The Role of NFkB in Intervertebral Disc Matrix Homeostasis in Response to Mechanical Signaling

Sowa, GA1,2; Wang, D1; ... proteoglycan  total collagen
Response to 24 Hours of mechanical Stretch at 6% 0.1 Hz
Control
Stretch
Stretch + ACHP
ORS 2011 Annual Meeting
Fund of The Pittsburgh Foundatio
Acknowledgements:
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After four hours of mechanical stretch at 6% 0.1 Hz, annulus
RESULTS normalized to total DNA and reported as percent compared to unstressed cells
Intervertebral disc annulus fibrosus cells were isolated from New Zealand White Rabbits immediately after sacrifice and cultured in F-12, 10% FBS, 1% P/S at 37°C/ 5% CO2 until 90% confluence. Cells were then exposed to tensile stress at 6% 0.1 Hz for either 4 or 24 hours using the Flexercell® Tension Plus System (Stretch) and compared to unstressed cells. Cells were also pre-incubated for 1 hour with 1nM 2-amino-6-(2-cyclopentylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile (ACHP), a chemical inhibitor of IKK-2, involved in the phosphorylation and activation of NFkB. Total proteoglycan and total collagen synthesis was measured via incorporation of 35S and 3H by pulse labeling for 24hours after completion of the stretching regimen and normalized to total DNA and reported as percent compared to control cells.

METHODS
Intervertebral disc annulus fibrosus cells were isolated from New Zealand White Rabbits immediately after sacrifice and cultured in F-12, 10% FBS, 1% P/S at 37°C/ 5% CO2 until 90% confluence. Cells were then exposed to tensile stress at 6% 0.1 Hz for either 4 or 24 hours using the Flexercell® Tension Plus System (Stretch) and compared to unstressed cells. Cells were also pre-incubated for 1 hour with 1nM 2-amino-6-(2-cyclopentylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile (ACHP), a chemical inhibitor of IKK-2, involved in the phosphorylation and activation of NFkB. Total proteoglycan and total collagen synthesis was measured via incorporation of 35S and 3H by pulse labeling for 24hours after completion of the stretching regimen and normalized to total DNA and reported as percent compared to control cells.

RESULTS
After four hours of mechanical stretch at 6% 0.1 Hz, annulus fibrosus cells demonstrate an increase in the production of total proteoglycan and collagen. However, inhibition of the NFkB pathways using ACHP demonstrates loss of this anabolic effect (Figure 1). On the contrary, prolonged (24 hours) of mechanical stretch at 6% 0.1 Hz results in a decrease in total proteoglycan and collagen production, which is not altered by inhibition of the NFkB signaling pathway by ACHP (Figure 2). Cells exposed to ACHP alone showed increase in proteoglycan and collagen synthesis after 4 hours, and decrease after 24 hours.

Acknowledgements: This work was supported by NIH/NIAMS 1R21 AR055681 and The Albert B. Ferguson, Jr. MD Orthopaedic Fund of The Pittsburgh Foundation.

DISCUSSION
These results suggest that beneficial levels of loading, such as that observed after 4 hours of moderate (6%) mechanical stretch, may in part signal through the NFkB pathway. However, these data also suggest that catabolic durations of mechanical stretch do not involve the NFkB pathway. These findings contribute to the understanding of the role of mechanical loading in matrix homeostasis. In addition, this contributes to understanding the mechanisms through which mechanical loading demonstrate a time dependent threshold. These data are critical to predicting how mechanical loading can be expected to affect the disc matrix homeostasis. This enhanced understanding of the interactions of mechanical and inflammatory signaling has the potential to lead to management strategies which would exploit key interactions between the pathways that control intervertebral disc matrix homeostasis in vivo.

References