MECHANICAL STIMULATION ALTERS PLEIOTROPHIN EXPRESSION OF IVD CELLS AND INFLUENCES ENDOTHELIAL CELL MIGRATION BY CONDITIONED MEDIA

ABSTRACT INTRODUCTION:
Disc degeneration leads to considerable alterations of their mechanical function due to the decreased hydration capacity of the disc matrix that is mainly caused by degradation of matrix proteoglycan such as aggrecan. Moreover, ingrowth of blood vessels and nerves into degenerated discs has been observed. The purpose of the present study was to investigate on a cellular level whether both tissue alterations might be related and influenced by mechanical forces. The presence of pleiotrophin (PTN, HB-GAM), an angiogenic and neurotrophic factor, has been detected in degenerated human intervertebral discs (IVD) by immunohistochemical investigations. Recent findings suggest that PTN expression in human IVDs is associated with tissue vascularisation. In other cell systems, it has been shown that PTN expression is influenced by mechanical stimulation. The present study investigated the effects of mechanical loads on PTN and aggrecan (Agg) expression in IVD cells and determined how conditioned media (CM) of disc cells exposed to mechanical loads influenced the migration and adhesion of a human endothelial cells (HMEC-1).

METHODS:
Disc cells isolated separately from the annulus and nucleus of human disc biopsies (n=9) were preincubated in three-dimensional collagen type-I gels (300,000 cells/ml gel) and stimulated by the respective physiological mechanical load: cyclic tensile strain (4%,1Hz) for annulus cells and intermittent hydrostatic pressure (0.25 or 2.5MPa, 0.1Hz) for nucleus cells as recently described. One hour after mechanical stimulation was terminated, gene expression of pleiotrophin was analyzed in relation to the unstimulated control cultures by real-time RT-PCR. Aggrecan expression was determined 1 hour and 24 hours after end of mechanical stimulation. Serum-free conditioned media of the cell loading experiments were added to DMEM (+25% CM, +ITS) of a human endothelial cell line (HMEC-1). 48 hours later, endothelial cell migration through 8µm cell culture inserts was assessed. Statistical evaluations were performed on ΔCT values, normalized to the housekeeping gene GAPDH. The ΔCT of each stimulated sample was related to the respective ΔCT of each control sample. A Wilcoxon signed rank test was performed to detect differences between the stimulated and associated control group. Multiple testing was considered by adjustment of the p-value appropriately.

RESULTS:
Application of mechanical loads altered gene expression of PTN and aggrecan as well as the release of factors influencing endothelial cell migration depending on the applied load.

Cyclic tensile strain and hydrostatic pressure tended to increase PTN expression of IVD cells (Figure 1). Annulus cells increased PTN expression significantly in response to tensile loading (+50%, p=0.004), but there was a lower and not significant increase of PTN expression in nucleus cells (+29%, p=0.09) in response to hydrostatic pressure. Aggrecan expression was increased significantly in response to cyclic tensile strain (+39%, p=0.03) but decreased significantly in response to 2.5 MPa hydrostatic pressure (mean -21%, p=0.03). Low hydrostatic pressure (0.25MPa) did not influence gene expression compared to the unstimulated control cultures.

DISCUSSION:
The present study suggests that trophic growth factors such as PTN might be involved in disc vascularisation. Mechanical loading influences PTN expression and possibly, therefore, the degree of vascularisation, which is known to increase in disc degeneration. The expression of aggrecan, the predominant disc matrix proteoglycan is also influenced by mechanical factors. Recent data suggest that intact proteoglycans may inhibit ingrowth of nerves and blood vessels into disc matrix, therefore mechanical stimuli might also play a role in the regulation of matrix biosynthesis by alteration of gene expression of matrix forming and possibly degrading proteins. Thus this study demonstrates one mechanism whereby altered loading of the intervertebral disc could influence the pathogenesis of disc degeneration.

REFERENCES:

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