Proteinases in Degeneration of the Intervertebral Disc: Cause and Effect.

S Roberts, J Menage, EH Evans, SM Eisenstein

Centre for Spinal Studies, Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry and ISTM, Keele University, UK

Proteinases are necessary for the homeostasis of connective tissues, including the intervertebral disc. Increased proteinase activity, however, has been implicated in disorders of the disc, including disc degeneration, herniation and scoliosis. Proteinases reported in the disc are many and varied, including the serine proteinases, the matrix metalloproteinases (MMPs) 1,2,3,7,8,9,13 and the aggrecanases[1]. Between them, this array of enzymes is capable of degrading all the matrix components in the disc. There may be a chronological expression of enzymes, with different ones being produced at different stages of the disease[2], as is the case for MMPs in articular cartilage during osteoarthritis. Similarly different groups of enzymes may have a relevance to different stages of disc degeneration; aggrecanases to date appear to be less prominent in disc degeneration than MMPs, but this may reflect tissue being obtained for study predominantly in late stage disease.

There are many ways in which enzyme activity, the important factor physiologically, can be modulated. MMPs, for example, are produced as inactive precursors. In addition there are at least 4 endogenous inhibitors, TIMPs, which when bound to the MMPs render them inactive.

Immunohistochemistry has shown that these enzymes and their inhibitors can be produced not only by blood vessel cells, when present, in the disc, but also by the disc cells themselves[1]. Certainly disc cells *in vitro* can synthesise MMPs and TIMPs. MMP production and activity, both *in vitro* and *in vivo*, can be altered by loading, depending on the type, size and frequency of the load and the source of the cells, whether annulus or nucleus. For example, annulus cells up regulate MMPs 3, 13 and ADAMTS-4 differentially in response to the magnitude of load, irrespective of the frequency applied, whereas nucleus cells respond to different frequencies and loads[3].

MMP production is very sensitive to cytokines, with IL-1 or TNFα stimulating disc cells to produce more MMPs. Many cytokines and other potentially stimulatory molecules such as iNOS, thromboxane, monocyte chemoattractant protein (MCP-1) and TSG-6 (TNFa Stimulating Gene), are produced in degenerate discs, again by both disc and blood vessel cells, and are likely to influence proteinase production and activity. Disc cells closest to blood vessels are often more strongly immunopositive for MMPs than those further from them[1], suggesting that they may be produced in response to stimulation by cytokines within the blood vessel.

The well known effects of proteinases are the denaturation or degradation of the extracellular matrix, with some enzymes being more effective against certain molecules. For example, the collagenases, MMPs 1,8 and 13 are necessary to cleave the collagen triple helix *in vivo*, with MMP13 being particularly effective at denaturing type II collagen. Once the cycle of degradation has begun, it can self perpetuate in more ways than one. Fragments of matrix components created by enzyme activity can stimulate the production of more enzymes and some enzymes, eg serine proteinases and MMP2, can activate other MMPs. In addition to the matrix effects, MMPs may influence cell activity. MMP1 has been shown to confer resistance to apoptosis in some cell types[4]. It remains to be seen if any of the proteinases could influence disc cells in a similar manner.

It is important to understand the mechanism of disc degeneration, which factors are key players and what parameters they are dependent on. Only with this knowledge can there be correct targeting of future therapies such as pharmaceutical intervention or gene modification.

REFERENCES: [1] S. Roberts, B. Caterson, J. Menage, et al (2000) *Spine* **25**:3005-13. [2]C.Weiler, A.G. Zipperer, B.E. Bachmeier and N. Boos (2002) *Eur Spine J* **11** :308-320. [3] J.J. MacLean, C.R. Lee, M. Alini and J.C.Iatridis (2004) *J Orth Res* **22:**1193-1200. [4]G.A. Limb, K. Matter, G. Murphy et al. (2005) *Am J Path* **166**:1555-63.