INTRODUCTION: The functions of the intervertebral disc (IVD) as a unit, and of the annulus fibrosus (AF) and nucleus pulposus (NP) individually, are determined by the molecular composition of their extracellular matrices (ECM). The outer- and inner-AF consists of large collagen fibers in oblique layers, whereas the central NP contains a loose matrix rich in proteoglycans (PGs). Small-PGs (biglycan, decorin, lumican, and fibromodulin) bind to collagens, growth factors and other matrix components thereby regulating the assembly of the ECM and repair after injury. Loss by proteolytic fragmentation of large and small PGs may induce or inhibit essential inflammatory response pathways altering the ability of the ECM to maintain its structural integrity.

Aging is considered a primary factor in symptomatic IVD degeneration. Since age-related changes in ECM molecules may influence matrix quality potentially contributing to IVD degeneration, the purpose of this study was to quantify the age-related changes of a select group of ECM molecules, including collagen and PGs, in the AF and NP of human IVDs.

METHODS: IVD specimens obtained (through the Gift of Hope Organ and Tissue Donor Network) from both male and female thoracolumbar spines (T11-L5) represent a range of ages from 32-80. Samples used for this study met the approval of the Institutional Review Board. All spines (T11-L5) represent a range of ages from 32-80. Samples used for this study met the approval of the Institutional Review Board. All specimens were analyzed by decade of life. Only specimens that were classified as Thompson Grade II according to MR evaluation were analyzed in this study.

IVDs were dissected and the AF and NP were separated. DNA (Hoechst assay), proteoglycan (DMMB assay) and collagen (OH-proline HPLC assay) contents were determined for all samples. Select sets of samples (5-8 samples per decade of life) were processed for protein extraction [1,2]. Levels of biglycan, decorin, fibromodulin and lumican were semi-quantified by comparative Western blotting [1,2] by measuring the density of the immunoreactive bands on the transferred membranes with comparison to an internal control. Standard deviations within each decade and the significance of change during aging were calculated using ANOVA analysis.

RESULTS:

Changes in Collagen and total PG content
Collagen content (normalized to DNA) decreased with aging in both AF and NP. The lowest concentration was observed in the 70-80 year range (p <0.05) in both AF and NP. Proteoglycan content (normalized to DNA) showed a similar decrease. The lowest content of PG occurred in the 70-80 year range (p < 0.05). (Figure 1)

In the inner-AF, biglycan demonstrated an increasing concentration with aging (p < 0.05), while the levels of other small PGs did not change significantly. All populations of biglycan (intact, degraded, and total) reached their peak values during the 70-80 year range.

NP: Biglycan demonstrated a similar variation in concentration as in the inner-AF. The total and intact component of biglycan increased with aging with the highest concentration occurring in the 70-80 year range (p < 0.05). The degraded component of biglycan showed a decreasing trend in concentration with aging, though this was not statistically significant (p=0.12). (Figure 2)

DISCUSSION: The primary limitation of this study is related to specimen availability. Only grade II degenerated discs were analyzed, thereby controlling for metabolic changes associated with varying degrees of disc degeneration. These two factors, age and Thompson’s criteria, limit sample size; however, a more stringent analyses of age- related metabolic changes can be undertaken.

Our work demonstrates that the concentration of collagens and PGs decrease with aging in both the AF and NP. The overall decrease in total PG and collagen concentration with aging may be suggestive of a reduction in cellular biochemical activity and ECM production.

Additionally, the data presented here suggests that small-PGs undergo metabolic variation with aging. Small PGs demonstrated increased levels in either the AF (lumican and fibromodulin) or NP (biglycan). Biglycan appears to be the only small-PG in the NP that is up regulated with advancing age. A coordinated role of fibromodulin and lumican during collagen fibrillogenesis has been implicated as a factor in the degenerative cascade. The fragmentation and release of fibromodulin elicits an inflammatory response. Biglycan, on the other hand, has a crucial role in the formation of the pericellular matrix and acts as a regulator of growth factor activity [3]. Thus, the presence of these small PGs in increased concentrations in aging IVD tissues may affect the quality of the tissue.

In conclusion, these results may imply that the decrease in the levels of matrix components in the AF, and to some extent in the NP, during aging may be a contributing factor to the weakening of the collagenous network. Age-related alterations in PGs and collagens may make older tissues more susceptible to degeneration when biomechanically loaded. Additionally, we hypothesize that the increase in biglycan and fibromodulin in older tissues may further contribute to the failure of the proper cellular reparative processes.


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