A FIBRONECTIN FRAGMENT CAUSES INTERVERTEBRAL DISC DEGENERATION
-- A NEW IN VITRO AND IN VIVO MODEL
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Introduction. Various animal models of disc degeneration have been developed, however, most models are limited in that they involve an artificial injury to the disc to promote a degenerative response (1). Our aim was to develop a non-traumatic model of disc degeneration based on a physiologically relevant molecular mediator. We have characterized the radiographic, histological and gene expression changes in vivo and in vitro in response to amino terminal 30kDa fibronectin fragment (FN-f) exposure.

Methods. In Vitro After obtaining Institutional Animal Care and Use Committee approval, cells were harvested from both the annulus fibrous (AF) and nucleus pulpos (NP) of New Zealand white rabbits and suspended in 1.2% alginate and cultured in DMEM/F-12 medium. Experimental cultures were exposed to 1 µM FN-f. The cells were harvested at the 4 day time point and gene expression studies were performed for the expression of MMP-9, 13, type II collagen, aggrecan, and Fas mRNAs using semi-quantitative RT-PCR with an 18s ribosomal RNA control.

In Vivo 28 male New Zealand white rabbits weighing 3-4 kg underwent random injection of 5 or 6 lumbar discs with either 1 µM FN-f or a control substance (1µM 45kDa carboxyl terminal fibronectin, 1µM rabbit albumin or PBS) into the central region of the disc. The animals were euthanized at the 2, 4, 8, 12 and 16-week time points and the spines were harvested at the 4-day time point and gene expression studies were examined with radiographs, histology and gene expression studies using RT-PCR.

Essential results.

In vivo, a single injection of 30kDa Fn-f into rabbit intervertebral disc induced radiographic and histological disc degeneration.

Discussion. Molecular studies of disc degeneration have been limited by existing animal models. Our goal was to develop an animal model based on a molecular mediator that could be used in vitro allowing rapid, well-controlled molecular studies to be performed and in vivo allowing validation of the in vitro data. FN-f was chosen as the mediator due to its known role in chondral degeneration (2) and due to the documented up-regulation of fibronectin expression (3) and fibronectin fragment accumulation (4) in degenerating discs. Our data suggest that progressive disc degeneration in the rabbit does occur following exposure to FN-f. The role of other molecular processes (e.g. apoptosis) and other mediators (e.g. nitric oxide) can easily be studied using this reproducible in vitro and in vivo system.

Reference: