Treatment with the NFkB Inhibitor Nemo-Binding Domain Peptide to Inhibit Disc Degeneration

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Mechanical overloading and activation of the inducible transcription factor nuclear factor kappa B (NFκB) are both critically involved in intervertebral disc (IVD) degeneration. Degeneration of the spinal disc is characterized by an increase in pro-inflammatory cytokines, matrix metalloprotease (MMP) and a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) levels in the nucleus pulposus, which are known to be NFκB downstream targets and to be mechano-sensitive. Yet clinically available procedures do not treat these underlying causes of IVD degeneration. NEMO binding domain peptide (NBD) has been shown to block cytokine-induced NFκB activation, to inhibit IVD degradation in a mouse model of accelerated aging by increasing disc proteoglycan synthesis, and is therefore an attractive candidate to treat disc degeneration. However, the ability of NBD to reduce NFκB-induced levels of cytokines/metalloproteases in human nucleus pulposus cells (hNPCs) under mechanical loading conditions that are known to occur in IVD degeneration, and to diminish cytokine/metalloprotease levels in a rat model for disc degeneration is unknown.

In this proposal, we hypothesize that NBD inhibits IVD degeneration processes by reduction of NFκB- and mechano-regulated cytokines/metalloprotease levels in hNPCs *in vitro*, and in an *in vivo* model of disc degeneration. The aims are to (1) demonstrate an up-regulation of inflammatory/catabolic genes in response to mechanical overloading of Il-1β-prestimulated hNPCs, simulating IVD degeneration *in vitro*, (2) demonstrate that a NBD peptide treatment decreases an up-regulation of cytokines/catabolic genes in these mechanically stimulated hNPCs, and (3) investigate the ability NBD to reduce *in vivo* degeneration, and decrease levels of cytokines/metalloproteases in rat punctured discs. Human NPCs will be isolated from lumbar discs of Pfirrmann Grade 3-5 and characterized for their NP marker expression. For an *in vitro* model for simulated IVD degeneration, Il-1β prestimulated hNPCs will be embedded in a three-dimensional matrix and exposed to high mechanical compression. Gene expression levels of MMP1, 2, 3, 7 and 13, ADAMTS4 and 5, and IL1β, 6 and TNFα in response to mechanical loading will be determined, which are known to be up-regulated during IVD degeneration. Next, different amounts of NBD will be added to identify the optimal concentration to reduce up-regulated cytokines and metalloproteases in the mechano- and Il-1β-stimulated hNPCs. Using the NBD concentrations identified *in vitro*, peptide injections of NBD into rat degenerated IVDs will be performed and their regeneration monitored with μMRI. To induce IVD degeneration, rat discs will be needle punctured prior to peptide injections. Finally, expression levels of MMP1, 2, 3, 7 and 13, ADAMTS4 and 5, and IL1β, 6 and TNFα in NBD-treated rat degenerated discs will be analyzed and compared to untreated controls.

At the end of this study, the efficacy of an NBD peptide treatment to reduce IVD degeneration by inhibiting cytokine/metalloprotease levels under detrimental mechanical conditions *in vitro* and in full biological complexity *in vivo* will be demonstrated. This may provide the basis for a new therapy to diminish the likelihood of the need of conventional surgical interventions, which are associated with risks and pain for the patient.