AGING OF THE RABBIT INTERVERTEBRAL DISC: LONGITUDINAL MRI STUDY, HISTOLOGY AND GENE EXPRESSION ANALYSIS

INTRODUCTION: Intervertebral disc (IVD) degeneration is increasingly viewed as a process distinct from aging. Although, there is a correlation between aging and onset of intervertebral disc degeneration (IDD), degeneration is not observed when secondary injuries do not occur. However, the cells of the IVD are continuously subject to hypoxia, low pH, low nutrition, and high pressure during the aging process. Thus the tissue undergoes continuing adaptation which will differ from the injury related cascade.

Previously, our group established a reproducible rabbit model of IDD induced by anterolateral “stab” of the annulus fibrosus, characterized by MRI, x-ray, histology, and mRNA expression.

The current study evaluates the discs from normally aging rabbits compare several parameters in the aging and degenerating IVDs.

METHODS: IACUC approval was obtained for the use of New Zealand white rabbits. MRIs of 4 skeletally mature female rabbits were obtained at 0, 12, 24, 60, 120 and wks using 1.5T clinical magnet with a commercial surface coil. T2-weighted mid-sagittal images of L1-2 (considering that the degeneration model ultimately exhibited nearly 70% decrease in MRI Index by 24 wks). T2-weighted mid-sagittal images of L1-2 (considering that the degeneration model ultimately exhibited nearly 70% decrease in MRI Index by 24 wks). The histological results, on the other hand, showed that dramatic cellular changes occurred in the aging model, though overall disc structure was well-preserved. The gene expression analysis demonstrated a decrease in the expression of collagen genes while the proteoglycan genes increased. This is in contrast to the observed decrease in both proteoglycan and collagen gene expression in the degenerative model. The increased production of BMP-2 and TIMP-1 gene expression overtime suggests an attempt of the aging disc to resist the catabolic cascade. This aging is in contrast to the degenerative model, which demonstrated decreased expression of these genes. The low SOX-9 expression in the young disc parallels the notochordal phenotype of the disc during development with a chondrogenic shift in phenotype occurring during maturation and aging process. The expression of TGF-β demonstrated a biphasic response, which was also present in the degenerative model, suggesting important variables in the control of this gene.

These data showed a remarkable difference between the aging model and IDD model in rabbits. Therefore the morphological and phenotypical changes of the aging might be considered a normal physiological process while those associated with degeneration could be considered as reaction to a injury.

RESULTS: Through 24 weeks, mean MRI Index decreased by <5%. However, by 60 and 120 wks, MRI Index had decreased 19% and 25%, respectively (Figure 1). The histological analysis showed a change in the tissue composition. The NP showed the presence of notochordal cells in the young, fibro-chondrocytes in the adult and hypertrophic chondrocytes in the aged rabbit disc. The extracellular matrix (ECM) of the NPs became more dense and fibrotic with aging. No osteophytes formation was observed in any of the animals.

The PCR analysis showed that collagen type Ia gene expression decreased with aging becoming undetectable at 120 weeks. Collagen type IIa mRNA was significantly higher in the young IVDs (p<0.01). In contrast, aggrecan was significant lower in the young (p=0.009). Biglycan mRNA was significantly lower in the young (p=0.04) and higher in the aged (p=0.07) compared with the adult. In the context of the anabolic growth factors, BMP-2 mRNA was significant lower in the young discs (p<0.0005) and higher with the adult. The TGF-β1 mRNA was significantly lower in the young (p=0.008) and old (p=0.01) compared with the adults. As measure of anticytobolic gene expression, TIMP-1 mRNA was significant lower in the young IVDs (p=0.001) compared with the adult. The chondrogenic transcription factor SOX-9 mRNA was significant lower in the young (p=0.04) (Figure 3). The mRNA expression of aggrecan, BMP-2 TIMP-1 and SOX-9 showed a trend toward decreased expression among aged discs, but this did not match statistical significance.

DISCUSSION: In this normal aging model, rabbit lumbar discs experienced 25% decrease in MRI Index over 120 wks—whereas our degeneration model, this magnitude of decrease had occurred within just 3-6 wks of annular stab. Age-related MRI changes thus occurred at a much slower rate than degeneration-related MRI changes. The magnitude of age-related MRI Index decrease was relatively modest (considering that the degeneration model ultimately exhibited nearly 70% decrease in MRI Index by 24 wks). The histological results, on the other hand, showed that dramatic cellular changes occurred in the aging model, though overall disc structure was well-preserved. The gene expression analysis demonstrated a decrease in the expression of collagen genes while the proteoglycan genes increased. This is in contrast to the observed decrease in both proteoglycan and collagen gene expression in the degenerative model. The increased production of BMP-2 and TIMP-1 gene expression overtime suggests an attempt of the aging disc to resist the catabolic cascade. This aging is in contrast to the degenerative model, which demonstrated decreased expression of these genes. The low SOX-9 expression in the young disc parallels the notochordal phenotype of the disc during development with a chondrogenic shift in phenotype occurring during maturation and aging process. The expression of TGF-β demonstrated a biphasic response, which was also present in the degenerative model, suggesting important variables in the control of this gene.

These data showed a remarkable difference between the aging model and IDD model in rabbits. Therefore the morphological and phenotypical changes of the aging might be considered a normal physiological process while those associated with degeneration could be considered as reaction to a injury.

REFERENCES:

AFFILIATED INSTITUTIONS FOR CO-AUTHORS:
+Department of Orthopaedic and Traumatology, Campus Bio-Medico University, Rome, Italy
+Department of Physical Medicine and Rehabilitation, University of Pittsburgh School of Medicine, Pittsburgh, PA USA