 2**): Treatment with IL-1β significantly up-regulated IL-1β, TNF-α and IL-6 mRNA expression by AF and NP cells. The addition of p38i (5 nM and 10 nM) to IL-1-stimulated cultures significantly down-regulated the mRNA levels of these molecules in AF and NP cells. Expression of ADAMTS4 and ADAMTS5 (**Fig. 2**): IL-1β significantly stimulated the expression of mRNA for ADAMTS4 and ADAMTS5 by AF and NP cells. The stimulatory effects of IL-1β were significantly inhibited by treatment of AF and NP cells with p38i. Expression of NGF and COX-2 (**Fig. 2**): IL-1β increased levels of expression of NGF and COX-2 mRNA. However, p38i effectively suppressed the IL-1β-stimulated gene expression.

DISCUSSION: Our results show that p38i, SCIO-469, significantly suppressed the IL-1β-induced expression of cytokines and aggrecanase *in vitro*. It also decreased the amount of TNF-α and total nitrite levels in the culture media and suppressed the expression of NGF and COX2, two markers related to pain generation. The results may suggest that the *in vivo* use of p38i has a clinical benefit and should be tested to determine if the injection of p38i retards the progression of disc degeneration by blocking cytokine pathways. Detailed pharmacokinetic and safety studies will also be required to show efficacy and safety.

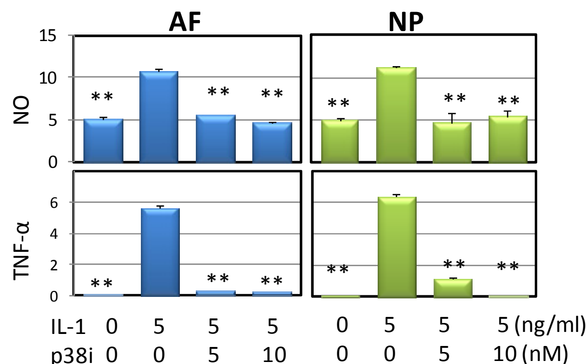

Fig. 1: NO and TNF-α levels in the media (p<0.01, vs. IL-1)**

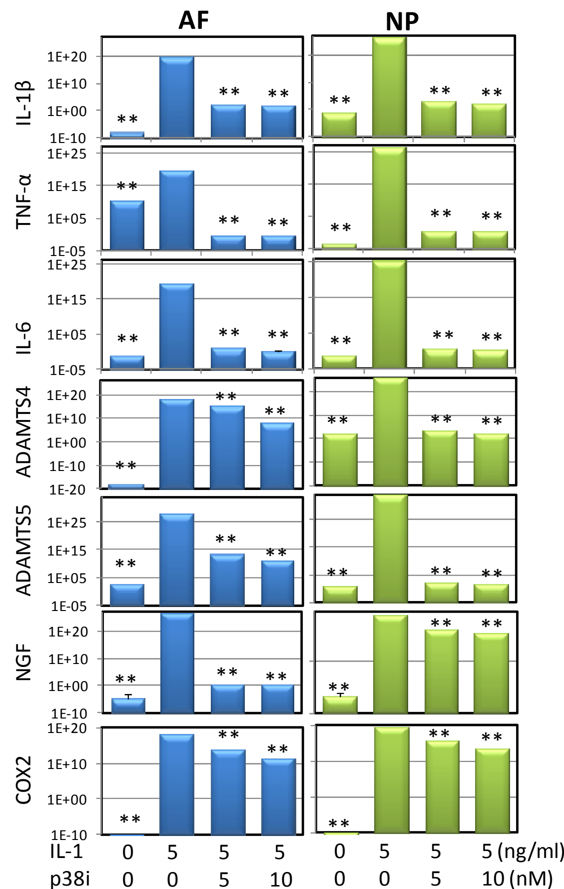

Fig. 2: Cytokines, ADAMTS and COX2 mRNA Expression (p<0.01, vs. IL-1)**

REFERENCE

[1]Freemont AJ+, J Pathol 2002;196(4):374-9 [2]Abe Y+, Spine 2007; 32:635-42; [3]Studer RK+, Spine 2007;32:2827-33

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