Molecular Changes in a Goat Intervertebral Disc Degeneration Model

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Introduction: One of the first signs of disc degeneration is the loss of proteoglycans from the nucleus pulposus (NP). The subsequent loss of water is hypothesized to result in a loss of pressure, leading to a collapse of the IVD and clinical signs including decreased disc height and increased signal intensity on T2-weighted magnetic resonance imaging. On a molecular level, the amount of proteoglycans in the NP decreases while the amount of collagen increases, especially type I collagen. Glycosaminoglycans (GAG) and hydroxyprolines (Hyp) are components of proteoglycans and collagens respectively, and can serve as indicators for their presence. The ratio of these substances was shown to correlate well with the severity of disc degeneration. 3

The cause of these changes in the extracellular matrix (ECM) however remains unclear. Degeneration is characterized by matrix degradation. Amongst others, matrix metalloproteases (MMPs) are held responsible for this process. Also, the production of matrix molecules is changed in degenerated discs. In the NP, collagen type I increases, replacing collagen type II by type I. Biglycans are produced in degenerated NPs while aggrecan is the main ECM product in normal discs.4

Recently we described a slowly progressive intervertebral disc degeneration in the goat5 in which degeneration was induced by means of injecting 0.25 U/ml Chondroitinase ABC (CABC), a proteoglycan side chain degrading enzyme. The chemically induced loss of proteoglycans might mimic the process of human disc degeneration. To further characterize the changes observed in this model and their analogy to human disc degeneration we determined the GAG/Hyp ratio, the gene expression of collagen type I and II, aggrecan, biglycan and MMP-13.

Materials and Methods: The samples used in this study were obtained from goats used in previous studies5,6. All research protocols have been approved by the Animal Ethics Committee. In total, 109 lumbar intervertebral discs of eighteen skeletally mature female Dutch milk goats (at least 3.5 years old) were used. Paramidsagittal slices were obtained from all discs which were firstly scored according to a modified human macroscopic disc degeneration score.6 The discs were scored either as normal (grade I-II), mildly degenerated (Grade II-III) or severely degenerated (Grade IV-V).

Next, samples were obtained from both the NP as well as the AF which were solubilized using a digestion buffer. Spectrophotometric analysis was used to measure the amount of GAGs (Bly dye; Biocolor Ltd., Newtownabbey, Northern Ireland) and Hyp (dimethylaminobenzaldehyde). The ratio of these values was subsequently calculated. Other NP and AF samples were homogenized with ceramic beads in a lysis solution by MagNalyser (Roche Diagnostics, GmbH) and total RNA was measured using the automated MagnaPure robot and the RNA tissue isolation kit II (Roche Diagnostics, Brussels, Belgium). Finally, the relative gene expression of collagen type I and II, aggrecan, biglycan and MMP-13 was determined by dividing their expression by the weighted mean of 18S and YWHAZ (housekeeping genes).

Results: In the NP of the normal, non-degenerated discs the GAG/Hyp ratio was 30:1 (see fig. 3). The ratio dropped significantly to 15 in the mildly degenerated discs (p<0.0001) and even further to 7 in the severely degenerated discs (p<0.0001 compared to normal and p<0.05 compared to mildly degenerated discs). A similar effect was seen in the AF (see fig. 1A) in which the ratio was 3:1 in normal AFs, 1.7:1 in mildly and 1.1:1 in severely degenerated discs.

The relative gene expression of collagen type I and MMP-13 increased in relation to the degeneration grade in the NP. The expression of aggrecan however significantly decreased in degenerated discs (see fig. 1B-D). The gene expression of collagen type II and biglycan did not change significantly (data not shown).

Discussion: Changes in the GAG/Hyp ratio of chemically induced degeneration in goat intervertebral discs resembles the changes seen in humans remarkably well. Also, the gene expression profile of the genes measured matches the pattern of human disc degeneration. Also, the gene expression changes are in line with the actual biochemical changes observed in the discs, corroborating the reliability of the observations.

In conclusion, we have observed biochemical and biological changes, both mimicking human disc degeneration, in a promising large animal model of disc degeneration. Previous and present data suggest that the CABC-induced intervertebral disc degeneration in the goat model resembles human intervertebral disc degeneration and therefore can be used as a valid model


Acknowledgements: This study was financially supported by Cytori Therapeutics Inc, San Diego, California, USA.

Prof. PJJM Wuisman has passed away on 25 July 2007.