AGE RELATED CHANGES IN APOPTOTIC RATE IN THE MURINE INTERVERTEBRAL DISC

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RELEVANCE TO MUSCULOSKELETAL CONDITIONS:
The role of aging in the etiology of disc degeneration is not fully understood. Apoptosis (programmed cell death) may play an important role in this process. The current study examines the relation of aging to the apoptotic index and the distribution of cells undergoing apoptosis in intervertebral discs.

INTRODUCTION:
Intervertebral discs undergo age-related degenerative changes. There are numerous factors involved in disc degeneration, such as declining nutrition, loss of viable cells, cell senescence, post-translational modification of matrix proteins, accumulation of degraded matrix molecules, and fatigue failure of the matrix to name a few. Apoptosis may be a major cause of disc degeneration and also a possible "common pathway" for other effecting processes. Therefore, we chose to study the trends of apoptotic index in relation to age in an animal model.

METHODS:
Intervertebral discs from the tails of sixteen C57BL/6 male mice were divided according to age (1, 12, 22 and 36 months) with four animals in each group. After euthanasia, tail segments were dissected, fixed, decalcified, embedded in paraffin and sectioned. Hematoxylin and Eosin staining visualized morphological and apoptotic changes. Programmed cell death was visualized by in-situ TdT-mediated nick end labeling of DNA fragments with fluorescein-dUTP (In situ cell detection kit, Promega). Three serial sections from each segment were analyzed with an imaging system and evaluated for the percent of cells undergoing apoptosis (Zeiss imaging software). The percent apoptotic cell index (ACI%) was determined by the following formula: ACI% = (ΣA1/ΣA2) X100%, where A1 is the sum of all cells positive with fluorescein-dUTP within a defined area, and A2 is the sum total of all cells in the specified area.

RESULTS:
Programmed cell death was detected in cells from the nucleus pulposus (NP) and the inner annulus of the discs (Figure 1). An increase in ACI% was observed up to 22 months of age in the NP. In contrast, in the AF, ACI% did not increase considerably (Figure 2). The intervertebral discs of 36-month-old mice showed a near total absence of cells in the AF and the NP. The results were supported by the lack of nuclear staining with H&E and lack of staining for programmed cell death using the Tunel method. Statistically, the ACI% data for both the NP and the AF at 1, 12, and 22 months were treated by a one-way analysis of variance. Results were considered significant for p-values of less than 0.05. The data were first examined to ensure that normality and equal variance tests were satisfied. For the NP specimens, there was a statistically significant change in ACI% between 1, 12, and 22 months (p<0.01). The power of the test was 1.0. For the AF specimens, there was not a statistically significant change in ACI% (p>0.618). The power of the test was low.

DISCUSSION:
To the best of our knowledge there is only one previous report of apoptosis in intervertebral discs retrieved from human cadaveric and surgical samples. We present here a model which enables studying apoptosis in intervertebral discs in a controlled, consistent, and reproducible manner. The findings are consistent with previous data concerning the progressive decrease in cellularity of the NP with aging. It suggests a possible explanation of this phenomenon of decreasing cellularity, which may be related to the high ACI% observed in younger specimens. The apoptosis occurring in intervertebral discs is probably part of the normal physiological homeostatic mechanism. It may also be related to disc degeneration through factors that increase the rate of apoptosis, thus possibly inducing premature aging and degeneration. The current model and its findings will serve as a basis for further investigation into different factors affecting apoptosis in the intervertebral disc.

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