Regulation of gene expression in intervertebral disc cells by low and high hydrostatic pressure

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INTRODUCTION: Intervertebral disc structures are exposed to wide ranges of intradiscal hydrostatic pressure during different loading excercises reaching a minimum during lying or relaxed sitting and a maximum during lifting weights with a round back[1]. We hypothesize that these different loading magnitudes influence intervertebral disc (IVD) cell metabolism, causing either anabolic effects or degenerative processes depending on their magnitudes. Therefore the aim of this study was to assess changes in gene expression of human nucleus and annulus cells after the application of low hydrostatic pressure (0.25 MPa) and high hydrostatic pressure (2.5 MPa).

METHODS: IVD cells isolated from human disc biopsies (n=23) were seeded into three-dimensional collagen-type-I matrices and exposed to the different loading magnitudes by specially developed pressure chambers. The lower pressure range (0.25 MPa, 30 min, 0.1 Hz), was applied with a recently published device by using an external compression cylinder[2]. For the application of higher loads (2.5 MPa, 30 min, 0.1 Hz) the cell-loaded collagen gels were sealed into sterile bags with culture medium and stimulated in a newly developed water-filled compression cylinder by using a loading frame. These methods allowed the comparison of loading effects in a wide physiological range under equal three-dimensional culture conditions. Cells were harvested 24h after end of stimulation and changes in the expression of genes known to influence IVD matrix turnover (collagen-I, collagen-II, aggrecan, MMP1, MMP2, MMP3, MMP13) were analyzed by real-time RT-PCR. A Wilcoxon signed-rank Test and a Wilcoxon 2-sample Test were performed to detect differences between stimulated samples and control samples and differences between low and high hydrostatic pressure. Multiple testing was considered by adjusting the p-value to 0.007.

RESULTS: Both regimes of hydrostatic pressure influenced gene expression in nucleus and annulus cells but differences in responses magnitude-to-magnitude and region-to-region were detectable. Low hydrostatic-pressure (0.25 MPa) tended to increase collagen-I expression of both annulus and nucleus cells ($p<0.05$) and aggrecan expression of nucleus cells ($p=0.031$) but significantly decreased nucleus cells MMP3 expression ($p=0.001$). The effects on all other catabolic target genes tended to decrease in both annulus and nucleus cells. High hydrostatic pressure (2.5 MPa) tended to decrease gene expression of all anabolic proteins with significant effects on aggrecan expression of nucleus cells ($p=0.004$) and a strong tendency of decreased collagen-I expression of both annulus and nucleus cells ($p=0.016$). MMP1, MMP3 and MMP13 expression tended to increase in both annulus and nucleus cells with strong tendencies for nucleus cells ($p=0.02$ for MMP13) and annulus cells ($p=0.016$ for MMP1).

DISCUSSION & CONCLUSIONS: These results demonstrate that hydrostatic pressure as one of the physiological stimuli of the intervertebral disc may regulate matrix turnover in a magnitude dependent way. Low hydrostatic pressure (0.25MPa) tends to result in anabolic effects, whereas high hydrostatic pressure (2.5MPa) tends to cause catabolic effects. Therefore, hydrostatic pressure may play an important role in the maintenance of intervertebral disc matrix but also in its degradation.

REFERENCES: [1] *Wilke HJ, Neef P, Caimi M, et al:* New in vivo measurements of pressures in the intervertebral disc in daily life. *Spine* **24:** 755-762, 1999 [2] *Neidlinger-Wilke C, Würtz K, Liedert A, et al.* A three-dimensional collagen matrix as a suitable culture system for the comparison of cyclic strain and hydrostatic pressure effects on intervertebral disc cells. *J Neurosurg* **2**: 457-465, 2005

ACKNOWLEDGEMENTS: This work was supported by the EU-project EURODISC (QLK6-CT-2002-02582)